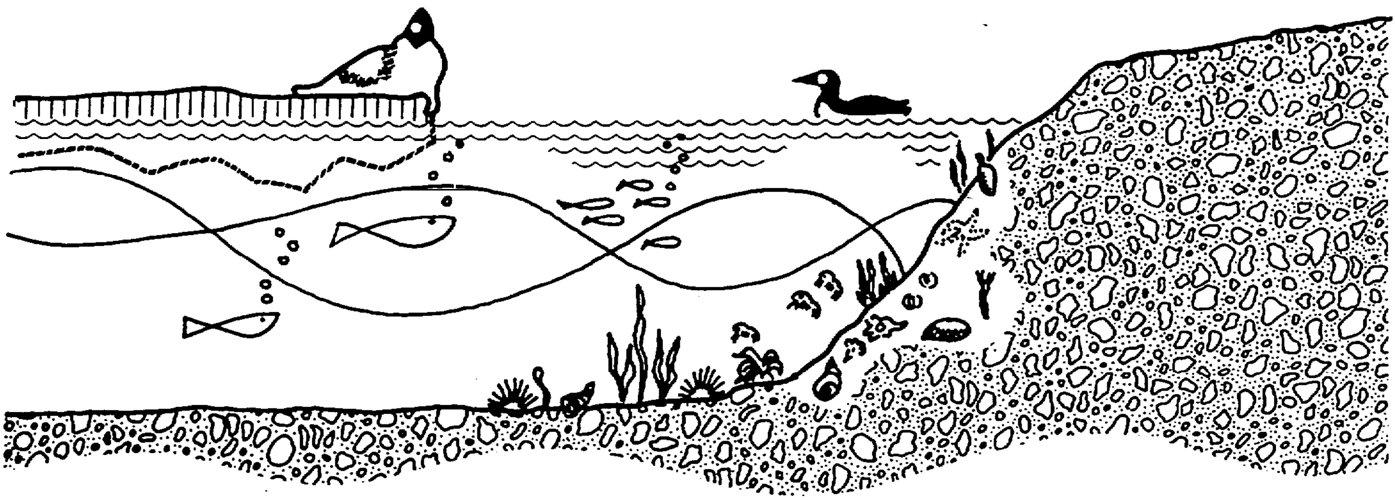
# MICROBIOLOGY

## 2. Biodegradation of Oil



**Baffin Island Oil Spill Project**

**WORKING REPORT SERIES**

BIOS CAPE HATT

1982 STUDY RESULTS

# The Baffin Island Oil Spill Project

## OBJECTIVES

The Baffin Island Oil Spill (BIOS) Project is a program of research into arctic marine oil spill countermeasures. It consists of two main experiments or studies. The first of these, referred to as the Nearshore Study, was designed to determine if the use of dispersants in the nearshore environment would decrease or increase the impact of spilled oil. The second of the two experiments in the BIOS Project is referred to as the Shoreline Study. It was designed to determine the relative effectiveness of shoreline cleanup countermeasures on arctic beaches.

The project was designed to be four years in length and commenced in 1980.

## FUNDING

The BIOS Project is funded and supported by the Canadian Government (Environment Canada: Canadian Coast Guard; Indian and Northern Affairs; Energy, Mines & Resources; and Fisheries & Oceans), by the U.S. Government (Outer Continental Shelf Environmental Assessment Program and U.S. Coast Guard), by the Norwegian Government and by the Petroleum Industry (Canadian Offshore Oil Spill Research Association; BP International [London] and Petro-Canada).

## WORKING REPORT SERIES

This report is the result of work performed under the Baffin Island Oil Spill Project. It is undergoing a limited distribution prior to Project completion in order to transfer the information to people working in related research. The report has not undergone rigorous technical review by the BIOS management or technical committees and does not necessarily reflect the views or policies of these groups.

For further information on the BIOS Project contact:

BIOS Project Office
#804, 9942 - 108 Street
Edmonton, Alberta
T5K 2J5

Phone: (403) 420-2592/94

Correct citation for this publication:

Eimhjellin, K., Nilssen, 0., Sommer, T. and Sendstat, E., 1983, Microbiology: 2. Biodegradation of Oil - 1982 Study Results. (BIOS) Baffin Island Oil Spill Working Report 82-6: 41 p.

# BAFFIN ISLAND OIL SPILL PROJECT

MICROBIAL DEGRADATION OF OIL

## MEASUREMENTS IN RAGGED CHANNEL TEST BAYS AND ENHANCED BIODEGRADATION EXPERIMENTS IN Z-LAGOON, A POSTSPILL SURVEY,

Kjell Eimhjellen, Ole Nilssen and Tor Sommer
Department of Biochemistry, Norwegian Institute of Technology,
N-7034 Trondheim - NTH, Norway

Erling Sendstad
The Foundation of Scientific and Industrial Research at the
Norwegian Institute of Technology (SINTEF),
N-7034 Trondheim - NTH, Norway

March, 1983

Participation sponsored by
The Royal Ministry of Environment, Norway
through their research programs:
The Norwegian Marine Pollution Research and Monitoring Program (FOH)
Oil Pollution Control Research and Development Program

## SUPMARY

This report summarizes the results from the first postspill sampling after the experimental oilspills in 1981 with the objective to monitor the possible longterm effects of oil on the activity of the microbial degradation of petrogenic hydrocarbons in the nearshore waters and sediments. At the same time the results from a number of experiments on enhanced biodegradation o-f oil in backshore sediments are reported. The evaluations are based essentially on the results from one set of samples taken in the period August 18 to September 4, 1982.

Relative to 1981 the waters of the test bays appeared to have somewhat increased levels of generally heterotrophic as well as oildegrading bacteria. The rates of degradation (per unit volume of water) of weathered Lago Medio crude oil (assessed by tritiated Lago Medio) were close to or above the highest activity recorded in 1981. And in the same water samples the rates of biodegradation of n-hexadecane was 2-5 times higher than normally observed in 1981. These results seem to indicate a change in the biochemical aptitude of the bacterial populations of the water in handling petrogenic hydrocarbons. Some quantitative variation between the bays were observed, the water of Bay 10 having the lowest activity.

The oiled sediments of the tidal zone in Bay 11 (the site for the surface oilspill in 1981) had very high numbers of oil degrading bacteria (ODB), in the range $7 \cdot 10^6$ - $7 \cdot 10^7$ ODB per ml sediment. This may explain the high numbers of ODB occasionally found throughout the water column in Bay 11.

The sediments of Bay 7 and 11 seemed to have populations of bacteria and biochemical activity for oil degradation that were typical for the situation in 1981. In Bay 9 and 10, however, the bacterial counts as well as the assessed specific rate of degradation of n-hexadecane were noticeably higher than in the sediment of the two other bays. According to the analyses of Erco'the sediment from these two bays were the only ones containing low, but significant amounts of petrogenic hydrocarbons immediately after the dispersed oilspill in 1981. Chemical analyses of 1982 samples showed increased levels of oil in the sediments, particularly in Bay 11, but gave no strong indications of biodegradation.

The test plots for enhanced biodegradation of oil emulsion laid down in 1981 in Bay 102 were relocated and sampled after the flooding incident in late August 1981. The results from the microbiological analyses seem to indicate quite clearly that the microbial activity within the buried oil-sediment layer was very high and, apart from a contamination of the unfertilized control plots by fertilizer from the neighboring plots, the fertilizer dependent level of bacteria observed in 1981 was still apparent. The oiled sediment having received the highest concentration of fertilizer had $1.3 \cdot 10^8$ ODB per ml of sediment; in the other fertilized plots the counts of ODB were lower by a factor of 10 to 50. No direct measurements of carbon dioxide production from the sediments were made.

After two days the unfertilized plots in Bay 102 had counts for ODB of $0.7.10^5$ (oil-emulsion) and $7 \cdot 10^5$ (crude oil) per ml sediment, indicating essentially no change since 1981.

A new set of test plots for an enhanced oil biodegradation experiment in the backshore of a low energy beach was laid down in Bay 106 inside the Z-1agoon, with the hope that these oil-sediment surfaces will remain exposed. After 16 days the fertilized oil-emulsion plots contained ODB in excess of $10^8$ per ml sediment, the sediment with crude oil an order of magnitude less. The unfertilized control plots had counts of ODB factors of 20-40 lower than their fertilized counterparts.

ACKNOWLEDGEMENT

We are grateful to the crew from Woodward-Clyde Consultants that relocated and remarked our experimental plots for the enhanced biodegradation experiment in Bay 102. Thanks to the great help and cooperation from Project Manager Peter Blackall we were able to establish the new experimental plots in the backshore of the low energy beach in Bay 106. As usual we owe great thanks to our microbiology colleagues from the Arctic Biological Station, Montreal and to the personnel at the Project Office and of Camp Cape Hatt.

We like to acknowledge the financial support from the Royal Ministry of Environment, Norway, through their research programs PFO and FOH.

TABLE OF CONTENTS

## LIST OF FIGURES



## LIST OF TABLES

# 1. INTI?ODUCTION

The Baffin Island Oil Spill Project (B1OS Project) is a study designed to evaluate the short-term and the long-term effects of oil spilled on arctic shorelines and in shallow waters. The study is devided into two projects, a near shore project for comparison of effect and fate of two different types of oil spills to extract the essential consequence of using a chemi-al dispersant in an arctic environment, and an on shore project aimed at assessing the effect of natural physical forces in the recovery of oilpolu-ted shorelines and to test practical cleaning techniques.

