A STUDY OF THE BIODEGRADATION OF A SOUTH LOUISIANA CRUDE OIL EMPLOYING COMPUTERIZED MASS SPECTROMETRY

J.D. Walker and R.R. Colwell
Department of Microbiology
University of Maryland
College Park, Maryland
and
L. Petrakis
Division of Physical Sciences
Gulf Research and Development Company
Pittsburgh, Pennsylvania

ABSTRACT

Inocula from oil-contaminated Colgate Creek and oil-free Eastern Bay sediment produced similar growth on a South Louisiana crude oil. The Colgate Creek inoculum was found to contain a wider variety of bacterial genera and to produce greater degradation of South Louisiana crude oil than the Eastern Bay sediment inocula. The resin fraction of the crude oil increased during growth and weathering. All of the hydrocarbon classes of the crude oil were susceptible to degradation by microorganisms present in the Colgate Creek inoculum, but not by those in the Eastern Bay samples.

INTRODUCTION

An important objective of the research underway in our laboratory is to gain an understanding of microbial degradation of petroleum, particularly as it occurs in situ. Low-resolution computerized mass spectrometry has been found to be an adequate method for quantitating biodegradation [5]. This technique has been successfully employed in comparing biodegradation of petroleum by microorganisms from oil-free and oil-contaminated environments [6,7,8,9]. Gas-liquid chromatography (GLC) also has proved useful, particularly in the assessment of the fate of n-alkanes during microbial degradation of petroleum [1,2]. Thus, the analytical methods employed in our laboratory have provided us with information concerning the fate of n-alkanes, as well as of other major groups of hydrocarbons, in petroleum undergoing microbial biodegradation.

Materials and methods

Methods of sampling, microbiological procedures, chemical analysis and description of the media and culture systems employed have been published in detail elsewhere [3,5].

Results and discussion

Samples were collected from Eastern Bay and Colgate Creek in Baltimore Harbor of Chesapeake Bay during November, 1973 (figure 1). Measurements taken at sampling stations in Eastern Bay and Colgate Creek were similar for chemical, physical, and microbiological parameters, except that a larger quantity of benzene-extractable material was observed to be present in Colgate Creek (table 1). Results of computerized low-resolution mass spectrometry revealed that the benzene-extractable material in Colgate Creek sediment was oil and that Eastern Bay sediment did not contain detectable levels of oil.

Results of growth studies utilizing Eastern Bay and Colgate Creek sediment inocula in flasks held at 15°C in shaken culture with South Louisiana crude oil as substrate are shown in figure 2. Growth curves obtained for the inocula were similar. A number of bacterial genera were present during growth of the cultures and these were detected by the plate count procedure employed (table 2). None of the bacterial species was observed to be present throughout any one of the growth cycles monitored over a 49-day period. Eastern Bay sediment inocula yielded three *Pseudomonas* spp., whereas the Colgate Creek sediment inocula yielded two *Pseudomonas* spp., a *Vibrio* sp., and two coryneforms. Thus, despite similar growth curves, the Eastern Bay and Colgate Creek sediment bacteria showed significant differences in number and type of bacterial isolates recovered from each environment. These differences were observed to be significant in terms of type and amount of petroleum hydrocarbon degradation for which the bacteria were responsible.

During the seven week incubation period of the experiments undertaken in this study, the total petroleum residue recovered from the weathered samples oscillated above and below 50 mg, i.e., the level observed in the control (figure 3). Eastern Bay sediment bacteria produced a decrease of 28% in the total residue at three weeks, but the concentration of residue increased to the original level at seven weeks. Colgate Creek sediment bacteria produced a 40% decrease in residue at two weeks but the amount of residue in the Colgate Creek culture also increased to 24% of the initial concentration at seven weeks. It was clear from the results obtained in this study that the total amount of residue removed via microbial degradation was greater for Colgate Creek than for Eastern Bay sediment cultures.

