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Evaluation of Petroleum-Degrading Potential of Bacteria from Water and Sediment

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Bacteria from water and sediment of an oil-polluted harbor were examined for ability to degrade petroleum. Water samples contained a greater variety of bacterial species capable of degrading petroleum than sediment. Cultures from both water and sediment contained *Pseudomonas* and *Acinetobacter* spp. Bacteria present in the water samples produced significantly greater degradation of 2-, 3-, 4-, and 5-ring cycloalkanes and mono-, di-, tri-, tetra-, and pentaaromatics compared with bacteria in sediment samples.

Several studies have been reported which provide an evaluation of the potential of bacteria in the marine environment for petroleum degradation. However, few studies have been done in which bacteria in the water column have been compared with sediment isolates in petroleum-degradative capability. Since oil spilled in the marine environment reaches the water column and the sediment in a shallow aquatic habitat, especially during migration of the oil (2, 5), it is important to know the comparative biodegradative potential of the water and sediment bacteria. The objective of this study was, therefore, to examine bacteria present in the water and sediment in an oil-contaminated environment for ability to degrade petroleum.

One-hundred-milliliter portions of salts solution supplemented with nitrate and phosphate (7) in 250-ml Erlenmeyer flasks were inoculated with 1 ml of Colgate Creek water or 1 ml of a 10^{-2} dilution of Colgate Creek sediment (difference in optical density <0.1 μm). The inoculated solutions were overlaid with 0.1% (vol/vol) sterile South Louisiana crude oil. Duplicate flask cultures and uninoculated controls (weathered oil samples) were incubated on a shaker (60 1.25 in strokes/min) for 28 days at 19 ± 1 C. Microbial growth, monitored by plate count, emulsification (i.e., absorbance at 600 nm), and release of acidic products (i.e., pH) were monitored at weekly intervals. Bacteria growing in flask cultures were presumptively identified to genus by Gram reaction, morphology, motility, flagella stain, glucose utilization, and catalase and oxidase tests.

Flasks were extracted using 100 ml of $CHCl_3$ at day 0, to determine amount of oil present at the start of the experiment, and at day 28, to quantify weathering and biodegradation. Extracts were dried over Na_2SO_4 and concentrated to constant weight under N_2 at 80 C. Remaining total residue was tared and fractionated on ion-exchange and alumina columns to yield saturate, aromatic, resin, and asphaltene fractions (3). The saturate fraction was analyzed using computerized gas-liquid chromatography and mass spectrometry, and the aromatic fraction was analyzed by computerized mass spectrometry, as described previously (8).

Colgate Creek in Baltimore Harbor, continuously exposed to oil from accidental spills, terrestrial runoff, etc. (6), was selected as a sampling site for comparing water and sediment bacteria and petroleum degradation capability. South Louisiana crude oil was selected for study, since studies in other laboratories have been conducted using Louisiana crude oil. Hence, comparison of data from our study with work of others may provide useful information.

Bacteria in the water and sediment samples tested were found to produce comparable growth on crude oil. Bacteria in the water samples produced greater emulsification of the oil than did bacteria in the sediment samples. However, the sediment bacteria produced a lower pH (Fig. 1).

Two *Acinetobacter* spp. and a *Pseudomonas* sp. were isolated from Colgate Creek sediment, whereas three *Acinetobacter* spp. and two *Pseudomonas* spp. were isolated from Colgate Creek water cultures.

Weathering of the total residue was found to be negligible (100.5 ± 5.9% of the control), whereas the sediment and water were found to degrade approximately 32 and 50% of the crude

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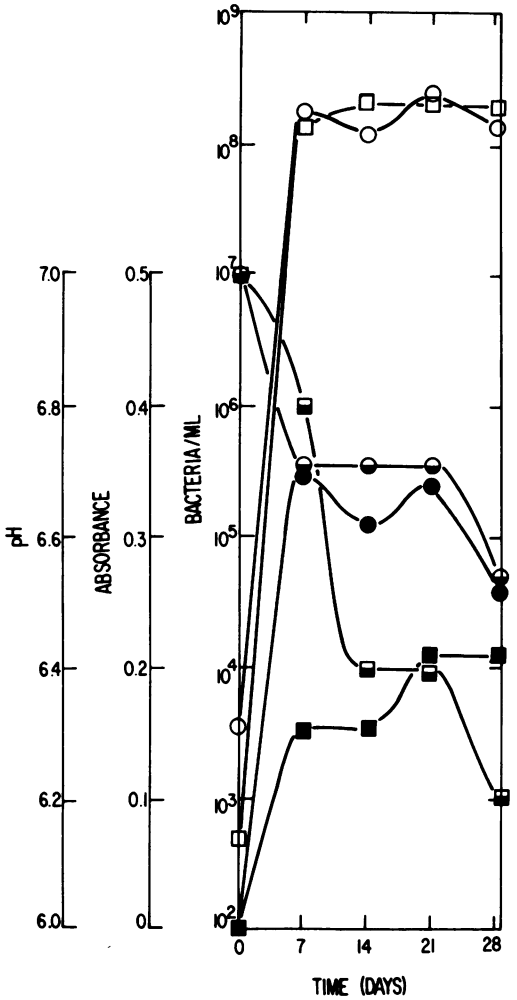