The Norwegian group of microbiologists has been involved in both projects. In the near shore project we have been concerned with the activity for microbial degradation of oil in the water column and in the near shore bottom sediments before and after the oil spills. A baseline investigation in 1980 and a prespill period in 1981 were designed to indicate initial activity of the microbial population potentially able to handle petrogenic hydrocarbons. The main objectives for the post spill studies are to monitor the possible effect of the discharged oil on this activity. In relation to the onshore oilspill countermeasures we have started a series of enhanced biogradation experimentsto testto whatextent artificial fertilizer may im-prove the natural rate of microbial degradation and weathering of oil that is washed up into the backshore sediments.

The 1982 field work had the following spesific objectives:

- to carry out a minimum of one set of monitoring analyses for oildegrading bacteria and total heterotrophic bacteria in the water and sediments of the BIOS test bays and to assess the rates of microbial degradation of radioactive Lago Medio weathered crude oil and some selected individual hydrocarbons in the same samples.

- to retrace the shoreline enchanced biodegradation test plots that were covered by sand in the fall of 1981 due to storm and high tides and if possible, to determine the level of microbial activity in all the test plots established in Bay 102 in 1980 and 1981.

to assess the microbial activity in the crude oil control plots at "Crude oil point". One segment of these plots were fertilized in September 1981.

- to establish 4 new test plots in Bay 106 for enhanced biodegradation experiments in the backshore of a low energy beach.

- to assess the microbial activity in the sediments of the tidal zone that was covered by oil during the surface spill in 1981.

This report summarizes the results of the 1982 work at Cape Hatt, August 16 to September 6.

# 20 MATERIALS AND METHODS

## 2.1. SAMPLING PROCEDURES

Mater samples at 0.1, 5 and 10 m depth and samples of beach sand a-rid bottom sediments were taken and handled as previously described (1, 2).

The water samples collected August 20, 23 and 30 coinsided with sampling cycles 3, 4 and 5 of the sampling program for the Canadian microbiology group (see Table 1 for the relationship between cycle and bay).

## 2.2. EXPERIMENTAL AND ANALYTICAL METHODS

### 2.2.1. Hydrocarbon mineralization experiments

The generally tritiated Lago Medio weathered crude oil ($^{3}H$-Lago Medio), 1-$^{14}C$-*n*-hexadecane and [1(4,5,8)-$^{14}C$]-naphthalene had the same spesific activity as referred to in (2). The radiochemicals were used undiluted in mineralization experiments carried out as described in (2).

### 2.2.2. Microbiological analyses

The most probable number techniques used to determine the counts for total viable heterotrophic bacteria (TVH) and oil-degrading bacteria (ODB) in water, sediment and beach sand samples are described in (l). For the water-samples collected in Bay 7 to 11 equal volumes from each depth at the two stations in each bay were mixed and two separate aliquots were drawn for parallel microbial analyses. The arithmetic mean value of the two analyses were used to represent the cell-count at each depth.

### 2.2.3. Procedures connected to the onshore enhanced biodegradation experiments

The 102 A-C and 102 D-k! plots established in 1980 and 1981 were flooded by a combination of excessive high tides and strong waveaction August 29 and 30 1981. A detailed description of the events as given in (4). The plots were relocated and remarked in early August 1982 by the Woodward-Clyde crew and sampled by the procedures outlined in (2).

As a partial replacement of the plots in Bay 102 assumed to have been lost, two segments of the crude oil control plot T 1 at "Crude oil point" in the Z-lagoon were selected for microbial surveillance. This plot had been set up in August 1980 to serve as a control for assessment of the natural physical and biological weathering of crude oil deposited in the backshore area of a medium energy beach. The sediments characterized as sandy pebble had been covered by 10 $kg/m^2$ s'ightly aged crude l-ago Medio. For detailed description of the site, see (3). It has to be stressed that the coarse sediment structure of the site was not very conducive for microbiological sampling. One of the segments were only marked to serve as unfertilized control (see Fig. 2). The other segment consisting of the outer westbound periferial area of T 1 (1 m by 4 m) was fertilized by 80 $g/m^2$ Norsk Hydro Fullgjødsel C (for declaration, see 2).

A new test plot area for enhanced biodegradation experiments was established in 1982 in teh supralittoral zone of Bay 106. This plot together with other plots laid in the same bay by Woodward-Clad served the purpose of testing natural and enhanced weathering of oil stranded in a low energy beach area. Two plots of 1 x 2 m were marked about 10 m up from the assumed swash line, see Fig. 2 for position and outline of the plots in Bay 106. One plot was covered by 10 $kg/m^2$ Lago Medio crude oil. The other plot was covered by oil-water emulsion (1:1). Due to very low adsorptive properties Of the sediments only about 10 $kg/m^2$ of the oil-emulsion could be applied. Each plot was divided in half and one half of each plot was fertilized by 100 $g/m^2$ Norsk Hydro Fullgjødsel C. The fertilizer was evenly spread by hand.

The plots were oiled August 19, 1982. The plots are obviously smaller than we wanted, but the shortage of oil limited their size.

# 3. RESULTS AND COMMENTS

For our group the post-spill monitoring work in 1982 took place during the 3 weeks of August 16 to September 6. Partly due to the time required' for the analyses that had to be carried out at Cape Hatt and partly due to the number of tasks that had to be done, the sampling program for each project is rather limited. With a few exceptions only one sample was taken in each sampling area. A composit sample was normally used when sampling sediment and sand. This would to some extent improve the sampling statistics inherent to such a limited sampling program. For water samples the situation is different. The single set of analyses of water is compared with the results from the more extensive sampling program in 1981 in an attempt to evaluate the post spill situation in 1982. The implication of these facts should be born in mind when reading this report.

## 3.1. NEAR SHORE PROJECT

The two major tasks of the BIOS Project, the surface spill in Bay 11 and the dispersed-oil spill in Bay 9 were very successfully carried out during the latter part of August 1981. Prior to the spills two full cycles of microbiological and biochemical monitoring work were completed, i.e analyses of two water-samples from all stations and depths and two bottom sediment samples from most bays. After the spills the possible short term effects of petrogenic hydrocarbons on the microbial activity connected to the degradation of hydrocarbons were monitored for another 4 sampling cycles.

Except for a possible slight build-up of oil degrading bacteria noticeable at 5 m depth in Bay 9, we could not detect any effect, positive or negative, from dispersed oil on the microbial activity related to hydrocarbon degradation. In the bay of the surface spill a reduced level of total heterotrophic bacteria were observed in the surface water immediately after the spill; the normal level of bacteria was re-established after about 2 weeks. During the same period about a 10 fold increase in oildegrading bacteria occurred in the surface water. A similar increase, but less marked, was observed at 5 m depth. In Bay 7 (control bay) and in Bay 11 the oil-concentration of the bottom sediments remained essentially uncontaminated for weeks following the oilspills.

A very slow possible increase may have been observed in Bay 11 late in the season. The sediments of Bay 9 and Bay 10 definitely received oil subsequent to the dispersed oil spill. On the average the oil-concentration in Bay 9 sediments were elevated an order of magnitude to 5-10 ppm. Immediately following the spill levels of 2-5 ppm could be detected in the sediments of Bay 10, but subsequent sampling revealed values only 1-3 times the general background level (5). In all cases the oil concentrations found in the bottom sediments have to be considered very low. The microbiological and biochemical analyses of the sediments showed no sign" ficant effects of the petrogenic hydrocarbons during the 3-4 post spill weeks.

The post-spill investigations in 1982 an( 1983 have the purpose to monitor the long-term effects of the oil released into the environment in 1981. For financial reasons these investigations had to be rather limited. The program for the 1982 near shore studies consisted of one full cycle of microbiological analyses of water samples from 3 depths at all stations (8 in number) in the 4 test bays; Bay 11 was actually sampled twice, one week apart. From each station one composit sediment sample was taken for microbiological analyses. The biochemical analyses for degradation of radioactive oil and hydrocarbons had to be limited to one 5 m watersample from each bay and one sediment sample from each microbiology station.

### 3.1.1. Microbiological analyses of water and bottom sediments

The data for total viable heterotrophic bacteria and oildegrading bacteria in the water column at 0.1, 5 and 10 m in Bay 7, 9, 10 and 11 are given in Table 1. To improve the statistics of the analysis, keeping the amount of work constant, the watersamples from the same depth at both stations in each bay were mixed and subsampled for analysis. The table gives for each depth the arithmetic mean of two analytical figures.

The findings are summarized and compared to the results in 1980 and 1981 in Table 7. For 1982 and 1980 all available data are used, for 1981 we have taken the data for the corresponding period in late August.

As found previously there is a considerable variation in the bacteria population at the various depths, indicating a pronounced stratification of the with the bacterial population in the surface water. At times the situation may be different, as illustrated by the analyses of samples from Bay 11 in cycle 5 (August 30). That day fairly equal and high numbers of oil degrading bacteria were found throughout the water column. Except for two figures obtained in 1980 the numbers found in these watersamples from Bay 11 represent the highest level of oil degrading bacteria recorded in Ragged Channel during the BIOS Project. And particularly noteworthy is the high proportion of oildegrading bacteria. A few days earlier the count for the same bacteria was equal to the level found in the other test bays. This may indicate a substantial influence of the sampling time with the tidal cycle. The occasional high levels of bacteria and particularly of the oildegrading type in the water of Bay 11 may be caused by a flux from the oiled tidal zone which contains high numbers of the same bacteria in the sediments (see Table 3). Unfortunately we have only biochemical analysis of one of these 5 m watersamples, the one having the low bacterial count of August 20.

