Effect of Environmental Parameters on Bacterial Degradation of Bunker C Oil, Crude Oils, and Hydrocarbons

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Received for publication 22 April 1974

Mixed microbial cultures, previously enriched on Bunker C fuel oil, grew on and degraded Bunker C fuel oil at temperatures ranging from 5 to 28 C. At 15 C, 41 to 85% of the benzene-soluble components of Bunker C disappeared after incubation for 7 days; at 5 C the values ranged from 21 to 52% after 14 days of incubation. A Nocardia sp. isolated from a culture enriched on Bunker C oil grew on Venezuelan crude oil, Bunker C, hexadecane, and a hydrocarbon mixture at temperatures of 5 and 15 C. The 10-C decrease in temperature resulted in an average 2.2-fold decrease in generation time of the bacteria. Gas-liquid chromatographic measurements of Venezuelan and Arabian crude oils which had been incubated with the Nocardia sp. showed significant degradation of the n-alkane portion and the chromatographically unresolved components of the oils. The concentration of elemental nitrogen required to bring about the disappearance of 1 mg of hexadecane by the Nocardia sp. was 0.5 mg. The results confirm suggestions that the rate of natural biodegradation of oil in marine temperate-to-polar zones is probably limited by low temperatures and phosphorus concentrations, but suggest that the concentrations of nitrogen occurring naturally are probably not rate-limiting factors.

In February of 1970 the oil tanker Arrow, carrying 108,000 barrels of Bunker C fuel oil, ran aground in Chedabucto Bay, Nova Scotia. Approximately 100 miles (ca. 160.9 km) of the surrounding shoreline was polluted with oil within a short time of the accident (1).

Natural effects which can be responsible for the clean-up of oil-polluted waters and shorelines include physical effects such as wave action, radiant energy, and the extremes of weather (wind, storms) as well as biodegradation, which over the long term is believed to be important in the cleansing of chronically polluted waters (6). This last effect has led some investigators to suggest seeding oil spills with bacteria as a means of cleaning up accidental spills (7, 13, 17). It was well known at the time of the Chedabucto Bay oil spill that bacteria could degrade crude oils (8, 14, 17); however, it was not so clear that bacteria could act as effectively on heavy fuel oils such as Bunker C, especially under conditions of low water temperatures (a minimum of -1 C) known to exist in Chedabucto Bay.

The oil spill in Chedabucto Bay afforded an opportunity to study naturally occurring bacterial populations which presumably had been enriched in situ with a Bunker C substrate. The purpose of this study was to determine the action of these populations on the degradation of Bunker C oil and to assess quantitatively the effect of nutrient levels and certain environmental parameters on the rate of growth, on hydrocarbon substrates, of a representative microbial species isolated from an oil-polluted beach in Chedabucto Bay, Nova Scotia.

MATERIALS AND METHODS

The inorganic liquid minimal medium used throughout the study, except where modifications are noted, consisted of the following (in grams per liter): K_2HPO_4, 4.74; KH_2PO_4, 0.56; MgSO_4, 0.50; NaCl, 28.4; CaCl_2, 0.1; NH_4NO_3, 2.5; and 1 ml of a trace element stock (10) adjusted to a final pH of 7.1.

Total viable bacterial numbers were determined by the drop plate method (9) on Trypticase soy agar (TSA; BBL) plates containing 3% NaCl incubated at 15 C for 7 days.

The method used to determine percent hydrocarbon-utilizing (HCU) bacteria and the hydrocarbon mixture (naphthalene, 0.1 g; anthracene, 0.1 g; dibenzothiophene, 0.1 g; decalin, 5 ml; hexadecane, 5 ml; hexadecene-1, 5 ml; octadecane, 0.1 g; dodecane, 5 ml; and iso-octane, 5 ml) were described previously (10). Bacteria which grew in the stoppered air-tight tubes of liquid minimal medium without an added carbon source were used as a blank correction; their
numbers were subtracted from the totals of HCU bacteria prior to calculating the percentages.