Of the several fractions examined, the asphaltenes showed a significant increase during the seven week incubation period (figure 4). The asphaltenes increased 271% after weathering for one week, then decreased to a level approximating the original amount of asphaltenes present in the control at seven weeks. Asphaltenes in the Eastern Bay cultures increased to 328% of the control at three weeks, then decreased to a value ca. 14% greater than the control at seven weeks. Asphaltenes in the Colgate Creek culture increased 100% at one and five weeks, but the data indicates ca. 70% degradation of the asphaltenes occurred at three weeks. At seven weeks, approximately 85% of the asphaltenes remained in the Colgate Creek culture. Thus, during weathering and biodegradation, the asphaltene fraction, comprising phenolic acids, carboxylic acids, ketones, esters, and porphyrins, can increase at various stages of

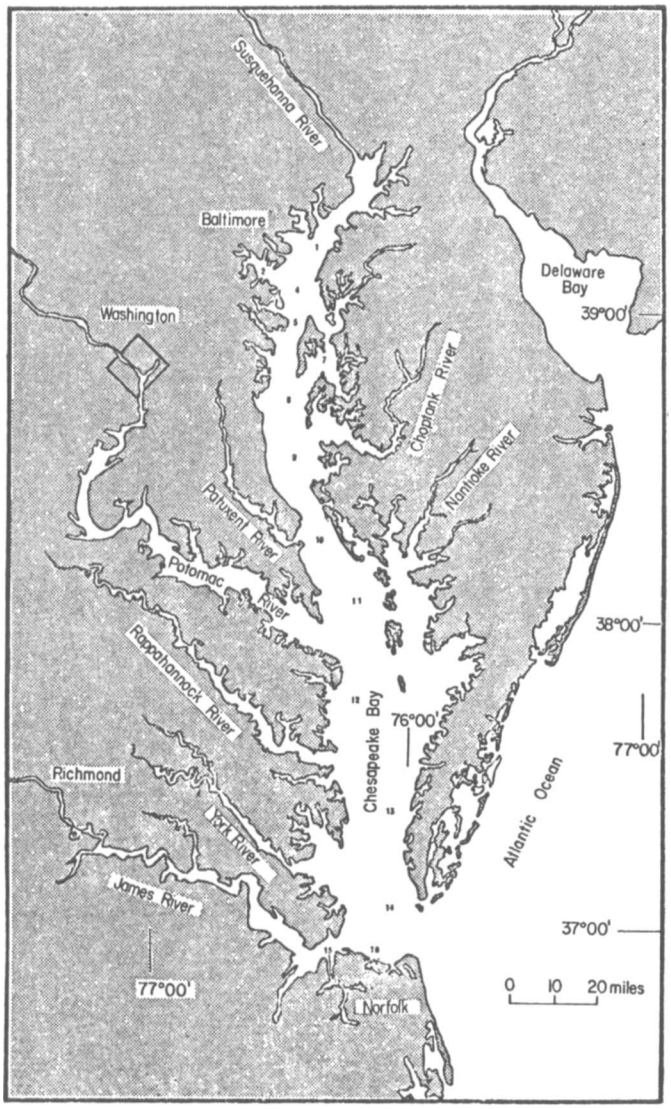

Figure 1. Map of Chesapeake Bay showing locations of the stations in Colgate Creek (2) and Eastern Bay (7) from which samples were collected.

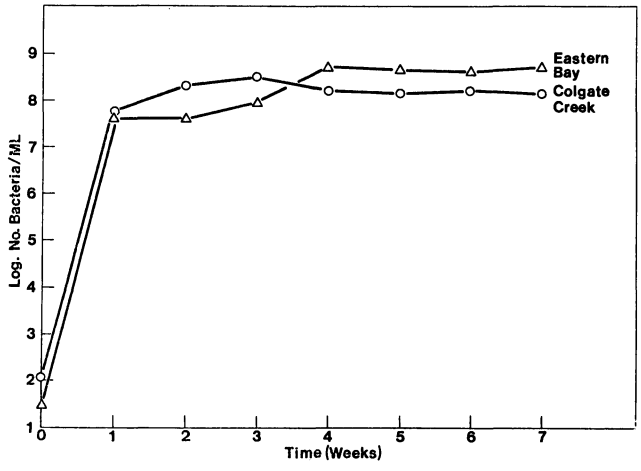

Figure 2. Growth of sediment bacteria from Eastern Bay (△) and Colgate Creek (○) on a South Louisiana crude oil, measured by plate counts (Walker and Colwell, 1973).