Boehm (6) finds a pronounced patchiness of the oil in the bottom sediments of Bay 11 and a notable degree of biodegradation of the residual oil in areas with the highest concentration of oil. This may, in his opinion, suggest transport of biodegraded oil from the intertidal zone to the bottom sediments of the Bay, and our data may support this contention.

In general the level of bacteria appears to be higher in the water samples from 1982 compared to 1980 and 1981. This apply to both groups of bacteria. The conclusion is mainly based on the high figures recorded in 1982, but the general average figures for all depths leaves the same impression. The biochemical analyses substantiate this conclusion. Apart from the single observation for Bay 11 on August 30 which may be directly associated with its oiled beach, there seems to be no significant difference between the water from the various bays. The general tendency for an increased activity of biodegradation of oil may possibly be linked to the observed low, but significant level of petrogenic hydrocarbons in the Ragged Channel (6).

The results from the microbiological analyses of the bottom sediments of the test bays are given in Table 2. The figures for each bay is averaged and a comparison with the average figures for the sediments from the same bays in 1980 and 1981 is presented in Table 8.

In no case does the level of oil degrading bacteria show any appreciable increase as a possible consequence of the small amounts of oil found in the sediment in some of the testbays, particularly Bay 9 and 10. On the average Bay 9 and 10 appear to have higher levels of total heterotrophic bacteria compared to the situation in the previous two years and also to possess increased levels of oildegrading bacteria, but the maximal figures do not exceed any single high figures observed in 1980 and 1981. Bunch (private communication) using a different method of enumeration, has observed an increase in oleoclasts in the Bay sediments from 1981 to 1982. Their absolute numbers are lower than our figures, but the noticeable increase is not inconsistent with our combined microbiological and biochemical observations (see 3.1.2.).

### 3.1.2. Mineralization of weathered Lago Medio crude oil and defined hydrocarbons

As usual the 5 m depth water sample at the microbiological stations were used to represent the waterbody in the experimental bays in the biochemical assessment of the capacity for mineralization of radioactive hydrocarbons. In an attempt to improve the statistics of the measurements, equal volumes of water from the two stationsin each bay were mixed and used in several parallel experiments. The results of the experiments from one set of samples from Bay 7 to 11 taken August 20-23 are given in the Tables 9 A-C.

Table 9 A and 9 B summarize the data from the experiments with tritiated weathered Lago Medio crude oil ($^{3}$H-Lago Medio) and $^{14}$C-n-hexadecane. The results are presented in two ways. In a number of experiments for each sample the test system contained 10 μg of hydrocarbon substrate. The quantitative results from these experiments are expressed as cpm of the mineralized product which accumulated over the entire incubation period (13 days) and the time related accumulation of products is used to calculate the rate of mineralization. This rate is designated $V_{10}$.

For $^{3}$H-Lago Medio also a maximal rate of mineralization ($V_{max}$) was determined, by a kinetic evaluation of the results from a set of 5 experiments with increasing concentration of substrate from 1 to 20 μg. $V_{max}$ represents an expression of the maximal capacity of the microbial population of the sample for metabolizing oil at optimal substrate concentration. The results from the mineralization experiments with n-hexadecane did not lend themselves to this mathematical treatment.

The conversion of naphthalene was too slow to permit any rate assessment within the incubation period of 13 days. Therefore only the cpm's of $^{14}CO_2$ from the radioactive substrate are given in Table 9 C as indications of biodegradation.

Based on -the average $V_{10}$-values for mineralization of $^3$H-Lago Medio the activity over the summer season in 1981. had a marked maximum during the first two weeks of August (approx. 35 µg/$m^3$, d). The activity decreased rapidly to a plateau of 15-20 µg/$m^3$,d for the rest of August and early September.

This trend was very much the same in all bays, except for some deviation in the activity pattern of the various bays towards middle of September (Fig. 12 and 13 in (2)). Due to the limited sampling program we have of course no way of telling whether the oildegrading activity followed a similar quantitative pattern in 1982.

The determinations of V,. for $^3$H-Lago Medio in the watersamples in late August 1982 had satisfactory standard deviations. Between the bays, however, the average values for V,. varied more than seen last year for a single set of analyses, from 11.8 to 47 µg/$m^3$,d. The latter value found for Bay 7 is close to 30% higher than the highest average value for all bays in 1981, indicating a reasonably marked increase in activity. In Bay 9 and 11 the activity was close to the maximal activity found in 1981, whereas in Bay 10 the activity was very much in the low range. The same qualitative picture of the variation between the bays can be seen from the cumulative levels of radioactivity in the $^3H_2O$.

The results from the mineralization of 14C-n-hexadecane confirm the considerable variation in activity between the bays, with Bay 7 and Bay 9 having the highest activity and again Bay 10 having the lowest. These experiments have an appreciable scattering in the results due to a rather long lag-period prior to the dissolution of the n-hexadecane into the waterphase at this

1 ow temperature, which will again affect very much the availability of the substrate.' Nevertheless, the average values for $V_{10}$ are higher than recorded earlier, emphasizing a change in the potential for oil degradation in the bacteria of the water of Ragged Channel. This may be in line with the comments on the microbiological data and the observed low levels of petrogenic hydrocarbon in the Channel.

The results from the biodegradation of naphthalene in the watersamples can only be evaluated qualitatively. All the 21 experiments give detectable levels of radioactivity in $^{14}CO_2$ after 13 days of incubation period. In 1981 only-about 20% of the watersamples gave positive results. This again indicates a raised level of activity for biological mineralization of hydrocarbons in this environment.

8 sediment samples, one from each of the 8 microbiology stations were also analyzed for their ability to degrade hydrocarbons. Each sample were analyzed in triplicate and the 6 results for each bay are grouped together and the results presented in Table 10 A.

Due to an unfortunate mistake the preparation of the experiments 1 µg instead of 10 µg $^{3}$H-Lago Medio was used in most of the analyses. We have no direct comparison for the Vi-values obtained from these experiments. The $V_{10}$-value found for Bay 9 is equal to the highest rates measured in 1981 for the bay sediments (Table 5 in (2)). Bay 9 and 10 have clearly higher rates for $^{14}$C-hexadecane degradation than 7 and 11. Altogether this may be interpreted as a positive influence of the oil found in the sediment of the former bays. The degradation of naphthalene is however very weak; only in 10 out of 24 tests (6 per bay) could very low, but significant level of radioactivity in the carbon dioxide be detected. This result seems to be at variance with the results of Boehm (6). In the bottom sediments of Bay 9 and 10 the residual low amounts of oil show no change in the phytane/n-$C_{18}$ ratio, indicating no biodegradation. For Bay 11 the analyses of the sediments from the microbiology stations indicate increasing levels of oil, compared to 1981, but the very low phytane/n-$C_{18}$ ratios give no basis for assuming microbial degradation of the alkanes. Sediment samples from the same bay, but collected in more shallow water, do contain oil that has an increased phytane/n-$C_{18}$-ratio. The oil content in these samples exhibits pronounced patchiness and greater extent of biodegradation in the samples with the highest concentration of oil suggests the biological process to be beach-related.

At the moment we can offer no explanation for the discrepancy between the definite verification of microbial mineralization of Lago Medio oil and n-hexadecane in sediments during experiments in the laboratory at *in situ* conditions of temperature and the apparent failure to detect any chemical change in the oil in the sediments *in situ* over the time span of one year.

### 3.1.3. Microbial activity in the oiled tidal zone of Bay 11.

The tidal zone of the test area in Bay 11 was covered by slightly pre-weathered Lago Medio crude oil during the experimental surface spill August 19, 1981. In 1982 the oil contaminated area was still very noticeable, although some of the excess oil which formed a more or less continuous surface layer over the sediments during the first post-spill weeks, had disappeared forming discernible "clean" spots. The oil had apparently either been stripped off by the repeated tidal cycles or washed out by fresh-water drainage from the shore. A distinct oily colour was still, however, characteristic for the top layer of these sediments.

Table 3 gives some analytical figures for total viable heterotrophic bacteria and oildegrading bacteria in various samples selected from spots with different sediment structure or variation in oiling intensity. Otherwise the samples were arbitrarily taken within the test area. A sample from the supra-littoral zone above the oiled beach was taken as a control of unoiled sediments.

Virginal tide sediments and sand in the backshore contain less than $10^3$ oil-degrading bacteria per ml sediment (Table 7 and 8, in (l)). The relatively high level of these bacteria found in the backshore sand of Bay 11 indicates that this area also had been contaminated by oil, possibly as a result of the logistic activity during the spill operation in 1981.

In the tidal sediments still containing heavy concentration of oil high levels of oildegrading bacteria have developed, up to $7x10^7$ per ml of sediment at places. This level of bacteria approach the situation in fertilized oiled sediments on the dry beach (see Table 4). The highest concentration of these bacteria appeared to occur in the loose sandy sediments where oil seemed to have penetrated deeply. In more compact fine sediments of clayish nature, the level of the same bacteria was very much lower.