Isolations of HCU bacteria were made on minimal medium plates to which 1.2% washed Ionagar no. 2 (Oxoid, London, England) was added. The carbon source consisted of 0.5 ml of hydrocarbon mixture added to sterile filter paper secured in the lids of the petri dishes, which were inverted and incubated at 3 C for 2 weeks. Colonies were then picked and inoculated into liquid minimal medium containing 1% hydrocarbon mixture. The purity of the cultures was assured by repeated streaking on minimal medium plus hydrocarbon mixture agar plates.

Identification of isolates (10) was carried out by using standard techniques with reference to Bergey's Manual of Determinative Bacteriology (4) and Skerman (12). The Nocardia isolate described in this paper was obtained from enriched culture MA13 and was chosen for study because of its superior growth rate on Bunker C oil compared with other isolates.

For the growth studies, the Nocardia sp. was grown at 15 C for 5 to 7 days on minimal medium agar plates with hydrocarbon mixture in the lid. Suspensions of the culture were then inoculated into minimal medium containing hydrocarbons or oils at a 1% (vol/vol) concentration and, unless otherwise stated, incubated at 15 C on a refrigerated rotary shaker at 120 rpm. At specified time intervals, samples were removed and the viable numbers were determined. Generation times were calculated from the exponential phase of the growth curves.

Beach and water samples were collected aseptically from Chedabucto Bay in November 1970, when the water temperature was 7 C. These samples were enriched by the following procedure. One gram of beach sample or 1 ml of water sample was added to minimal medium containing 0.125% Bunker C, and the flasks were incubated for 14 days at 20 C and 120 rpm on a refrigerated gyrotrary shaker bath (New Brunswick Scientific Co., Inc., New Brunswick, NJ). For experimental purposes the resulting enriched cultures were used as inoculum for the work on mixed cultures. Bunker C oil, obtained from the tanks of the Arrow, (this no. 6 fuel oil prepared at Amuay refinery, Venezuela, has been described comprehensively previously [1]), was steam sterilized at 121 C and 15 lb/in² for 15 min in tightly capped flasks to prevent evaporation. Bunker C was used in experiments at a concentration of 0.125% by volume. Pure liquid hydrocarbons and crude oils were sterilized by being passed, under pressure, through a 0.45-μm membrane filter unit equipped with a prefiter and checked for sterility prior to use. Solid hydrocarbons were steam sterilized in tightly capped flasks at 121 C and 15 lb/in² for 15 min. Pure hydrocarbons (99% purity or practical grade; Matheson, Coleman and Bell, Cincinnati, Ohio) were added to liquid minimal medium to give a concentration of 1% by volume.

Bunker C degradation, measured as the disappearance of benzene-soluble components, was determined gravimetrically. The contents of an entire culture flask (50 ml) were extracted with 5 ml of benzene three times. The benzene extracts were pooled and placed in a preweighed bottle, the benzene was evaporated at 80 C, and the weight of the bottle and contents was recorded. This procedure recovered 85% of the Bunker C oil (the asphaltenes, approximately 10% of this oil [1], were not soluble in benzene). Percent degradation, measured as the disappearance of benzene-extractable components in comparison with an uninoculated control flask (weathering control) was calculated by: mg of Bunker C/100 ml/mg of Bunker C (control) × 100.

