Microbial Petroleum Degradation: Use of Mixed Hydrocarbon Substrates

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Methods of examining hydrocarbons to estimate the microbial degradation of petroleum are compared. Gas-liquid chromatography with a mixed hydrocarbon substrate has been shown to be useful in evaluating microbial potential for degradation of a number of hydrocarbons.

INTRODUCTION

A research program under way in our laboratory is designed to study the microbial ecology of petroleum utilization in Chesapeake Bay (13). A number of techniques have been proposed by various investigators and applied in the evaluation of microbial degradation of petroleum. These include: (i) oxygen consumption (3); (ii) biochemical oxygen demand (2); (iii) dry weight and dispersion (10); (iv) carbon dioxide evolution and gas-liquid chromatography (GLC) (1); (v) GLC (9); and (vi) column chromatography combined with GLC of n-alkanes (5). A comparative study using a mixed hydrocarbon substrate and available techniques for evaluating petroleum degradation has been undertaken. Results of such a study, employing a mixed hydrocarbon substrate and a commercially available motor oil, are reported, with the use of a mixed hydrocarbon substrate shown to be a valid estimator of microbial degradation of petroleum hydrocarbons.

MATERIALS AND METHODS

Microbiological methods. Sediment collected from an oil-polluted creek at Baltimore Harbor in Chesapeake Bay was used as the inoculum. One hundred milliliters of estuarine salts (13), in 250-ml Erlenmeyer flasks, supplemented with 2% (v/v) motor oil or mixed hydrocarbon substrate were inoculated with 1 ml of a 10⁻² dilution of the sediment. Inoculated flasks, as well as uninoculated controls, were incubated at 20°C on a reciprocal shaker set at 0 (1 1/4 inch) strokes per min. Growth was monitored by plate counts on an estuarine salts agar medium (13).

Extraction procedure. An internal standard, i.e., 0.1 g of dodecanol or n-octacosane, was added to flasks containing model petroleum prior to extraction. Each of the stationary-phase cultures and the uninoculated controls were extracted twice with 50 ml of benzene. The extracts were combined, dried over 20 g of Na₂SO₄, for 24 h at ambient temperature, filtered through Whatman no. 1 filter paper, and concentrated to ca. 8 ml at 35°C by rotary flask evaporation under nitrogen.

Analytical procedure with model petroleum. The 8-ml sample of mixed hydrocarbon substrate was further concentrated to 1.5 ml under nitrogen and analyzed by GLC. Chromatograms were obtained on a Shimadzu Model GC-4BMFP gas chromatograph (American Instrument Co., Silver Spring, Md.) equipped with a single-flame ionization detector. Two types of glass columns were used: a 3 mm by 1.5 m column packed with 3% OV-17 on 80/100 mesh Chromosorb P and a 3 mm by 1.5 m column packed with 3% OV-1 on 80/100 Chromosorb C. The carrier gas, nitrogen, was run through the columns at a rate of 40 ml per min. Temperature was programmed from 60 to 300°C at 5°C per min, and individual hydrocarbons were quantitated using a Hewlett-Packard Model 3373 B Integrator. Column efficiency and detector response, measured with a mixture of C₆₋₅-C₃0 saturated and unsaturated hydrocarbons (Applied Science mixture No. 19251), yielded a relative error of less than 2.5% for all components.

Analytical procedure with motor oil. After microbial degradation, 20-weight, non-detergent motor oil was fractionated on activated Silica Gel G by eluting sequentially with hexane, benzene, and methanol, using a procedure similar to that described by Kator, Oppenheimer, and Miget for fractionating Louisiana crude oil (6). Each of the fractions prepared from the degraded motor oil, as well as the controls, was subjected to column chromatography separation, using ion-exchange resins and activated Silica Gel G, in order to determine percentage of resins, saturates, and aromatics, as described by Jewell et al. (4). Saturates were analyzed by computerized GLC and saturates and aromatics by low-resolution mass spectrometry, as described by Robinson and Cook (11) and American Society for Testing and Materials methods D2786 and 5217-CR.