The total population of bacteria in oiled beach was characteristically dominated by the bacteria which analytically are classified as being of the oildegrading type; the analytical figure for total heterotrophic bacteria were in two cases actually lower than the analytical figures for oildegraders. But this did not hold for the other samples, particularly for situations with-high absolute figures for oildegrading as well as generally heterotrophic bacteria.

It may be assumed from these observations that the tidal zone possesses levels of nutrients sufficient to establish a substantial population of bacteria when an external and excessive source of carbon and energy is supplied. The number of bacteria in these sediments are 10-100 times the number found in the bottom sediments of the nearshore waters containing only 1-10 ppm of oil.

Due to the excessive amounts of oil in the sediments the biochemical activity of the biodegradation of oil could not be assessed by the use of the radioactive hydrocarbon method. Direct measurements of the carbon dioxide production or the rate of oxygen consumption would of course have given a much better basis for assessing the rate of biodegradation in this environment, than assumptions derived the determination of size of the bacterial populations. Based on the experience of the enhanced biodegradation experiments at Bay 102 (2) it is not, however, unjustified to assume that an increased level of bacteria, and in particular oildegrading bacteria, strongly indicates a real enhancement of biochemical activity for oildegradation. But, of course, we have no way of determining the absolute figures.

## 3.1.4. Conclusions

The microbiological and biochemical analyses of watersamples and sediment samples from the test bays of Ragged Channel in 1982 have not exposed any drastic changes in the parameters which have been monitored in the period 1980 to 1982.

The analyses appear to indicate somewhat higher levels of bacteria in the water, particularly in the inner part of the Ragged Channel, from 1981 to 1982. These findings are further substantiated by indication of increased biochemical capasity for mineralizing radioactive weathered Lago Medio crude oil and _n_-hexadecane. Pronounced variations in the parameters are, however, seen between the bays. In Bay 11 high levels of bacteria, and in particular of the oildegrading type, may possibly be caused by drainage from the oiled beach. For the differences between the other bays we can so far offer no other explanation than patchiness due to stratification of the water column.

For the sediments we can detect no obvious change in the microbial activity in Bay 7 and 11 compared to the prespill and postspill situation in 1981. The conditions in the sediment of Bay 9 and 10 in 1982 appear to be somewhat different. They clearly contain higher numbers of total heterotrophic as well as oildegrading bacteria than the sediment of the other bays sampled at the same time and the measured rate of degradation of _n_-hexadecane is also higher, on the average 2 to 3 times. It is not unlikely to assume that this difference in general microbial activity as well as specific activity linked to oildegradation may be caused by the oil detected in sediment of Bay 9 and 10, but not in 7 and 11. This conclusion is not inconsistent with the observed decreased level of oil in the same sediments from 1981 to 1982 (Boehm (6)), but the measured increased level of biochemical activity for oil degradation in the sediments is drastically at variance with chemical evidence for the biological stability of the oil hydrocarbons.

The average values found for these parameters in Bay 9 and 10 are higher than the average figures for all bays in 1981 [see Tables 2, 3 and 6 in (2)1, but the maximal figures found in 1982 do not exceed the maximal figures observed in single samples analysed in 1981. Consequently the possible change in the level of activity is only marginal, which might be expected from the degree of oil contamination of the sediments caused by the oil spills.

A few samples of the sediments from the intertidal test area of Bay 11 have been taken. The oiling of the sediments has clearly caused a dramatic increase in the bacterial population of this environment. This indicate that substantial amounts of nutrients must be available and a positive effect of these bacteria on the self-cleansing of the tidal sediments ought to be expected.

## 3.2. ON SHORE PROJECTS
## ENHANCED BIODEGRADATION EXPERIMENTS

The test plots for the enhanced biodegradation experiments were established on the backshore of the high energy beach of Bay 102 in early August 1981. The position of the test plots and the technical data for the oiling procedure is very instructively described in the report of Owens et al (4). To mimic a spill situation the experimental site was chosen to be close to, but definitely above the highwater line. After completing 3 sampling sequences, including one before the oil was applied, excessive high tides occurred on two successive days (August 29 and 30). On the second day the high tide coinsided with heavy waves. This caused complete burial of the test plots under 5 to 20 cm of sand and gravel (see Owens et al. (4) for further description). Part of the oil was clearly removed from the plots by the wave action and oil sediment patches could be found distributed at various places along the beach. During subsequent sampling in 1982 no trace of plot D could be detected. The test plot I02A and 102B laid in 1980 to function as unfertilized controls and which had survived the previous year very well, were also partly covered by sand. But in contrast to the other plots, they could be easily remarked and sampled. Some of the oil-sand lumps transported by the waves had ended up in the unoiled control plot and ruined further sampling.

In the fall of 1981 it was decided that further pursuit of these plots should await the outcome of the following winter. Any work to uncover the plots in 1981 might easily have been in vain and further sampling was also postponed until next year.

On arrival at Cape Hatt in August 1982 the plots 102 D to 102 H were still buried and showed no indications of having been reworked by waveactions in the intervening period. The plots had already been relocated and remarked by the Woodward-Clyde crew and sampled for chemical analyses by the Seakem chemists. The oil layer appeared to be relatively intact and we sampled for microbiological analyses from the same holes used by the chemists. A new unoiled control area was selected west of 102 D. No attempt to uncover the test plots were made. We have decided to use the plots in their present state. The results of the analyses from this year would provide information to what extent the original intension of the experiment is still fulfilled.

In an attempt to redeem some of the damage assumed to be irrevocable for the experimental plots 102 D to H, a simple contingency experiment was set up in September 1981. By consent from the Shoreline Countermeasure Technical Committee a small part of the crude oil control plot T 1 at the "Crude oil point" was fertilized. T 1 had been sprayed with approx. 10 $kg/m^2$ crude Lago Medio in August 1980 and devided up into 1 x 1 segments for sampling in 1981-83. 1 m areas at the edges were not intended for sampling [see Fig. 3.1 in (4)] and put to our disposal. The whole area has very coarse sediments, mostly pebbles, and the area to the west was found most suited for sampling. This area was fertilized (see Fig. 2). An unoiled area close to the fertilized plot was chosen for control sampling.

As a consequence of the bad luck in 1981 and partly due to the interest in testing enhanced biodegradation procedures on oiled backshore sediments in a low energy bay area, two new test plots were established in 1982 in Bay 106 within the Z-lagoon. Woodward-Clyde had already established two test plots in the same area to assess the natural recovery rates. These plots were found too small to be included in our fertilization experiments. By kind cooperation with the Project Manager a separate section was set aside for the testplots as indicated in Fig. 2. The plots are quite a bit smaller than we wanted them, but lack of oil enforced limitation. We realize that the observation time for these plots will only be about a year, since the BIOS Project terminates in 1983. But we take the belief that a return to Cape Hatt sometime in the period 1985-95 for further inspection and analyses somehow might be poss·ble.

This report gives the results for the microbiological analyses for all the test plots. The them cal analyses of samples taken from the same plots are reported by Woodward-Clyde (7).

Except for the new testplots in Bay 106 (sampled 6 and 10 days after oiling) only one set of samples were taken from each plot. In 1980 and 1981 the rate in carbon dioxide production in the various test plots was also determined in an attempt to assess quantitatively the effect of treatment on the biodegradation rate. Unfortunately shortage of time and personnel did not permit measurements of this kind in 1982.

## 3.2.1. Microbial activity in the test plots

### 3.2.1.1. Test plots in Bay 102

The results of the microbiological analyses of samples from test plots 102 A - 102 H in August - September 1982 are given in Table 4. The sampling time is assumed to be close to the annual peak in microbial activity which may be attainable during the artic summer. Unfortunately practical problems during transport from Cape Hatt ruined the opportunity for correct reading of the analytical values for total viable heterotrophic bacteria (TVH), for that reason the numbers of TVH for some samples are gross underestimates.

In Table 12 the numbers found for oildegrading bacteria are compared to the maximal values found in the same plots in 1980 and 1981.

For all oiled plots the level of bacteria are still very high relative to the original levels in virgin sand. The havoc caused by the disturbances of the oil plots has clearly made it difficult to find a new dependable uncontaminated area on the beach. The new control area was unmistakenly contaminated as indicated by the high number of oildegrading bacteria (ODB). On the other hand, among the various oiled test plots there still exist differences in the levels of bacteria that would be expected from the initial treatment given to the plots and from the results observed last year. The forces that caused the burial of the plots have not, as it seems, changed the conditions within each plot to any large degree.

The oldest plots (102 A and 102 B, unfertilized} have after two years populations of $10^5$ - $10^6$ ODB per ml of sand. Initially the microbial development in the oil-emulsion plot was very much faster than in sand having received unmodified crude oil (1). This difference disappeared ofter one year and apparently remained so after the second year. The difference in ODB observed in 102 A and 102 B in 1982 may not be significant.

The test area with oil-emulsion established in 1981 had originally two unfertilized plots. One of them was partly covered by an absorbent (102 D) and another received no treatment (lu2 F). During the 4 weeks of observation in 1981 no significant difference in the microbial development could be detected between these two plots. In 1982 plot 102 D had a level of ODB expected for an unfertilized oiled beach (based on experience from the development in 102 A-B), whereas 102 F had a count of ODB similar to or even higher than the fertilized plots 102 E and 102 H. Plot 102 F is situated right between these two fertilized plots and a contamination of 102 F by fertilizers from 102 G and or 102 E seems very likely to have happened.