Hexadecane and crude oils were extracted from the appropriate cultures by a modification of the procedure outlined by Soli and Bens (13). To 40 ml of culture fluid, 0.5 ml of 12 N H₂SO₄ and sufficient NaN₂ to give a concentration of 10⁻² M were added and mixed. The contents of the flask and 2 ml of heptane were transferred to a centrifuge tube. After capping and mixing, the contents were centrifuged at 0 C for 15 min at 28,700 x g; the heptane layer was removed, and the extraction was repeated using an additional 2 ml of heptane. The supernatant extract was placed in a capped glass vial and kept at –10 C until chromatographed (subsequent repeated extractions of the culture fluid did not produce significant amounts of additional material; consequently, the extraction as outlined was considered virtually complete). The gas chromatographic analyses were carried out on a Victoreen series 4000 gas chromatograph (The Victoreen Instrument Co., Cleveland, Ohio). The 90- by 1.2-cm stainless-steel column was packed with 3% silicone OV-1 on 85-100 BS mesh acid-washed dimethylchlorosilane-treated Chromosorb G. Nitrogen was used as the carrier gas at a flow rate of 40 ml/min. For hexadecane analyses the column temperature was 175 C, the injection block temperature was 300 C, and the flame ionization detector temperature was 350 C. The recorder sensitivity was 6 x 10⁻⁸ A. Analyses of Venezuelan and Arabian crude oils were performed under similar conditions with the column held isothermally for 5 min at 80 C, programmed from 80 C to 290 C at 10 C/min, and then held isothermally at 290 C for 10 min. The recorder sensitivity was 1.5 x 10⁻⁸ A. Two methods of measuring the extent of degradation are reported. First, peaks tentatively assigned to the n-alkane series of hydrocarbons by comparison of retention times with pure analytical standards (Polyscience Corp., Niles, II.) were measured, and percent degradation was determined by comparing peak heights obtained for a test extract with the peak heights of a control which had been incubated in the presence of 10⁻² M NaN₂. The second method was that outlined by Zafiriou et al. (15) for measuring the background of unresolved substances in the chromatogram. This method involves measuring the distances from the baseline of the chromatogram (obtained from a solvent run) to the valleys immediately preceding the n-C₁₆ and n-C₁₄ peaks and expressing the two values as a ratio. Percent degradation of unresolved substances was determined by comparing the ratio obtained for a test extract with that of the control.

RESULTS

Mixed culture studies. All beach and water samples taken from different locations of Che-
dabucto Bay yielded populations of bacteria capable of degrading Bunker C (Table 1). Each mixed culture brought about a different amount of Bunker C degradation depending on the area from which the sample was taken, whether or not it was a beach or water sample, and on the temperature at which the culture was incubated. There appeared to be a correlation between the amount of Bunker C degraded and the visible appearance, with regard to the presence of polluting oil, of the beach areas from which the samples were taken. All beach areas sampled had been oiled as a result of the Arrow spill; however, at the time of sampling a certain amount of natural clean-up had taken place. There was no visible evidence of oil on the beaches in area 2, whereas oil was visible along certain parts of the beaches in area 3. Oil was very much in evidence along the beaches of area 1. Of the three areas sampled, the enriched cultures obtained from area 1 brought about the greatest percentages of Bunker C degradation at all three temperatures. Cultures derived from area 2 and MA36, samples which were obtained at or in waters adjacent to clean beaches, yielded the lowest values for the percentages of Bunker C degraded. It was also noted that beach samples from each area yielded higher degradation values than corresponding water samples after incubation at 15 and 10 C. These results strengthened the assumption that the presence of Bunker C on beaches resulted in an enrichment of organisms capable of degrading Bunker C. The values obtained for Bunker C degradation by the mixed cultures were comparable after 7 days of incubation at temperatures of 15 and 10 C. (If the time course of degradation had been followed, it would be expected to show a faster rate initially at 15 C than at 10 C followed by a decline, thus accounting for the similarity of the final values reported for 10 and 15 C, or alternatively different microorganisms in the mixed cultures could have been favored at each temperature.) Incubation at 5 C for 14 days produced different values for all cultures; the values for degradation at 5 C for 14 days were 21 to 70% less than values obtained at the 10-C incubation carried out for 7 days.

The enriched cultures grew on a wide variety of hydrocarbon substrates. Aromatic compounds, straight-chain alkanes and alkenes, and cycloalkanes, as well as Venezuelan crude and Bunker C oils, served as carbon sources for growth of these cultures (Table 2). There was no apparent correlation between ability to grow on aromatics and cycloalkanes (Table 2) and increased ability to degrade Bunker C (Table 1).