RESULTS AND DISCUSSION

A mixed saturated hydrocarbon substrate, i.e., a model petroleum, was used successfully to evaluate the microbial potential for hydrocarbon degradation in Chesapeake Bay water and sediment (Table 1, Fig. 1). It was efficiently extracted using the procedure outlined above (Table 2). The model petroleum which was used
TABLE 1. Composition of mixed saturated hydrocarbon substrate

| Hydrocarbon         | Percent | Elution sequence on OV-1 | OV-17* |
|---------------------|---------|---------------------------|--------|
| Normal alkanes      |         |                           |        |
| Decane              | 7.80    | 5                         | 4      |
| Undecane            | 7.80    | 6                         | 6      |
| Dodecane            | 7.80    | 8                         | 7      |
| Tridecane           | 7.80    | 9                         | 8      |
| Tetradecane         | 7.80    | 10                        | 10     |
| Pentadecane         | 7.80    | 11                        | 11     |
| Hexadecane          | 7.80    | 12                        | 12     |
| Heptadecane         | 7.80    | 13                        | 14     |
| Octadecane          | 7.80    | 15                        | 15     |
| Nonadecane          | 7.80    | 16                        | 16     |
| Eicosane            | 7.80    | 18                        | 17     |
| Branched alkanes    |         |                           |        |
| Pristane            | 3.90    | 14                        | 13     |
| Cyclic alkanes      |         |                           |        |
| Cyclohexane         | 3.90    | 2                         | 2      |
| Aromatics           |         |                           |        |
| Cumene              | 3.90    | 4                         | 5      |
| Naphthalene         | 0.66    | 7                         | 9      |
| Phenanthrene        | 0.66    | 17                        | 18     |
| Polynuclear aromatics|       |                           |        |
| 1,2-Benzanthracene | 0.39    | 19                        | 19     |
| Perylene            | 0.39    | 20                        | 20     |
| Pyrene              | 0.39    | 21                        | 21     |

* See Fig. 1.

was designed to represent the major classes of hydrocarbons of petroleum and to permit measurement of the degradation of a number of simple and complex hydrocarbons (8).

A number of methods are used to study microbial degradation of petroleum. Tech-

TABLE 2. Efficiency of the method employed for extracting hydrocarbons comprising model petroleum

| Hydrocarbon        | Replicate (% recovery) | 1   | 2   | 3   | Avg         |
|--------------------|------------------------|-----|-----|-----|-------------|
| Cyclohexane        |                        | 97.65 | 98.07 | 96.97 | 97.56 ± 0.26 |
| n-Decane           |                        | 96.31 | 97.94 | 95.24 | 96.49 ± 0.64 |
| Cumene             |                        | 97.77 | 96.74 | 96.51 | 97.00 ± 0.31 |
| n-Undecane         |                        | 97.42 | 96.58 | 96.53 | 96.89 ± 0.23 |
| n-Dodecane         |                        | 97.34 | 97.26 | 96.57 | 97.05 ± 0.19 |
| n-Tridecane        |                        | 96.78 | 96.60 | 96.68 | 96.68 ± 0.04 |
| Naphthalene        |                        | 97.42 | 96.66 | 96.63 | 96.90 ± 0.21 |
| n-Tetradecane      |                        | 97.03 | 96.72 | 96.92 | 96.89 ± 0.07 |
| n-Pentadecane      |                        | 97.44 | 96.80 | 96.43 | 96.89 ± 0.24 |
| n-Hexadecane       |                        | 96.71 | 96.79 | 96.89 | 96.79 ± 0.04 |
| n-Octadecane       |                        | 97.63 | 96.87 | 97.03 | 97.17 ± 0.18 |
| n-Nonadecane       |                        | 98.41 | 97.11 | 98.43 | 97.98 ± 0.35 |
| n-Eicosane         |                        | 97.31 | 97.03 | 97.25 | 97.19 ± 0.06 |
| Phenanthrene       |                        | 97.61 | 97.32 | 97.61 | 97.51 ± 0.07 |
| 1,2-Benzanthracene|                        | 97.57 | 97.37 | 97.42 | 97.45 ± 0.04 |
| Perylene           |                        | 98.88 | 98.39 | 97.65 | 98.30 ± 0.29 |
| Pyrene             |                        | 97.32 | 96.24 | 97.23 | 96.93 ± 0.28 |

* Quantitated using OV-17 column programmed to run from 60 to 300 C at 5 C per min and an internal standard of octacosane.