During the inspection of the plots and sampling for chemical analyses the Seakem chemists could not find any oil in the area originally occupied by 102D (7). The erosions caused by the combined action of high tides and waves in the fall of 1981 had apparently completely removed all visible oil from the surface and subsurface at this spot. The microbiological analyses, however, indicate that the sediments remaining on the site are still sufficiently contaminated by oil to maintain an enhanced population of oil degrading bacteria. The similar situation clearly exists for the sediment area chosen as a new assumed uncontaminated control plot.

The initially fertilized plots (including the possibly contaminated 102 F) have all very high populations of ODB, ranging from $3 \cdot 10^6$ to $1.3 \cdot 10^8$ ODB per ml sediment. By far the highest count was found in plot 102 H which had been given the highest level of - ertilizer. Its number of ODB was 30-40 times the population of ODB in 102 G which received one tenth the amount of fertilizer and in 102 E wh·ch was tilzed after having been given the same amount of fertilizer as 102 H. This is very much the same situation as found in 1981, lending support to the assumption that tides and waves in 1981 have not done any severe damage to the plots. These plots may therefore still give useful information.

Based on the correlation between carbondioxide production rates and the bacterial populations found for the various plots in 1981 it seems justified to assume that fertilization has maintained an enhanced rate of biodegradation of oil, even with the oil buried under sand.

The biodegradation ratio calculated from the analytical figures for high molecular weight alkanes and the microbiologically more stable isoprenoid hydrocarbons (ALK/ISO ratio) do not seem to support this assumption very strongly (7). In plot I02E which was mixed with a rotatiller after application of oil and fertilizer the residual oil had an ALK/ISO ratio of 1.9 in 1981, a change from the initial value of 2.5 in the slightly aged Lago Medio oil. This gives a fair indication of biodegradation. In the other plotsF, G and H,no change in the biodegradation ratio could be observed.

The samples taken for hydrocarbon analyses represent the entire vertical profile of the oil containing sediment layer, which varied between 6 and 10 cm in the varous plots. Changes in the oil due to preferential biodegradation at the very top surface of the oiled sediment would therefore very likely be scrambled by the unchanged oil in the deeper layers of the sediment and left unnoticed by these analyses. This might particularly be the case for plot I02F to H, where the oil was layered on top of the sediment. The same kind of discrepancy in the sampling procedures may be inferred to affect the microbiological analyses, but to a lesser degree since in general a more narrow sediment layer; O-2 cm for surface sample, was used as basis for these analyses. In plot 102E the oil and fertilizer were well mixed into the top

10-15 cm of beach sediment giving a drier and more aerated sediment-oil composit compared to the more stratified situation in the other plots. This could have permitted active microbial oxidation of oil throughout the oil bearing sediment. The mixing caused the concentration of fertilizer to be somewhat lower, may be even considerably lower, than in the top surface of the other plots and giving rise to a lower population of oil degrading bacteria, compared to the situation in plot I02H which received the same amount of fertilizer per unit area. In this situation it is not unlikely that an extensive biodegradation of oil in plot 102E may have taken place, indicated by the ALK/ISO-ratio, under conditions of a moderate population of oil degrading bacteria. On the other hand extensive degradation of oil may have taken place at the top surface of 102G and I02H, as assumed by the high figures for TVH and ODB in these plots, without giving any appreciable change in the average ALK/ISO-ratio.

This explanation of the microbiological and hydrocarbon chemical analyses of plot 102E to H would mean that mechanical mixing of oiled sediments may have a beneficial effect on the biodegradation of the oil. The 1983 analyses of the mixed and unmixed plots of crude oil and oil emulsion control plots IMC and IME laid by Woodward-Clyde in Bay 106 in 1982 (7) may give information of value for further consideration of this situation.

3.2.1.2. Test plots in Bay 106

Fig. 2 gives the location of the testplots in Bay 106 relative to the high water line and the test plots established by Woodward-Clyde. The plots are mimicking the situation of oil stranded by high tide and we have the hope that these plots will remain exposed for the entire observation period.

The simplest possible experimental set-up was used to test the effect of one concentration level of fertilizer (100 g/$m^2$ Fullgjødsel C) on the biodegradation of crude oil and oil-emulsion. This called for 4 test-plots. A 5th area was chosen for an unoiled control.

The results of the analyses indicating the microbial development during the first 16 days after depositing the oil are given in Table 5.

The test plots were not sampled prior to the oiling. The selected area for the unoiled control had much higher levels of ODB than normally found for backshore sediments, indicating that we had chosen a precontaminated site. Nevertheless, addition of oil on the sediment caused an appreciable further increase in ODB (106 NCC and 106 NCE) and the microbial development seen in 1980 repeated itself. In the sediment covered by the oil-emulsion the count of oildegrading bacteria as well as total viable heterotrophic bacteria rose faster and to a higher level than in the sediment receiving the crude oil. A factor of 20 in difference was observed in the level of bacteria in these plots. The addition of fertilizer increased the level of ODB another 20-40 times and the relative difference between the two plots still remained.

In the span of 16 days populations of approx. $3*10^7$, respectively $3\cdot10^8$ ODB/ml sediment developed in the fertilized sediments oiled with crude oil respectively oil emulsion, with population of TVH 5-20 times higher. This verified very much the experiences of last year and demonstrated the amazing speed with which bacteria may grow under these conditions when provided with ample sources of nutrients and carbon/energy. Without added nutrients a substantial population of bacteria is established at the expense of the nutrients in the oil or in the sediments themselves, but the latter components clearly become limiting very soon. By addition of an external supply of nitrogen-phosphor-containing fertilizer the level of bacteria may be raised another one to two orders of magnitude. The results from 1982 do not permit any definite assumption as to the maximum level of bacteria which may be obtained under the set conditions, but it is not unlikely to assume that the observed populations are close to those attainable during the short arctic summer.

### 3.2.1.3. Test plots at "Crude oil point"

In an attempt to remedy to some extent the misfortune at Bay 102 in late August 1981, the Technical Committee for the Shoreline Countermeasures kindly consented to make use of the oiled plot at "Crude oil point" for an improvised fertilization experiment. Plot T 1 [see Fig. 4.3 in (3)] was oiled with aged (8%) Lago Medio crude oil (10 kg/$m^2$) in August 1980. A similar plot (T 2) was oiled with an emulsion (1:1) of the same oil. These plots will be sampled once a year in the postspill period as control of the physical and biological weathering of the two types of oil. The plot area is located in the supralittoral zone and consisted of gravelly sand including lumps of moss and stunted willows which suggested that the area had not been flooded recently (4).

Two segments (T 1 CC and T 1 FC, see Fig. 2) were selected to give maximal opportunity for sampling in sand and one (T 1 FC) was fertilized by the usual Norsk Hydro Fullgjødsel C (2). These experimental plots were not sampled for microbiological analyses in 1981.

The results of one set of samples taken in 1982 are given in Table 6. The fertilized plot did have a higher level of oildegrading bacteria (ODB) compared to the unfertilized plot (about 5 times), but for both plots the count of ODB was low compared to the values found in similar plots in Bay 102 (Table 4). This may reflect the fertilization of already weathered oil (4) at a late stage. More likely, however, the explanation of the rather low number is a physical one. The sediment structure of T1 is very coarse and although the sampling was carried out among the stones and pebbles, the samples consisted of mainly coarse sand, making quantitative comparison with other beach situations very difficult.

Nevertheless, the results appear to show that fertilization of an oiled beach, even one year ofter the oil is "stranded", has a positive effect on the microbial population, at least on the specific population of oildegrading bacteria. In this particular instance the analyses of total viable heterotrophs are not valid.

Chemical analyses (4) indicate that appreciable weathering by evaporative actions has taken place in the intervening period between 1980 and 1981. The calculated ratio between alkane and isoprenoid fractions are too inconsistent to make a definite assumptions as to the extent of biodegradation during the first year. The total evaluation of the data (4) indecates that biological weathering has not been significant in reducing the oil content of the sediments.

### 3.2.2. Conclusions

Judged by the microbiological analyses only minor damages to the experimental plots at the high energy beach 102 was caused by the flooding incident in 1981. The sand covered oiled plots still had very high counts of oildegrading and generally heterotrophic bacteria., As in 1981 the highest count was found in the oiled sediment which had received the highest concentration of fertilizer and otherwise left untouched. The plot having received the same amount of fertilizer mixed into the sediment by a rotating tiller, had approximately one fifth the level of bacteria. The latter treatment seem to offer no advantage above a simple fertilization with one tenth of the maximal amount (400 $g/m^2$) used in this experiment. Chemical analyses, however, seem to indicate that this contention may be wrong. The tilled plot was the one among the 102 plots to have oil with a pronounced reduction in the biodegradation ratio ALK/ISO.

After two years the number of oildegrading bacteria still remained relatively high in unfertilized oiled plots at Bay 102, 1-2 orders of magnitude lower than in plots treated with low amounts (40 $g/m^2$) of fertilizer. Any significant difference in biodegradation between stranded crude oil or oil emulsions cannot 'yet be ascertained.

The small experiment on "Crude oil point" seems to varify that fertilization of oiled sediments one year after the spill may still be helpful. In a plot oiled in 1980 and fertilized in-1981, a 5 times increase in the count of oildegrading bacteria was found.