To better understand the process of enrichment, measurements were made on the course of Bunker C degradation, the total viable bacte-

### Table 1. Effect of temperature on the biodegradation of Bunker C by mixed cultures in minimal medium

| Culture | Area | Description of sample and area | % Bunker C disappeared at 15 C | % Bunker C disappeared at 10 C | % Bunker C disappeared at 5 C |
|---------|------|--------------------------------|------------------------------|-------------------------------|------------------------------|
| MA13    | 1    | Oiled beach; pebbles taken from high tide area. | 85                           | 82                            | 52                           |
| MA17    | 1    | Oiled beach; water taken 4 ft (ca. 122 cm) from shore. | 68                           | 61                            | 48                           |
| MA24    | 2    | Clean beach; pebbles taken from intertidal area. | 54                           | 70                            | 21                           |
| MA28    | 2    | Clean beach; water taken 6 ft (ca. 183 cm) from shore. | 50                           | 68                            | 29                           |
| MA34    | 3    | Mud sample taken from intertidal area. | 77                           | 88                            | 32                           |
| MA36    | 3    | Clean beach; water taken 3 ft (ca. 91 cm) from shore. | 41                           | 44                            | 30                           |
| MA310   | 3    | Water taken from stagnant pond above high tide; oil on bottom. | 61                           | 60                            | 36                           |

* Incubated for 7 days.
* Incubated for 14 days. Duplicate trials using 0.125% Bunker C.

### Table 2. Hydrocarbon substrate specificity of mixed cultures incubated for 2 weeks at 15 C

| Mixed culture | Naphthalene | Anthracene | Dibenzothiophene | Phenyl-cyclohexane | Decalin | Hexadecane | Hexadecene 1 | Venezuelan crude oil | Hydrocarbon mixture | ”Arrow” Bunker C | Oil | Control (no carbon source) |
|---------------|-------------|------------|-------------------|--------------------|--------|------------|--------------|---------------------|-------------------|-------------------|-----|--------------------------|
| MA 13         | +           | +          | ±                  | ±                  | -      | +          | +            | +                   | +                 | +                 | -   | -                        |
| MA 17         | +           | -          | ±                  | ±                  | -      | +          | +            | +                   | +                 | +                 | -   | -                        |
| MA 24         | ±           | -          | -                  | ±                  | -      | +          | +            | +                   | +                 | +                 | -   | -                        |
| MA 28         | -           | -          | -                  | -                  | ±      | +          | ±            | +                   | +                 | +                 | -   | -                        |
| MA 34         | -           | -          | -                  | -                  | -      | +          | ±            | +                   | +                 | +                 | -   | -                        |
| MA 36         | -           | -          | -                  | -                  | -      | +          | +            | +                   | +                 | +                 | -   | -                        |
| MA 310        | +           | -          | ±                  | ±                  | +      | +          | +            | +                   | +                 | +                 | -   | -                        |

* +, Profuse growth; ±, moderate growth as evidenced by turbidity; -, no growth. Duplicate trials.
rial population as counted on TSA, and the HCU and blank correction bacterial fractions of the population during the enrichment of sample MA36 at 28 C. Figure 1 shows that maximal values of Bunker C degradation coincided with the appearance of maximal numbers of HCU bacteria. When maximal values for Bunker C degradation were attained, HCU bacteria declined in number. At day 14, the presence of organisms which could grow in the basal medium without an added carbon source was noted. The bacterial population increased 10° times in 4 days of incubation, indicating that Bunker C provides a good source of carbon for growth and that intermediate products, toxic to the bacteria, apparently were not formed. When this experiment was repeated at 15 C, extended lag phases were observed for both the growth curve and the course of Bunker C degradation, and there was a decrease in total growth (Fig. 2) compared with the values obtained at 28 C (Fig. 1).

**Pure culture work.** The pure culture was isolated from enriched culture MA13 (see Table 1). The isolate appeared as a long, slender, gram-positive rod during the first 3 days of incubation on TSA, but by day 6 it had assumed a gram-positive coccobacillus-like form. The isolate was not acid fast, produced filiform cream-colored growth on TSA, used nitrate as sole nitrogen source, did not liquefy gelatin, and grew on TSA at 3 C but not 37 C. The organism was nonmotile, made litmus milk alkaline, and produced slight amounts of H2S. When grown on minimal medium plates plus hydrocarbon mixture, the colonies were circular, undulate, pale orange in color, and 2 mm in diameter. A developing culture on an agar slant was observed to form a mycelium from the coccobacilli-like inoculum. This mycelium later broke up into a multiplicity of coccobacilli-like forms. The isolate was classified as a *Nocardia* sp.