FIG. 1. Gas chromatographic tracing of hydrocarbons comprising model petroleum separated on an OV-17 column programmed to run from 60 to 300 C at 5 C per min. See Table 1 for identity of compounds numbered according to elution sequence.
niques such as oxygen consumption and biochemical oxygen demand provide a measure of the rate of petroleum degradation. Dispersion and dry weight data indicate the degree of emulsification of petroleum and the total amount of petroleum degraded. CO₂ evolution and GLC offer information on the rate of petroleum mineralization, as well as the extent of the hydrocarbon degradation. GLC provides a means to identify those hydrocarbons which are degraded and to gain information on the rate of degradation of the hydrocarbons, if applied at regular time intervals during growth of microorganisms on petroleum. Column chromatography combined with GLC of n-alkanes yields information on the class of compounds degraded, as well as detailed information on the degradation of the n-alkanes.

Since fractionation of petroleum by column chromatography yields information on classes of hydrocarbons, the degraded motor oil was fractionated in this study by column chromatography. A saturate fraction (hexane), an aromatic fraction (benzene), and an asphaltic fraction (methanol) were thus obtained. These fractions may be contaminated by compounds other than

### Table 3. Classes of hydrocarbons comprising each fraction as measured by column chromatography of degraded motor oil

| Fraction          | Wt of residue (mg) | Component (% of residue) |
|-------------------|--------------------|--------------------------|
|                   |                    | Asphaltenes | Resins | Saturates | Aromatics |
| Hexane (saturates) | 377.6              | 0           | 2.4    | 61.6      | 36.0      |
| Benzene (aromatics)| 15.0               | 0           | 10.6   | 15.2      | 74.2      |
| Methanol (asphaltenics) | 4.0*              | 0           | ND*    | ND        | ND        |

*Samples smaller than 15 mg did not provide enough material for fractionation.

*ND, Not determined.

![Fig. 2. Gas chromatographic tracing of hydrocarbons from hexane fraction of motor oil separated as described in American Society for Testing and Materials method 5217-Cr. Numbers indicate chain length of n-alkanes.](image-url)
and it also showed differences in the amount of each type of aromatic hydrocarbon (Table 4). Column chromatography of petroleum before and after microbial degradation provides valuable information concerning the asphaltenes, resins, aromatics, and saturates in petroleum. However, it may be useful to remove the asphaltic and resin fractions of the oil prior to column chromatography on Silica Gel G and to monitor the column eluate as described by Jewell et al. (4) to determine the purity of the fractions.

Analysis of n-alkanes is necessary because they are prominent hydrocarbons in most crude oils and may be responsible for the repressed degradation of other hydrocarbons present in the oil (M. P. Pirmik, R. M. Atlas, and R. Bartha, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, p. 170). However, direct GLC of petroleum, after microbial degradation, often yields a large envelope containing unknown hydrocarbons which may be refractory to degradation (Fig. 4). Certain aliphatic hydrocarbons detected by GLC can be identified (Table 5). However, the base envelope contains many unidentified aliphatic hydrocarbons. The fate of these compounds during petroleum degradation cannot be assessed by GLC. Although microbial degradation of polynuclear aromatic hydrocarbons has been reported (12), these compounds remain unresolved, being in the