A new experiment for enhanced biodegradation has been set up in Bay 106 inside the Z-lagoon to provide conditions characteristic for a low energy beach. The amount of fertilizer is one forth of the maximum concentration used in the similar experiment in 1981 at Bay 102. So far the microbial development has proceeded as expected from previous experience. In the fertilized oil emulsion plot population of $10^8$ oildegrading bacteria per ml of sediment is established in 2 1/2 weeks; for reasons so far unknown the crude oil seems to offer a less congenial basis for growth irrespective of the presence or absence of added fertilizer. The same pattern of microbial development was seen in 1980 in the unfertilized plots in Bay 102 (102A and B).

REFERENCES

(1) Eimhjellen, K., Sommer, T. and Sendstad, E., 1981, Microbiology: II Biodegradation of Oil - 1980 Study Results. (BIOS) Baffin Island Oil Spill Working Report 80-6: 33 p.

(2) Eimhjellen, K., Nilssen, O., Josefsen, K., Sommer, T., Sendstad, E,, Sveum, P. and Hoddo, T., 1982, Microbiology: II. Biodegradation of Oil - 1981 Study Results, (BIOS) Baffin Island Oil Spill Working Report 81-6: 57 p.

(3) Woodward-Clyde Consultants, 1981, Shoreline Countermeasures - 1980 Study Results. (BIOS) Baffin Island Oil Spill Working Report 80-4: 83 p.

(4) Owens, E.H., Harper, J.R. and Foget, C.R., 1982, Shoreline Countermeasures - 1981 Study Results. (BIOS) Baffin Island Oil Spill Working Report 81-4: 123 p.

(5) Boehm, P.D., Fiest, D.L. and Hirtzer, P., 1982, Chemistry: 2. Analytical Biogeochemistry - 1981 Study Results. (BIOS) Baffin Island Oil Spill Working Report 81-2: 354 p.

(6) Boehm, P.D., 1983, Chemistry: 2. Analytical Biogeochemistry - 1982 Study Results. (BIOS) Baffin Island Oil Spill Working Report 82-2: 210 p.

(7) Owens, E.H., Harper, J.R. and Foget, C.R., 1983, Shoreline Countermeasures - 1982 Study Results. (BIOS) Baffin Island Oil Spill Working Report 82-4.

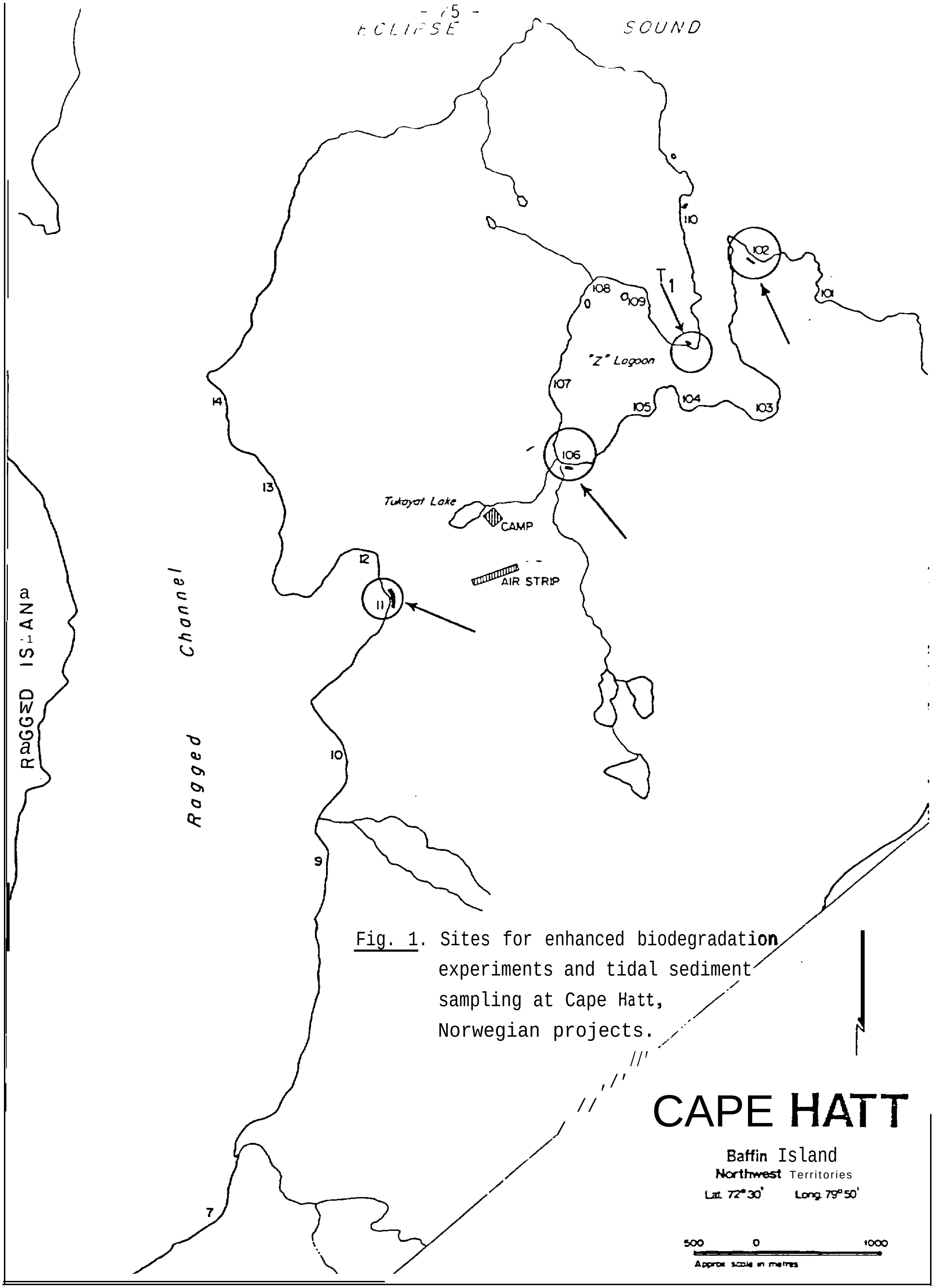
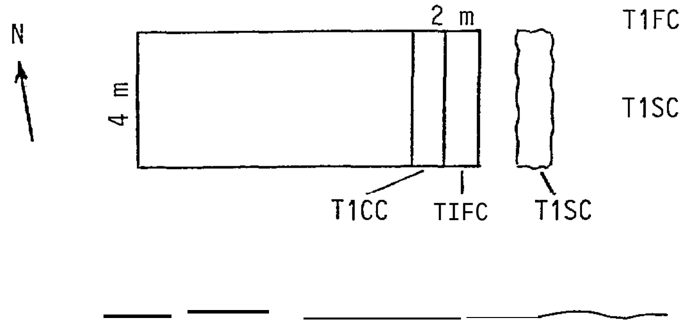



Fig. 1. Sites for enhanced biodegradation experiments and tidal sediment sampling at Cape Hatt, Norwegian projects.

Crude oil point. Plot T 1 with Norwegian sampling sites.

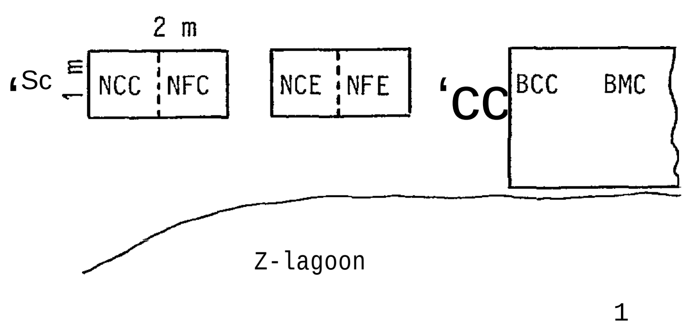



TICC - Control Crude

T1FC - Fertilized Crude (80 g fertilizer/m$^2$)

T1SC - Sand Control

Bay 106. Backshore Norwegian plots at low energy beach.




NSC - Sand Control

NCC - Control Crude

NFC - Fertilized Crude ( 100 g fertil izer/m$^2$)

NCE - Control Emulsion

NFE - Fertilized Emulsion (100 g fertilizer/m$^2$)

} Norwegian plots

BCC - Control Crude

BMC - Mixed Crude

} Woodward-Clyde plots

Fig. 2 Norwegian test plots for enhanced biodegradation experiments at "Crude oil point" and Bay 106.