The *Nocardia* sp. grew on hexadecane, the hydrocarbon mixture, glucose, and Venezuelan crude oil at similar rates (Table 3). The generation time when grown on Bunker C oil was slightly greater, whereas the organism grew only slowly on naphthalene. Decreasing the temperature of incubation from 15 to 5 C increased the generation time by factors ranging from 1.7 to 2.4, with an average value of 2.2. This factor is in agreement with that given by ZoBell (17) who estimated a two- to threefold increase in generation time for each 10-C decrease in temperature. When incubated at 15 C for 14 days, the *Nocardia* sp. utilized 94% of the n-alkane fractions of Arabian and 77% of Venezuelan crude oils.

The culture also carried out partial degradation of the unresolved gas-liquid chromatographic portion of the oils (Table 4).

The *Nocardia* sp. was isolated from a beach sample. Thus, it was not surprising to find that the organism grew well on hexadecane without the addition of salt or with up to 3% NaCl added (Table 5). A concentration of 5% NaCl greatly increased the generation time. In all other experiments the concentration of NaCl was 2.8%.

Hexadecane concentrations of 1 to 10% (vol/vol) were tested, and no significant difference in generation times was found (Table 6), suggesting that the microbiological system is saturated
TABLE 4. Degradation of the n-alkane and unresolved fractions of Arabian and Venezuelan crude oils by the Nocardia sp. after incubation at 15 C for 14 days*

| Oil            | Degradation (%) | n-Alkanes (C11-18 avg) | Unresolved fraction |
|----------------|-----------------|-------------------------|---------------------|
| Venezuelan     | 77.0            | 10%                     | 13.0                |
| Arabian        | 94.0            | 13%                     | 35.0                |

* Duplicate trials.

TABLE 5. Effect of NaCl concentration on the generation time of the Nocardia sp.*

| Sodium chloride (%) | Generation time (h) |
|---------------------|----------------------|
| 0                   | 14.4                 |
| 1                   | 12.0                 |
| 2                   | 10.3                 |
| 3                   | 14.4                 |
| 5                   | 64                   |

* One percent hydrocarbon mixture served as carbon source and incubation was at 15 C. Duplicate trials.

TABLE 6. Effect of hexadecane concentration on the generation time of the Nocardia sp. growing at 15 C*

| Hexadecane (%) | Generation time (h) |
|----------------|----------------------|
| 0              | No growth            |
| 1              | 11.0                 |
| 3              | 12.0                 |
| 5              | 12.0                 |
| 10             | 11.0                 |

* Duplicate trials.

with hydrocarbon at the 1% level. It is noteworthy that the rather high concentration of 10% hexadecane was not inhibitory to the growth of the organism.

The organism grew on hexadecane equally well at initial pH values of 7.0, 7.5, 8.0, and 8.5 (Fig. 3). There was a decrease in pH concomitant with growth of the organism. In this experiment, 0.05 M tris(hydroxymethyl)aminomethane (Tris) was used as an additional buffering agent; the presence of Tris plus phosphate decreased the generation times (Table 7) compared with those derived from growth media buffered with phosphate alone (Table 8). Tris was not used as a carbon source for growth.

In the marine environment, nitrogen and phosphorus, nutrients which are required for bacterial growth, are present in limited amounts. The maximal concentrations in the sea, as given by ZoBell (16), are nitrogen, 0.7 mg/liter, and phosphorus, 0.1 mg/liter. It has been suggested (13, 17) that these nutrients would limit biodegradation in a situation such as an oil spill in a marine area.

Experiments were carried out to answer the following questions: (i) to what extent do the various concentrations of nitrogen and phosphorus affect the rate of growth and maximal populations of the organism, and (ii) what amount of elemental nitrogen is required to bring about the disappearance of 1 mg of n-alkane using Nocardia sp. as a representative HCU microorganism?