Table 4. Comparison of aromatic hydrocarbons in the hexane and benzene fractions of degraded motor oil

| Hydrocarbon | Hexane fraction | Benzene fraction |
|-------------|----------------|-----------------|
|             | mg  | %   | mg  | %   |
| Monoaromatics |     |     |     |     |
| Alkyl/benzenes | 2.9 | 6.3 | 0.9 | 6.0 |
| Naphthene benzenes | 2.3 | 5.0 | 0.8 | 5.6 |
| Dinaphthene benzenes | 2.4 | 5.1 | 1.1 | 7.4 |
| Diaromatics |     |     |     |     |
| Naphthalenes | 0.9 | 2.0 | 0.5 | 3.4 |
| Acenaphthenes | 1.2 | 2.7 | 1.2 | 7.8 |
| Fluorenes | 1.3 | 2.8 | 1.5 | 9.9 |
| Triaromatics |     |     |     |     |
| Phenanthrenes | 0.6 | 1.3 | 0.9 | 6.9 |
| Naphthene phenanthrenes | 0.6 | 1.3 | 0.9 | 5.9 |
| Tetraaromatics |     |     |     |     |
| Pyrenes | 0.4 | 0.8 | 0.8 | 8.3 |
| Chrysenes | 0.2 | 0.5 | 0.4 | 2.9 |
| Pentaaromatics |     |     |     |     |
| Perylenes | 0.1 | 0.3 | 0.3 | 2.2 |
| Dibenzanthracenes | 0.1 | 0.3 | 0 | 0 |
| Sulfur aromatics |     |     |     |     |
| Benzothiophenes | 0.8 | 1.7 | 0.5 | 9.3 |
| Dibenzothiophenes | 0.4 | 0.8 | 1.0 | 6.1 |
| Naphthobenzothiophenes | 0.2 | 0.5 | 0.6 | 3.7 |
| Unidentified aromatics | 2.2 | 4.7 | 1.1 | 7.1 |

Table 5. Some aliphatic hydrocarbons of motor oil identified by GLC

| Peak no.* | Hydrocarbon             | Retention time (min) |
|-----------|-------------------------|----------------------|
| 2         | Cyclohexane             | 1                    |
| 3         | n-Decane                | 6                    |
| 4         | n-Undecane              | 9                    |
| 5         | n-Dodecane              | 12                   |
| 6         | n-Tridecane             | 14                   |
| 8         | n-Tetradecane           | 17                   |
| 11        | n-Pentadecane           | 19                   |
| 14        | n-Hexadecane            | 22                   |
| 17        | n-Heptadecane and pristane | 25               |
| 21        | n-Octadecane            | 27                   |
| 24        | n-Nonadecane            | 29                   |
| 28        | n-Eicosane              | 31                   |
| 31        | n-Heneicosane           | 33                   |
| 33        | n-Docosane              | 35                   |
| 35        | n-Tricosane             | 37                   |
| 38        | n-Tetracosane           | 38                   |
| 41        | n-Pentacosane           | 40                   |
| 43        | n-Hexacosane            | 42                   |
| 44        | n-Heptacosane           | 43                   |
| 46        | n-Octacosane            | 45                   |
| 48        | n-Nonacosane            | 46                   |

* Refers to Fig. 2.
envelope. Their fate cannot be assessed by GLC.

The use of model petroleum in studies of microbial petroleum degradation provides several unique advantages: (i) number and type of components can be altered to permit examination of different classes of hydrocarbons and to represent the natural petroleum being investigated, i.e., to serve as a model, or test, system for degradation studies; (ii) individual components can be analyzed quantitatively using a single GLC column; (iii) microbial potential for degradation of given hydrocarbons not detected by GLC can be assessed, e.g., cyclic alkanes, aromatics, and polynuclear aromatics; and (iv) degradation of components often considered
refractory, viz., pristane, can be measured. Model petroleum is not intended as a substitute for natural petroleum, but it can be used for a parallel analysis in those studies employing natural petroleum to estimate the potential of microorganisms in degrading unresolved components of the base envelope.