FIG. 1. *Growth of microorganisms from Colgate Creek water (circles) and sediment (squares) obtained from an enrichment culture with Louisiana crude oil added and incubated at 19 ± 1 C. Growth was measured by plate count (open figures) and absorbance (closed figures) and correlated with pH (semiclosed figures).*

oil, respectively (Table 1).

Zobell and Prokop (9) reported 67 to 78% oxidation of a Louisiana crude oil by sediment bacteria cultured in an enriched seawater medium for 30 days at 25 C. Miget et al. (4) observed that 35 to 51% of a Louisiana crude oil was oxidized by mixed bacterial cultures from polluted harbors, refinery ditches, and oil slicks after 60 h of culture in enriched seawater at 32 C. Also, Atlas and Bartha (1) reported 70% degradation of a Louisiana crude oil by mixed cultures of bacteria from seawater grown in enriched seawater for 18 days at 28 C. Unfortunately, comparison with other reports of degradation of fractions of crude oil cannot be made because fractionation of the oil was not reported in those studies with Louisiana crude oil.

In this study, mixed cultures of sediment and water microorganisms did not produce as great a degradation of Louisiana crude oil as reported by Zobell and Prokop (9), Miget et al. (4), or Atlas and Bartha (1), which can be explained as follows. The type of organisms in the inocula, incubation conditions, type of Louisiana crude oil, etc., were different. However, the results reported here clearly suggest that bacteria in the water column may possess greater capability of degrading crude oil than sediment bacteria. Degradation of saturates and aromatics, compared to weathering of saturates (22.5%) and aromatics (0%) noted here, also suggests this. Resins and asphaltenes (pyridines, quinolenes, carbozoles, sulfoxides, amides, phenolic acids, carboxylic acids, ketones, esters, and porphyrins) were found to increase to a greater extent as a result of degradation by bacteria in the water sample than the sediment bacteria, a reflection of differences in the two populations. Both fractions increased during weathering, namely, resins 123% and asphaltenes 200%.

A comparison of the degradative capability of water and sediment bacteria is shown in Fig. 2.

TABLE 1. *Average (± standard deviation) weight and percentage of fractions of South Louisiana crude oil remaining after biodegradation*

Fraction	Wt and % of fraction remaining after degradation by bacteria from			
	Sediment		Water	
	mg	%	mg	%
Saturates	13.0 ± 2.6	45.0 ± 4.5	3.5 ± 0.3	8.6 ± 0.8
Aromatics	14.1 ± 1.4	96.9 ± 2.4	8.9 ± 1.7	61.4 ± 2.7
Resins	6.0 ± 0.6	94.5 ± 8.8	11.3 ± 4.9	179.3 ± 6.2
Asphaltenes	0.9 ± 0.1	300.0 ± 33.3	1.6 ± 1.1	533.3 ± 36.7
Total residue	33.9 ± 2.1	67.8 ± 4.2	25.3 ± 4.2	50.6 ± 4.1