The effect of nitrogen concentration on the growth rate of the Nocardia sp. is shown in Table 8. Nitrogen concentrations ranging from 875 mg/liter to 0.875 mg/liter, (the minimal value roughly equivalent to that found in sea water), did not affect the generation time of the organism growing on any of the four carbon sources, hexadecane, hydrocarbon mixture, Venezuelan crude, and Bunker C. The varying nitrogen concentrations, however, did affect the size of the bacterial population resulting from

FIG. 3. Effect of pH on the growth of the Nocardia sp. at 15 C. Open symbols represent pH measurements; closed symbols represent the corresponding bacterial counts. Duplicate trials.
growth on each of the carbon sources; at lower nitrogen concentrations the stationary phase was reached at an earlier point in the incubation period, reflecting a depletion of nitrogen supply.

To answer the second question an experiment was set up so that the hydrocarbon (hexadecane) was not limiting (0.125%, vol/vol). Nitrogen concentrations ranging from 0.1 to 50 mg of nitrogen per liter were tested, and the reaction was stopped after 6 days while the growth of the Nocardia sp. at 15 C on a gyrotory shaker was still in the exponential phase. The microbial numbers and the amount of hexadecane were determined and used to construct the relationship illustrated in Fig. 4. From the straight-line portion of the curve representing hexadecane disappearance, it was deduced that approximately 0.05 mg of elemental nitrogen was required to bring about the disappearance of 1 mg of hexadecane in the presence of adequate supplies of phosphorous. Growth of the organism followed a curve similar to the hexadecane disappearance.

In contrast to the effect of nitrogen on growth rate, reduction of phosphorus concentration served to increase the generation time of the organism (Table 9). Since decreased concentrations of phosphorus resulted in lower pH values and consequent death, 0.05 M Tris was added as a buffering agent. A straight-line relationship existed between generation time and the log of phosphorus concentration, giving an exponential relationship which indicates a more complex situation than that which exists for nitrogen. The maximal population size was also affected by the concentration of phosphorus, indicative of a depletion of the available nutrient.

Table 7. Effect of Tris buffer* on generation time (G) and maximal population of the Nocardia sp. (per milliliter) grown at 15 C for 14 days on 1% hexadecane

| Initial pH | Final pH | G (h) | Maximal Population* |
|------------|----------|-------|---------------------|
| 7.0        | 5.1      | 9.9   | 1.0 x 10^4          |
| 7.5        | 5.9      | 9.5   | 3.2 x 10^4          |

*Tris buffer, 0.05 M, adjusted with HCl.

*Initial population was 4.8 x 10^9/ml. Duplicate trials.

Table 8. Effect of nitrogen concentration on the generation time (G) and maximal population of the Nocardia sp. (per milliliter) grown at 15 C for 14 days*

| Mg of N/liter* | Hydrocarbon mixture | Venezuelan crude oil | Bunker C | Hexadecane |
|----------------|---------------------|----------------------|----------|------------|
|                | G (h) | Maximal Population* | G (h) | Maximal Population* | G (h) | Maximal Population* | G (h) | Maximal Population* |
| 875.0          | 12.4  | 2 x 10^8           | 12.4  | 2.6 x 10^9           | 15.3  | 2.4 x 10^9           | 11.0  | 4.4 x 10^8           |
| 87.5           | 12.0  | 8 x 10^8           | 12.8  | 1 x 10^9            | 15.3  | 9.2 x 10^9           | 10.7  | 2.6 x 10^9           |
| 8.75           | 14.0  | 1.4 x 10^9         | 12.0  | 5.2 x 10^9          | 15.0  | 1.3 x 10^9           | 12.0  | 2.8 x 10^9           |
| 0.875          | 12.6  | 7.2 x 10^8         | 12.0  | 1.6 x 10^9          | 15.3  | 4.0 x 10^9           | ND    | ND                    |
| 0              | 7.2 x 10^8 |                   | 4.0 x 10^9 |                 | 1.6 x 10^9 |               | 2.0 x 10^8 |        |

*Trials in triplicate. Carbon source concentrations were 1% (vol/vol). ND, not determined.