The natural petroleum substrate used in the study reported here was motor oil. It produced similar cell yields when compared to growth on model petroleum. In order to compare microbial degradation of individual hydrocarbons, or hydrocarbon types, in both the model petroleum and motor oil, it was necessary to analyze the motor oil substrate by computerized GLC and mass spectrometry. A comparison of the microbial degradation of the aliphatic hydrocarbons in motor oil and in model petroleum revealed that 87 to 88% of the aliphatic hydrocarbons were degraded in each case (Table 6). A slight, but not significant, preferred degradation of even \( n \)-alkanes over odd \( n \)-alkanes above C-30 in motor oil was noted (Table 6). Inclusion of additional high-boiling \( n \)-alkanes in the model petroleum should be possible, providing retention times would not coincide with high-

| Hydrocarbon          | Motor oil* | % remaining | Model petroleum* | % remaining |
|----------------------|------------|-------------|------------------|-------------|
|                      | Control (mg) | Degraded (mg) | Control (mg) | Degraded (mg) |             |
| Normal alkanes       |            |             |            |             |             |
| Decane               | ND         | ND          | 124.8       | 14.46       | 11.59       |
| Undecane             | ND         | ND          | 124.8       | 17.43       | 13.97       |
| Dodecane             | ND         | ND          | 124.8       | 17.39       | 13.94       |
| Tridecane            | ND         | ND          | 124.8       | 17.19       | 13.78       |
| Tetradecane          | 0.210      | 0.0         | 124.8       | 17.12       | 13.72       |
| Pentadecane          | 0.356      | 0.0         | 124.8       | 17.17       | 13.76       |
| Hexadecane           | 0.678      | 0.084       | 124.8       | 17.62       | 14.12       |
| Heptadecane          | 1.482      | 0.167       | 124.8       | 18.12       | 14.52       |
| Octadecane           | 1.744      | 0.210       | 124.8       | 18.44       | 14.79       |
| Nonadecane           | 1.960      | 0.038       | 124.8       | 18.72       | 15.00       |
| Eicosane             | 1.820      | 0.091       | 124.8       | 18.64       | 14.94       |
| Heneicosane          | 1.216      | 0.057       | ND          | ND          |             |
| Docosane             | 0.262      | 0.089       | ND          | ND          |             |
| Tricosane            | 0.618      | 0.116       | ND          | ND          |             |
| Tetracosane          | 2.052      | 0.158       | ND          | ND          |             |
| Pentacosane          | 1.486      | 0.096       | ND          | ND          |             |
| Hexacosane           | 2.502      | 0.131       | ND          | ND          |             |
| Heptacosane          | 3.320      | 0.429       | ND          | ND          |             |
| Octacosane           | 3.910      | 0.368       | ND          | ND          |             |
| Nonacosane           | 2.544      | 0.189       | ND          | ND          |             |
| Branched alkanes     |            |             |            |             |             |
| Pristane             | ND         | ND          | 62.40       | 7.84        | 12.58       |
| Phytane              | ND         | ND          | ND          | ND          |             |
| Cyclic alkanes       |            |             |            |             |             |
| 1-ring (cyclohexane)*| 232.0      | 29.6        | 62.4        | 5.17        | 8.28        |
| 2-ring               | 170.5      | 22.8        | ND          | ND          |             |
| 3-ring               | 128.8      | 17.4        | ND          | ND          |             |
| 4-ring               | 103.1      | 14.4        | ND          | ND          |             |
| 5-ring               | 55.5       | 7.5         | ND          | ND          |             |
| 6-ring               | 38.6       | 4.3         | ND          | ND          |             |
| Total                | 751.640    | 96.223      | 1497.60     | 205.91      | 13.74       |

* Analyzed by ASTM methods D2786 and 5217-CR.
* Calculated from an OV-17 tracing, programmed from 60 to 300 C at 5 C per min to yield the amount remaining after degradation.
* ND, Not determined.
* Component of model petroleum used to represent the hydrocarbon type in motor oil.
boiling aromatic compounds. Such additions would provide a "synthetic" substrate more fully comparable to the "natural" substrate. The inability to resolve pristane-n-heptadecane and phytane-n-octadecane couplets in the analytic system employed for the motor oil is unfortunate, because the data would then have permitted an evaluation of the hypothesis of Pirnik et al. (reference above) that hydrocarbons in oil may repress degradation of isoalkanes. Failure to resolve this complex made it impossible to obtain precise measurement of the degradation of these compounds. Appropriate columns and temperature programming should allow separation of these couplets and, therefore, measurement of the extent of degradation of the isoalkanes. The ratio of pristane/heptadecane or phytane/octadecane as an internal standard for quantitation was not used. Such standards in studies of microbial petroleum degradation can give false results, since it is known that pristane is susceptible to microbial degradation (7). Separation and quantitation of pristane and phytane (not shown) is possible if model petroleum is the substrate and the hydrocarbons comprising model petroleum do not repress degradation of pristane.