Table 1

Enumeration of total viable heterotrophs (TVH) and oildegrading bacteria (ODB) in watersamples collected in Ragged Channel,Cape Hatt, August 1982. Equal amounts of water from both stations at each bay were collected, mixed and divided into 2 equal fractions. Subsequently 2 independent, parallel enumerations were performed and the arithmetic mean value calculated.

| Date | Bay | Depth (m) | TVH no.$L^{-1} \times 10^{-5}$ | ODB no.$L^{-1} \times 10^{-3}$ | % ODB |
|---|---|---|---|---|---|
| 08.23 | 7 | 0.1 | 60 | 85 | 1.4 |
| [cycle 4) | | 5 | 88 | 35 | 0.4 |
| | | 10 | 163 | 17 | 0.1 |
| 08.23 | 9 | 0.1 | 78 | 43 | 0.6 |
| (cycle 4) | | 5 | 45 | 17 | 0.4 |
| | | 10 | 43 | 3.5 | 0.1 |
| 08.20 | 10 | 0.1 | 68 | 25 | 0.4 |
| (cycle 3) | | 5 | 110 | 7 | 0.1 |
| | | 10 | 280 | 3.5 | 0.01 |
| 08.20 | 11 | 0.1 | 25 | 45 | 1.8 |
| (cycle 3) | | 5 | 350 | 52 | 0.1 |
| | | 10 | 150 | 2.5 | 0.02 |
| 08.30 | 11 | 0.1 | 750 | 950 | 1.3 |
| (cycle 5) | | 5 | 78 | 450 | 5.8 |
| | | 10 | 25 | 700 | 28.0 |

Incubation time TVH: 23 days

Incubation time ODB: 74-84 days

Tabel 2

Enumeration of total viable heterotrophs (TvH) and oildegrading bacteria (ODB) in sediments collected in Ragged Channel, Cape Hatt, August 1982.

Each sample represents a composit homogeneous mixture of 8 sediment samples taken individually by a diver using 50 ml sterile disposable syringes.

| Date | Station | TVH no.ml$^{-1}$ “10-5 | ODB no. ml$^{-1}$ “10-3 | $\frac{ODB}{TVH}$ 100 % |
|---|---|---|---|---|
| | Bay 7 | | | |
| 08.18 (Cycle 3) | 7 | 15 | 25 | 1.7 |
| 08.18 (Cycle 3) | 8 | 150 | 95 | 0.6 |
| | Bay 9 | | | |
| 08.19 (Cycle 3) | 5 | 150 | 450 | 3.0 |
| 08.19 (Cycle 3) | 6 | 150 | 45 | 0.3 |
| | Bay 10 | | | |
| 08.18 (Cycle 3) | 3 | 150 | 45 | 0.3 |
| 08.18 (Cycle 3) | 4 | 150 | 250 | 1.7 |
| | Bay 11 | | | |
| 08.18 (Cycle 3) | 1 | 45 | 25 | 0.6 |
| 08.18 (Cycle 3) | 2 | 45 | 25 | 0.6 |

Incubation time TVH: 17-18 days
Incubation time ODB: 69-70 days

Table 3

Enumeration of total viable heterotrophs (TVH) and oildegrading bacteria (ODB) of the upper 0-2 cm of the surface layer in the supralittoral and intertidal zones within the test area in Bay 11, Cape Hatt, August 1982. The "most probable number" (MPN) method was employed in assessing the bacterial populations. The surface oil spill was carried out 19 August 1981.

| Date | Sample | TVH no.$ml^{-1} \cdot 10^{-5}$ | ODB no.$ml^{-1} \cdot 10^{-3}$ | % ODB |
|---|---|---|---|---|
| 08.28 | S 11 A (sand 0-2 cm, supralittoral) | 2.8 | 13 | 4.6 |
| 08.28 | S 11 B (sand 8-10 cm, supralittoral) | 2.8 | 320 | 114 |
| 08.28 | S 11 C (sand under shallow, enclosed water, intertidal) | 435 | 72500 | 167 |
| 08.28 | S 11 D (grey sand, intertidal) | 73 | 7250 | 100 |
| 08.28 | S 11 E (sand covered by "dry" weathered oil, intertidal) | 4350 | 22000 | 5.0 |
| 08.28 | S 11 F (loamy soil, intertidal) | 13 | 73 | 5.6 |

Incubation time TVH: 18 days (S 11 E 9 days)

Incubation time ODB: 78 days

Table 4

Enhanced biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt. Results of the enumeration of total viable heterotrophs (TVH) and oil-degrading bacteria (ODE) upper 0-2 cm of the oil-containing layer.

Sample C (control), was taken from a spot about 4 meters west of plot 102 D. The test plots 102 A and 102 B were laid August 23 1980, the others August 1 1981, all plots initially receiving a load of 10 $kg/m^2$ crude Logo Medio. In plot 102 A and plots 102 D-H the oil was emulsified (oil/water 1:1).

| Date | Plot | TVH no.$ml^{-1}\cdot 10^{-5}$ | ODB no.$ml^{-1}\cdot 10^{-3}$ |
|---|---|---|---|
| 08.27 | 102 A (emulsion) | 7.3 | 73 |
| 08.27 | 102 B (crude oil) | 13 | 725 |
| 09.01 | 102 C (control) | 0.003 | 73 |
| 09.01 | 102 D (sorbent) | 0.13 | 73 |
| 09.01 | 102 E (fertilized and mixed) | 27.5 | 2750 |
| 09.01 | 102 F (oil control) | 1.3 | 13000 |
| 09.01 | 102 G (low level fertilized) | 44 | 4350 |
| 09.01 | 102 H (high level fertilized) | 1300 | 13000 |

Incubation time TVH: 102 A - B 9 days
102 c - H 5 days

Incubation time ODB: 71 - 77 days

Compared to a normal incubation time of about three weeks, the estimation of TVH will, according to experience, be too low by a factor of about 10-100, because of the short incubation time.

Table 5

Enhanced biodegradation experiment in the supralittoral zone of Bay 106, Cape Hatt. Results of the enumeration of total viable heterotrophs (TVH) and oil-degrading bacteria (ODB) of oil-containing sand of the upper O-2 cm of the surface layer. The oiled test plots were laid August 19 1982.

| Date | Plot | TVH no.ml$^{-1}$ “$10^{-5}$ | | ODB no.ml$^{-1}$ “$10^{-3}$ | % ODB |
|---|---|---|---|---|---|
| 08.25 | 106 NSC (sand control) | 13 | (3.2) | 130 | 10 |
| 09.04 | do. | 4.4 | | 275 | |
| 08.25 | 106 NCC (crude oil) | 32 | (7.3) | 73 | 2.3 |
| 09.04 | do. | 3.2 | | 725 | |
| 08.25 | 106 NFC (fertilized crude ) | 435 | (130) | 218 | 0.5 |
| 09.04 | do. | 320 | | 27500 | |
| 08.25 | 106 NCE (crude emulsi-fied) | 1300 | (220) | 2800 | 2.2 |
| 09.04 | do. | 320 | | 13000 | |
| 08.25 | 106 NFE (fertilized emulsion) | | (1300) | 130 | 0.1 |
| 09.04 | do. | 13000 | | 275000 | |

Incubation time TVH: 21 days (08.25) (results after 11 days given in parenthesis)

11 days (09.04)

Incubation time ODB: 71 days (08.25)

61 days (09.04)

Table 6

Enhanced biodegradation experiment in the supralittoral zone on Crude oil point (Z-lagoon), Cape Hatt. The oiled test plots were laid August 1980. One part of the plot (T1FC) was fertilized September 7 1981 (80 $g/m^2$). Results of the enumeration of total viable heterotrophs (TVH) and oil-degrading bacteria (ODB).

| Date | Sample | TVH<br>no.ml-'"10-5 | ODB<br>$no.ml^{-1}$ "10-3 | $\frac{ODB}{TVH} \cdot 100$<br>% |
|---|---|---|---|---|
| 08.29 | T 1 SC<br>(sand control) | 0.07 | 0.007 | 0.1 |
| 08.29 | T 1 CC<br>(crude control) | 130 | 27.5 | 0.2 |
| 08.29 | T I F C<br>(fertilized crude) | 7.3 | 130 | 17.8 |

Incubation time TVH: 8 days

Incubation time ODB: 74 days

Compared to a normal incubation time of about three weeks, the estimation of TVH will, according to experience, be too low by a factor of about 10, because of the short incubation time.

Table 7

Enumeration of total viable heterotrophic (TVH) bacteria and oildegrading bacteria (ODB) in the water of the BIOS test bays in Ragged Channel, Cape Hatt. A comparison of the microbial situation in the late August in 1980-82.

The values for 1982 are the arithmetic mean of 2 analyses of a single mixed sample from each depth at the 2 stations in each bay, August 20-30. The values for 1980 are arithmetic mean of the analytic results from 2 stations in each bay, August 26 - September 1. The figures given for 1981 represent the arithmetic mean of 4 analyses from each depth at the same stations for the period August 21 to 27.

| | | $TVH \cdot 10^{-5} \cdot L^{-1}$ | | | $ODB \cdot 10^{-3} \cdot L^{-1}$ | | |
|---|---|---|---|---|---|---|---|
| Bay | Depth | 1982 | 1981 | 1980 | 1982 | 1981 | 1980 |
| | 0.1 | 60 | 25 | - | 85 | 10 | |
| 7 | 5 | 88 | 130 | - | 35 | 6 | |
| | 10 | 163 | 50 | - | 17 | 3 | |
| | 0.1 | 78 | 140 | 14 | 43 | 4 | 150 |
| 9 | 5 | 45 | 45 | 42 | 17 | 5 | 137 |
| | 10 | 43 | 37 | 14 | 3 | 4 | 30 |
| | 0.1 | 68 | 45 | 60 | 25 | 6 | 14 |
| 10 | 5 | 110 | 27 | 35 | 7 | 8 | 7 |
| | 10 | 280 | 23 | 25 | 3 | 3 | 1 |
| | 0.1 | 378 | 19 | 475 | 500 | 12 | 107 |
| 11" | 5 | 214 | 70 | 200 | 250 | 12 | 5 |
| | 10 | 87 | 8 | 60 | 350 | 6 | 11 |

Table 8

The population of total viable heterotrophic (TVH) bacteria and oildegrading bacteria (ODB) in the bottom sediments of the BIOS test bays in Ragged Channel, Cape Hatt. A comparison of the microbial situation in late August to early September 1980-82.