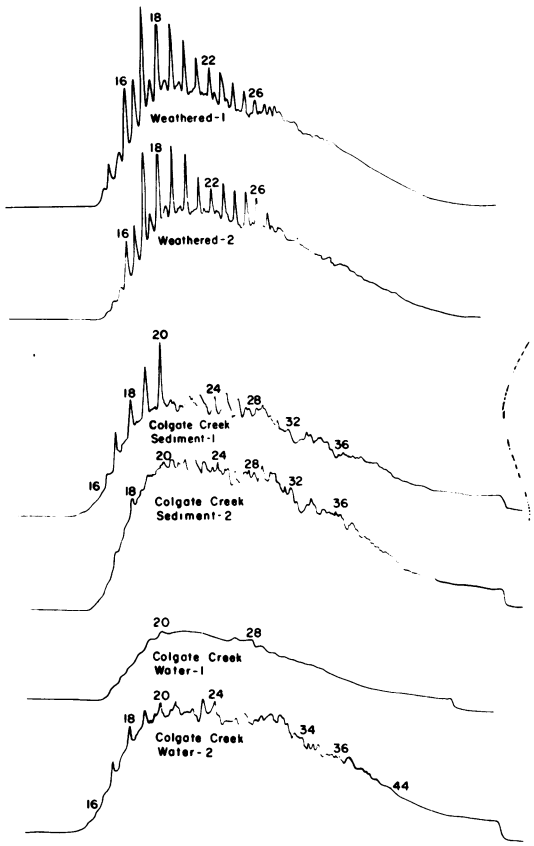

FIG. 2. *Gas-liquid chromatographic tracing of hydrocarbons comprising the saturate fraction of South Louisiana crude oil after weathering (top); degradation by bacteria from Colgate Creek sediment (middle) and water (bottom). Numbers for these samples correspond to replicates. Separation was accomplished using a stainless-steel column (2 mm by 1.5 m) of 3% SE-30 with 80/100 Chromosorb W. Attenuation was 256 × 10¹, and sample volumes were 1-μl injections. All gas-liquid chromatographic tracings were recorded by using the same method.*

Alkanes (C_{15}-C_{28}) were found to persist after weathering, with the differences between replicates noted not significant. Differences were observed among replicates, as seen in the gas-liquid chromatographic tracings of extracts of cultures inoculated with sediment, i.e., branched alkanes of C_{17}-C_{20} predominating in one of the replicates. Degradation of alkanes, as seen in the gas-liquid chromatographic tracings, was greatest in the case of the cultures which had received an inoculum from a water sample.

Quantitative analysis of hydrocarbons degraded by water and sediment bacteria revealed that the water-column bacteria were most effective in degrading South Louisiana crude oil (Table 2). Weathering removed 20, 17, 19, 15, 11, 25, and 60% of the alkanes and 1-, 2-, 3-, 4-, 5-, and 6-ring cycloalkanes, respectively, in the South Louisiana crude oil. Sediment bacteria removed 75, 70, 40, 37, 24, 48, and 62% of alkanes and 1-, 2-, 3-, 4-, 5-, and 6-ring cycloalkanes, respectively, compared with 82, 75, 65, 62, 52, 65, and 72% removal of these hydrocarbons by water bacteria. Although sediment bacteria removed greater quantities of saturated hydrocarbons than occurred via weathering, water bacteria removed significantly more 2-, 3-, 4-, 5-, and 6-ring cycloalkanes than the sediment bacteria. Similarly, cycloalkanes were found to degrade to a lesser extent as the ring number increased from 1 to 4. However, as the ring number increased from 4 to 6, the cycloalkanes were observed to be more susceptible to degradation. The reason for this observation is not known at the present time.

Weathering was not found to result in removal of any of the aromatic hydrocarbons. Again, the water bacteria were found to be more effective than sediment bacteria in degrading the aromatic hydrocarbons of South Louisiana crude oil. The sediment bacteria degraded monoaromatics (11%), pentaaromatics (25%), and sulfur aromatics (9%), whereas the water-column bacteria were found to degrade 38, 52, 27, 17, and 50% of the mono-, di-, tri-, tetra-, and pentaaromatics, respectively, but not the sulfur aromatics. Thus, addition of sulfur to an aromatic hydrocarbon rendered it less susceptible to degradation. Within the classes of aromatic hydrocarbons, certain trends were observed with respect to degradation. Addition of an alicyclic ring(s) to an aromatic ring (alkylbenzenes, naphthenebenzenes, and dinaphthenebenzenes) and the position of an alicyclic ring between two aromatic rings (fluorenes) altered biodegradability of the component.

In addition, less variability among replicates was noted for samples inoculated with water compared with the sediment samples.

Several possible explanations may be offered as to why sediment bacteria are less effective in degrading the hydrocarbon components of South Louisiana crude oil than water bacteria: (i) fewer kinds of bacteria, i.e., a smaller number of species, present in the mixed culture; (ii) anaerobiosis of the sediment; (iii) utilization of nutrients in sediments by the inoculum in preference to utilization of the oil; and (iv) differences in activity of the two populations towards petroleum hydrocarbons.

TABLE 2. *Average (± standard deviation) weight and percentage of hydrocarbons of South Louisiana crude oil remaining after biodegradation*