*Nitrogen source was NH_4NO_3.

*Initial population was 1.1 x 10^9 bacteria/ml.

*Initial population was 9.6 x 10^9 bacteria/ml.
DISCUSSION

Mixed microbial cultures from Chedabucto Bay which had been enriched from beach and water samples on Bunker C oil grew on and utilized Bunker C at temperatures as low as 5 C. During the enrichment of culture MA36 on Bunker C it was noted that decreased temperatures resulted in extended lag phases of both the growth curve and the Bunker C degradation curve (see Fig. 1 and 2). A similar lag in degradation has been noted by Atlas and Bartha (3) using Sweden crude oil. They attributed this effect to the presence of volatile toxic components, the rate of evaporation of which was reduced at lower temperatures.

ZoBell (17) listed the requirements for microbial attack of oils in the marine environment as (i) the presence of HCU bacteria in the marine environment and (ii) favorable environmental conditions for growth such as temperature, salinity, concentration of microbial nutrients, especially nitrogen and phosphorus, available oxygen, and the dispersion of oil in water. It was shown previously (10) that HCU bacteria were, for all practical purposes, ubiquitous in coastal and open waters from Frobisher Bay, N.W.T., Canada, along the eastern Canadian seaboard to Bermuda, thus meeting ZoBell's first requirement.

Nissen (11) placed emphasis on the importance of temperature in the degradation of oil and pointed out that it takes a long time for oil to disappear in Arctic regions. However, very little quantitative work has been published to show the effect of low temperatures on the bacterial degradation of hydrocarbons. In the study reported here, mixed and pure bacterial cultures were shown to be capable of growing on and degrading a wide variety of hydrocarbon sources, including the heavy fuel oil, Bunker C, at temperatures as low as 5 C. In the case of the Nocardia sp., the rate of growth on various hydrocarbons decreased, on the average, 2.2 times when the temperature was lowered from 15 to 5 C. Similarly, a mixed culture grew on and degraded Bunker C at reduced rates when incubated at 15 C rather than 28 C (Fig. 1, 2; Table 1). These results re-emphasize the importance of temperature in determining the natural rate of biodegradation in the marine environment, particularly in waters surrounding countries such as Canada, where water temperatures rarely exceed 15 C.

Literature reports (2, 17) have suggested that the nitrogen and phosphorus levels in the sea limit the rate of biodegradation of oil. However, in this study, the concentration of nitrogen, although it controlled the amount of growth, did not affect the rate of growth of the Nocardia sp. and the rate of degradation of oil (Fig. 1, 2, 4). In an open system such as the ocean, natural levels of nitrogen, although low, are being replenished constantly. Consequently, the supply of nitrogen should not be limiting with respect to natural rates of biodegradation or overall bacterial population size. In contrast, phosphorus concentration affected both the rate of growth of the Nocardia sp. and the bacterial population size.

These results indicate that the rate of natural biodegradation of oil pollutants will be limited by temperature and phosphorus concentrations but probably not by open sea nitrogen concentrations. The seeding of oil spills with bacteria presents a different situation, since in so doing an attempt is made to create a more efficient local system. It would be necessary in that event to add both nitrogen and phosphorus or accept real limitations to growth and rate of degradation imposed by their scarcity, thereby negating the effect of the seeding. Furthermore, it must be recognized that the addition of bacteria and nutrients still does not overcome the limitations which low northern temperatures impose. Finally, when enrichment of sea water with phosphorus is being considered, it should be kept in mind that the levels of phosphorus in the ocean are believed to be maintained at low levels by the insolubility of minerals of the apatite group or through the formation of struvite (5).

ACKNOWLEDGMENT

We thank R. M. MacKelvie of this laboratory for his constructive criticism of the manuscript.

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ERRATUM

Effect of Environmental Parameters on Bacterial Degradation of Bunker C Oil, Crude Oils, and Hydrocarbons

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Volume 28, no. 6, p. 915, abstract, line 13: "... of 1 mg of hexadecane by the Nocardia sp. was 0.5 mg. ... should read "... of 1 mg of hexadecane by the Nocardia sp. was 0.05 mg."