The values for 1982 are the arithmetic mean of the analyses of 2 sediment samples from each test bay (August 18-19) . The 1981 series represent the arithmetic mean of 7-10 sediment analyses from each testbay over the period August 8 to September 13. For 1980 only 5 sediment samples were analyzed with results given in the table. Each of the sediment samples from 1981 and 1982 represents a composit homogeneous mixture of 8 sediment samples from each site taken individually by diver using sterile disposable syringes.

| | | Cell count | | | |
|---|---|---|---|---|---|
| | | Bay 7 | Bay 9 | Bay 10 | Bay 11 |
| TVH $ml^{-1}\cdot10^{-5}$ | 1982 | 82 | 150 | 150 | 45 |
| | 1981 | 95 | 48 | 34 | 52 |
| | 1980 | | 12 | 95 | |
| ODB $ml^{-1}\cdot10^{-5}$ | 1982 | 0.60 | 2.5 | 1.5 | 0.25 |
| | 1981 | 0.85 | 1.1 | 1.8 | 0.7 |
| | 1980 | — | 0.5 | 0.75 | 0.14 |

Table 9 A

Mineralization of tritiated ($^3H$) weathered Lago Medio oil in the water of the test bays of Ragged Channel, Cape Hatt, in August 1982. Experimental rates were determined at 3.5 °C. $v_{10}$ designates the rate assessed in presence of 10 µg oil substrate in the test system (which correspond to 1 ppm if all substrate is dissolved in the waterphase) . The calculation of $V_{max}$ is based on the rates assessed in presence of 1, 2, 5, 10 and 20 µg of oil in the test system. Equal amounts of water from 5 m at both stations in each bay were mixed and aliquots used for the measurements.

| Date | Bay | Accumulated $^3H_2O$ in cpm/ml after 13 days incubation* | Rates of mineralization µg/m$^3$, d $v_{10}$* | $v_{max}$ | $r^2$ |
|---|---|---|---|---|---|
| 08.23 | 7 | 10300 * 1500 | 47 * 14.1 | 97.3 | 0.96 |
| 08.23 | 9 | 7900 ± 1200 | 27.5 ± 9.8 | 169.7 | 0.98 |
| 08.20 | 10 | 5700 * 400 | 11.8 * 2.7 | 28.7 | 0.99 |
| 08.20 | 11 | 9500 * 1400 | 32.2 * 8.3 | 50.6 | 0.96 |
| | sterile control | 3900 * 300 | | | |

* 4 parallel experiments with 10 µg $^3$H-Lago Medio

Table 9 B

Mineralization of $^{14}C$-hexadecane in the water of the testbays of Ragged Channel, Cape Hatt, in August 1982.

The experimental rates were determined at 3.5 $^{o}C$. $v_{10}$ designates the rate assessed in a test-system contaning 10 µg hydrocarbon and is calculated from 6 parallel experiments for each watersample (only 3 for Bay 7) . The watersample was composed of equal volumes of water from 5 m depth from both stations in each bay.

| Date | Bay | Accumulated $^{14}CO_2$ cpm/ml after 13-days incubation | Rate of mineralization µg/$m^3$, d $v_{10}$ |
|---|---|---|---|
| 08.23 | 7 | 43000 ± 18000 | 420 * 280 |
| 08.23 | 9 | 44000 ± 22000 | 270 ± 150 |
| 08.20 | 10 | 8900 * 7700 | 50 * 45 |
| 08.20 | 11 | 31000 ± 23000 | 180 ± 150 |
| | Sterile control | 10 * 10 | |

Table 9·C

Mineralization of $^{14}C$-naphthalene in the water of the test bays of Ragged Channel, Cape Hatt, in August 1982.

The measurements were carried out at 3.5 °C. 10 µg $^{14}C$-naphthalene was used as substrate in 6 parallel experiments for each watersample (3 parallel experiments for Bay 7). The watersample was composed of equal volumes of water from 5 m depth at both stations in each bay.

| Date | Bay | cpm/ml in $^{14}CO_2$ after 13 days incubation: average | range |
|---|---|---|---|
| 08.23 | 7 | 570 ± 810 | 30 - 1500 |
| 08.23 | 9 | 250 ± 250 | 130 - 750 |
| 08.20 | 10 | 120 * 190 | 25 - 500 |
| 08.20 | 11 | 160 ± 330 | 7 - 840 |
| | Sterile control | 10 * 10 | |

Table 10

Mineralization of tritiated Lago Medio crude oil and $^{14}C$-hexadecane in sediments of the test bays of Ragged Channel, Cape Hatt, in August 1982.

Experimental rates determined at 3.5 °C. ‘1 and ‘10 designate the rate assessed in presence of 1 µg, respectively 10 µg of substrate in the test system (which would correspond to 0.1 or 1 ppm if all substrate is dissolved in the water phase). Two samples (each a composit of 8 subsamples) were taken at each bay and from each sample normally 3 parallel measurements were carried out. The same samples were analyzed microbiologically (Table 2).

| | | | $^{3}H$-Lago Medio µg/L,d | | | $^{14}C$-n-hexadecane µg/L,d |
|---|---|---|---|---|---|---|
| Date | Bay | No exp. | ‘1 | ‘10 | No exp. | |
| 08.18 | 7 | 5 | 5.5 * 3.0 | | 6 | 14.7 * 4.9 |
| 08.19 | 9 | 1 | 8.3 | | 6 | 24.0 ± 9.0 |
| 08.19 | 9 | 6 | | 38.3 * 5.8 | | |
| 08.18 | 10 | 6 | 8.3 * 2.8 | | 6 | 24.5 ± 8.1 |
| 08.18 | 11 | 6 | 11.2 * 5.4 | | 6 | 10.9 * 3.5 |

Table 11,

Enumeration of oildegrading bacteria (ODB) in tidal sediments in bays of Cape Hatt. A comparison of results for uncontaminated Bay 103 (1980) and the oil contaminated Bay 11 in 1982.

| | | $ODB \cdot 10^{-3}$ $ml^{-1}$ sediment |
|---|---|---|
| Uncontaminated tidal zones | Bay 103 1980 | 0.45; 2; 3.5 |
| Oiled tidal zone of Bay 11 | watercovered sand | -73000 |
| | oiled sand | 7300 |
| | sand covered by weathered oil | 22000 |
| | loamy sediment | 73 |

Table 12

On shore biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt. A comparison of the maximal levels of oildegrading bacteria (ODB) observed in the various plots during t-he summer seasons of 1980-82.

The plots A and B were sprayed with oil emulsion, respectively crude oil (Lago Medio) in August 1980. The plots D to H were laid with emulsion (approx. 20 $kg/m^2$) in August 1981 and treated as decribed below [se also (2)]. The numbers give ODB $ml^{-1}$ “$10^{-5}$ and the number in parenthesis indicates the percentage of ODB relative to the count of total viable heterotrophic bacteria in the same sample.

| | A oil emulsion | B crude oil | c unoiled control | D emulsion + sorbent | E emulsion + fertilizer + tilting | F emulsion | G emulsion + low level fertilizer | H emulsion + high level fertilizer |
|---|---|---|---|---|---|---|---|---|
| 1980 | 150 | 0.95 | < 0.001 | | | | | |
| 1981*) | 1.3 | 0.73 | < 0.001 | 2.0 | > 73 | 1.4 | 68 | 730 |
| | (0.3) | (0.26) | | (0.4) | (> 70) | (1.4) | (0.16) | (lo) |
| 1982 | 0.73 | 7.3 | 0.73 **) | 0.73 | 27.5 | 130 | 43.5 | 130 |

*) The values for 1981 given for plots D-H represent arithmetic mean of the results from O-2 cm and 5-10 cm sample.

**) Unoiled control new and arbitrarily selected west of plot 102 D.

Table 13

Development of microbial activity in oiled beach sediments and the effect of added fertilizer. Enumeration of oildegrading bacteria (ODB) in experimental plots at "Crude oil point" and in Bay 106, Cape Hatt.

Plot T 1 at "Crude oil point" was sprayed with Lago Medio crude oil in August 1980 and a segment was fertilized in early September 1981 (80 g/m$^2$ Norsk Hydro Fullgjødsel C). Plots of emulsified (1:1) and crude oil in Bay 106 were laid in the supralittoral zone in August 1982. The fertilized parts received 100 g/m$^2$ Fullgjødsel C.

ODB ml$^{-1}$ "10-$^{5}$

| | CRUDE OIL POINT | | | BAY 106 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | unoiled control | crude oil | crude oil + fertilizer | days after oiling | unoiled control | crude oil | + fertilizer | oil - emulsion | + fertilizer |
| 1981 | | | | | | | | | |
| 1982 | 0.00007 | 0.27 | 1.3 | 5 | 1.3 (10) | 0.73 (2.3) | 2.2 (0.5) | 28 (2.2) | 1.3 |
| | | | | 16 | 2.7 | 7.3 | 275 | 130 | 2750 |

Number in parenthesis indicates % ODB of the total viable count for heterotrophic bacteria.