Hydrocarbon	Wt and % of hydrocarbon remaining after degradation by bacteria from			
	Sediment		Water	
	mg	%	mg	%
Alkanes	1.8 ± 0.1	25.7 ± 1.4	1.3 ± 0.6	17.9 ± 1.9
1-Ring cycloalkanes	1.9 ± 0.4	31.4 ± 6.0	1.5 ± 0.6	24.6 ± 4.3
2-Ring cycloalkanes	2.6 ± 0.3	60.5 ± 7.0	1.5 ± 0.4	34.9 ± 5.3
3-Ring cycloalkanes	1.9 ± 0.2	63.4 ± 6.6	1.2 ± 0.3	38.4 ± 4.4
4-Ring cycloalkanes	2.2 ± 0.2	75.9 ± 6.9	1.4 ± 0.4	48.3 ± 1.8
5-Ring cycloalkanes	1.5 ± 0.2	51.7 ± 6.9	1.0 ± 0.3	34.5 ± 1.4
6-Ring cycloalkanes	1.1 ± 0.2	37.9 ± 6.9	0.8 ± 0.1	27.6 ± 3.5
Monoaromatics	5.6 ± 0.4	88.9 ± 1.7	3.9 ± 0.6	61.9 ± 1.8
Alkylbenzenes	1.5 ± 0.1	75.0 ± 1.2	1.0 ± 0.2	50.0 ± 0.7
Naphthenebenzenes	1.7 ± 0.2	89.5 ± 2.7	1.3 ± 0.2	68.4 ± 1.0
Dinaphthenebenzenes	2.4 ± 0.3	104.3 ± 3.6	1.6 ± 0.2	69.6 ± 1.4
Diaromatics	4.5 ± 0.1	93.7 ± 2.1	2.3 ± 0.6	47.9 ± 1.5
Naphthalenes	1.3 ± 0	68.4 ± 6.2	0.6 ± 0.1	31.6 ± 1.0
Acenaphthenes-dibenzofurans	1.6 ± 0.1	114.3 ± 7.1	0.7 ± 0.1	50.0 ± 0.9
Fluorenes	1.6 ± 0.1	114.3 ± 7.7	1.0 ± 0.1	71.4 ± 1.3
Triaromatics	1.6 ± 0.1	116.7 ± 16.7	1.1 ± 0.4	73.3 ± 6.7
Phenanthrenes	1.1 ± 0.2	100.0 ± 7.1	0.8 ± 0.2	72.7 ± 5.8
Naphthenephenanthrenes	0.5 ± 0.1	125.0 ± 8.7	0.3 ± 0.1	75.0 ± 5.0
Tetraaromatics	0.8 ± 0.1	125.0 ± 8.3	0.5 ± 0.3	83.3 ± 4.7
Pyrenes	0.5 ± 0.1	125.0 ± 6.7	0.3 ± 0.1	75.0 ± 3.2
Chrysenes	0.3 ± 0.1	150.0 ± 7.8	0.1 ± 0	50.0 ± 1.8
Pentaaromatics	0.2 ± 0.1	75.0 ± 25.0	0.1 ± 0	50.0 ± 0
Perylenes	0.1 ± 0	100.0 ± 0	0.1 ± 0	100.0 ± 0
Dibenzanthracenes	0.1 ± 0	100.0 ± 0	0 ± 0	0 ± 0
Sulfur aromatics	0.6 ± 0.1	91.7 ± 8.4	1.0 ± 0.3	166.7 ± 9.3
Benzothiophenes	0.2 ± 0.1	66.7 ± 7.8	0.2 ± 0.1	66.7 ± 7.7
Dibenzothiophenes	0.3 ± 0.1	100.0 ± 1.4	0.3 ± 1.0	100.0 ± 1.4
Naphthobenzothiophenes	0.1 ± 0	100.0 ± 0	0.5 ± 0	500.0 ± 4.4

In conclusion, it appears that bacteria in the water column of an oil-contaminated environment can be more effective in degrading hydrocarbons of crude oil than sediment bacteria.

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LITERATURE CITED

1. Atlas, R. M., and R. Bartha. 1973. Abundance, distribution and oil biodegradation potential of microorganisms in Raritan Bay. Environ. Pollut. 4:291-300.
2. Conomos, T. J. 1975. Movement of spilled oil as predicted by estuarine nontidal drift. Limnol. Oceanogr. 20: 159-173.
3. Jewell, D. M., E. W. Albaugh, B. E. Davis, and R. G. Ruberto. 1972. Combination of techniques for characterization of residuals. Am. Chem. Soc. 17:F81-F91.
4. Miget, R. J., C. H. Oppenheimer, C. H. Kator, and P. A. LaRock. 1969. Microbial degradation of normal paraffin hydrocarbons in crude oil, p. 327-331. *In* API/FWPCA Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.
5. Voroshilova, A. A., and E. V. Dianova. 1950. Bacterial oxidation of oil and its migration in natural waters. Mikrobiologiya 19:203-210.
6. Walker, J. D., and R. R. Colwell. 1973. Microbial ecology of petroleum utilization in Chesapeake Bay, p. 685-690. *In* API/EPA/USCG Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.
7. Walker, J. D., and R. R. Colwell. 1974. Microbial degradation of model petroleum at low temperatures. Microb. Ecol. 1:63-95.
8. Walker, J. D., R. R. Colwell, and L. Petrakis. 1975. Microbial petroleum degradation: use of computerized mass spectrometry. Can. J. Microbiol. In press.
9. Zobell, C. E., and J. F. Prokop. 1966. Microbial oxidation of mineral oils in Barataria Bay bottom deposits. Z. Allg. Mikrobiol. 6:143-162.