Analysis of the base envelope, remaining at the end of the experiments designed to follow microbial degradation of motor oil (Fig. 4) by mass spectrometry, revealed the presence of 1- to 6-ring cycloalkanes (Table 6). These compounds as well as cyclohexane, the latter a component of model petroleum, are susceptible to degradation by mixed cultures of microorgan-

### Table 7. Comparison of degradation of aromatic hydrocarbons in model petroleum and motor oil

| Hydrocarbon                        | Motor oil* | Model petroleum* |
|------------------------------------|------------|------------------|
|                                    | Control (mg) | Degraded (mg) | % remaining | Control (mg) | Degraded (mg) | % remaining |
| Monoaromatics                      |            |                 |            |            |                 |            |
| Alkylbenzenes (cumene)             | 157.2      | 11.8            | 7.50       | 62.4       | 0.43            | 0.70       |
| Naphthene benzenes                 | 112.6      | 9.3             | 8.25       | ND         | ND              |            |
| Dinaphthene benzenes               | 124.5      | 9.6             | 7.71       | ND         | ND              |            |
| Diaromatics                        |            |                 |            |            |                 |            |
| Naphthalenes (naphthalene)         | 61.2       | 3.7             | 6.04       | 10.5       | 0.05            | 0.51       |
| Acenaphthenes                      | 88.0       | 5.1             | 5.79       | ND         | ND              |            |
| Fluorenes                          | 96.9       | 5.3             | 5.46       | ND         | ND              |            |
| Triaromatics                       |            |                 |            |            |                 |            |
| Phenanthrenes (phenanthrene)       | 49.3       | 2.3             | 4.66       | 10.5       | 1.27            | 12.12      |
| Naphthene phenanthrenes            | 37.4       | 2.4             | 6.41       | ND         | ND              |            |
| Tetraaromatics                     |            |                 |            |            |                 |            |
| Pyrenes (pyrene)                   | 36.4       | 1.5             | 4.12       | 6.5        | 0.80            | 12.40      |
| Chrysenes                          | 19.6       | 0.8             | 4.08       | ND         | ND              |            |
| Pentaaromatics                     |            |                 |            |            |                 |            |
| Perylenes (perylene)               | 13.0       | 0.6             | 4.61       | 6.5        | 0.71            | 11.00      |
| Dibenzanthracenes (1,2-benzanthracene) | 3.7  | 0.4             | 10.81      | 6.5        | 0.77            | 11.86      |
| Sulfur aromatics                   |            |                 |            |            |                 |            |
| Benzothiophenes                    | 40.4       | 3.1             | 7.67       | ND         | ND              |            |
| Dibenzothiophenes                  | 47.4       | 1.4             | 2.95       | ND         | ND              |            |
| Naphthobenzothiophenes             | 25.5       | 0.9             | 3.52       | ND         | ND              |            |
| Unidentified aromatics             | 73.1       | 8.8             | 12.03      | ND         | ND              |            |
| Total                              | 986.2      | 74.2            | 7.52       | 102.9      | 4.03            | 3.91       |

* Analyzed by the method of Robinson and Cook (11).
* See footnote b in Table 3.
* See footnote d in Table 3.
* ND, Not determined.
nisms. Cycloalkanes can be, and are, added to model petroleum, with the provision that they not interfere with the separation and identification of the other hydrocarbons of the mixture.

Representative aromatic hydrocarbons of each class are present in model petroleum and serve to permit comparison with "natural" petroleum. Not represented, however, are sulfur and unidentified aromatics (Table 7). Greater degradation of the mono- and diaromatics has been observed using the model petroleum, compared with motor oil (Table 7). On the other hand, greater degradation of polyaromatics in motor oil was observed (Table 7). The basis of such selective degradation is unknown at the present time. Co-oxidation of these compounds in motor oil may occur because a greater number of oxidizable substrates are provided.

Results obtained in this and other research carried out in our laboratory show that various mixed hydrocarbon substrates or model petroleums provide suitable mixtures of hydrocarbons for the study of microbial degradation of petroleum hydrocarbons. Degradation of individual hydrocarbons can be assayed quickly using a single GLC column. Further, model petroleums are ideal substrates for studies involving questions of sequential or preferential hydrocarbon utilization. At the present time, the only other method suitable for detailed analysis of microbial degradation of several types of petroleum hydrocarbons is fractionation by column chromatography followed by computerized GLC and mass spectrometry. However, most laboratories do not have access to the expensive and sophisticated apparatus necessary for computerized low-resolution mass spectrometry. Thus, the use of model petroleums to estimate microbial potential for degrading hydrocarbons offers a reasonable and reliable alternative (14).

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LITERATURE CITED

1. Atlas, R. M., and R. Barth. 1972. Degradation and mineralization of petroleum by two bacteria isolated from coastal waters. Biotechnol. Bioeng. 14:297-308.
2. Bridie, A. L., and J. Boc. 1971. Biological degradation of mineral oil in seawater. J. Inst. Petrol. 57:270-277.
3. Gibbs, C. F. 1972. A new approach to the measurement of rate of oxidation of crude oil in seawater systems. Chemosphere 3:119-124.
4. Jewell, D. M., E. W. Albaugh, B. E. Davis, and R. G. Ruberto. 1972. Combination of techniques for the characterization of residuals. Preprints. Div. Petrol. Chem., ACS 17:F81-F89.
5. Jobson, A., F. D. Cook, and D. W. S. Westlake. 1972. Microbial utilization of crude oil. Appl. Microbiol. 23:1082-1089.
6. Kator, H., C. H. Oppenheimer, and R. J. Miget. 1971. Microbial degradation of Louisiana crude oil in closed flasks and under simulated field conditions, p. 287-296. In American Petroleum Institute/Environmental Protection Agency/U.S. Coast Guard Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.
7. McKenna, E. J., and R. E. Kallio. 1971. Microbial metabolism of the isoprenoid alkane pristane. Proc. Nat. Acad. Sci. U.S.A. 68:1552-1554.
8. Mair, B. J. 1964. Hydrocarbons isolated from petroleum. Oil Gas J. 162:130-134.
9. Mechalis, B. J., T. J. Meyers, and R. L. Kolpack. 1972. Microbial decomposition patterns using crude oil. In D. G. Ahearn and S. P. Meyers (ed.). The microbial degradation of oil pollutants. Publication No. LSU-SG-73-01, Ctr. for Wetland Resources, Louisiana State University, Baton Rouge, La.
10. Reisfeld, A., E. Rosenberg, and D. Gutnick. 1972. Microbial degradation of crude oil: factors affecting the dispersion in sea water by mixed and pure cultures. Appl. Microbiol. 24:363-368.
11. Robinson, C. J., and G. L. Cook. 1969. Low-resolution mass spectrometric determination of aromatic fractions from petroleum. Anal. Chem. 41:1548-1554.
12. Sisler, F. D., and C. E. ZoBell. 1947. Microbial utilization of carcinogenic hydrocarbons. Science 106:521-532.
13. Walker, J. D., and R. R. Colwell. 1973. Microbial ecology of petroleum utilization in Chesapeake Bay, p. 685-691. In 1973 American Petroleum Institute/Environmental Protection Agency/U.S. Coast Guard Conference on Prevention and Control of Oil Spills, American Petroleum Institute, Washington, D.C.
14. Walker, J. D., and R. R. Colwell. 1974. Microbial degradation of model petroleum at low temperatures. J. Microbial Ecol. 1:59-91.