Table 1. Physical, chemical and microbiological parameters of Colgate Creek and Eastern Bay in Chesapeake Bay at the time of sample collection, November, 1973

Parameter	Colgate Creek	Eastern Bay
Water Temperature (°C)	10.6	8.8
Dissolved Oxygen (ppm)	12.4	11.4
Salinity (ppt)	10.0	13.4
pH	6.9	7.9
Air Temperature (°C)	10.0	12.0
Depth (m)	9.8	8.3
Transparency (m)	1.0	3.0
Benzene-extractables sediment (%,w/w)	0.05	0.006
Benzene-extractable: water (%, w/v)	0.00005	0.00001
Bacteria/ ml water	6.5×10^4	1.6×10^2
Bacteria/ g sediment	6.4×10^5	9.0×10^5
Fungi/ ml water	0.06	0.09
Fungi/ g sediment	1.0×10^2	1.5×10^1
Yeast/ ml water	1	0
Yeast/ g sediment	0	0

Table 2. Selected characteristics of bacteria isolated from Eastern Bay and Colgate Creek sediment samples inoculated into a South Louisiana crude oil medium

Tentative Genus	Isolate	Source of Inoculum			Characteristics												
		Eastern Bay	Colgate Creek	Days Observed	Gram reaction	Rod	Motile	Polar Flagella	Glucose acid	Glucose alkaline	Glucose fermented	Glucose gas	Catalase	Oxidase	Fluorescent pigment	Insoluble pigment	Soluble pigment
Pseudomonas	626	+	-	0-42	-	+	++	-	+	-	-	-	+	+	-	-	-
Pseudomonas	627	+	-	35-49	-	+	++	+	-	-	-	-	+	+	-	-	-
Pseudomonas	628	+	-	42-49	-	+	++	+	-	-	-	-	+	-	-	-	-
Coryneform	634	-	+	0-7	+	+	--	-	+	-	-	-	+	+	-	-	-
Coryneform	635	-	+	0-7	+	+	--	-	+	-	-	-	+	+	+	-	-
Pseudomonas	636	-	+	7-42	-	+	++	+	-	-	-	-	+	+	-	-	-
Vibrio	637	-	+	14-42	-	+	++	+	-	+	-	-	+	-	-	-	-
Pseudomonas	638	-	+	14-49	-	+	++	-	-	-	-	-	+	+	-	-	-

microbial biodegradation of South Louisiana crude oil. Nevertheless, Colgate Creek sediment bacteria are capable of degrading asphaltenes present in South Louisiana crude oil.

Resins increased in concentration during weathering by about 100% at three weeks, decreased to the initial concentration at six weeks, and increased to 61% above the control at seven weeks (figure 5). Eastern Bay sediment bacteria removed 61% of the resins at three weeks, whereas Colgate Creek bacteria removed 61% at two weeks. The Colgate Creek culture revealed an increase of 61% in the resins at six weeks, then a decrease to 16% above the control at seven weeks. Eastern Bay bacteria produced a 100% increase in the resin concentration at seven weeks. Resins were degraded by Eastern

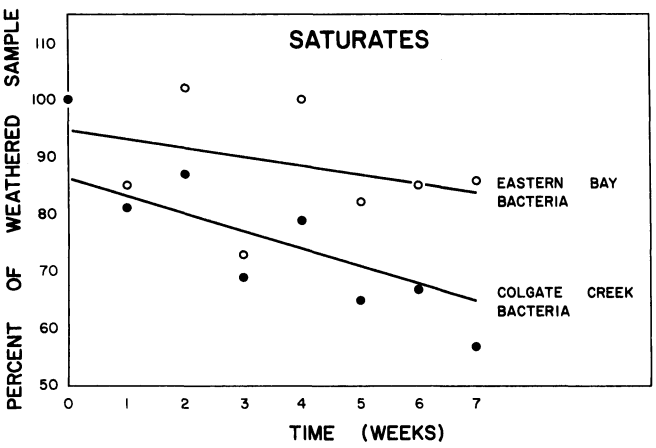

Figure 3. Saturate degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

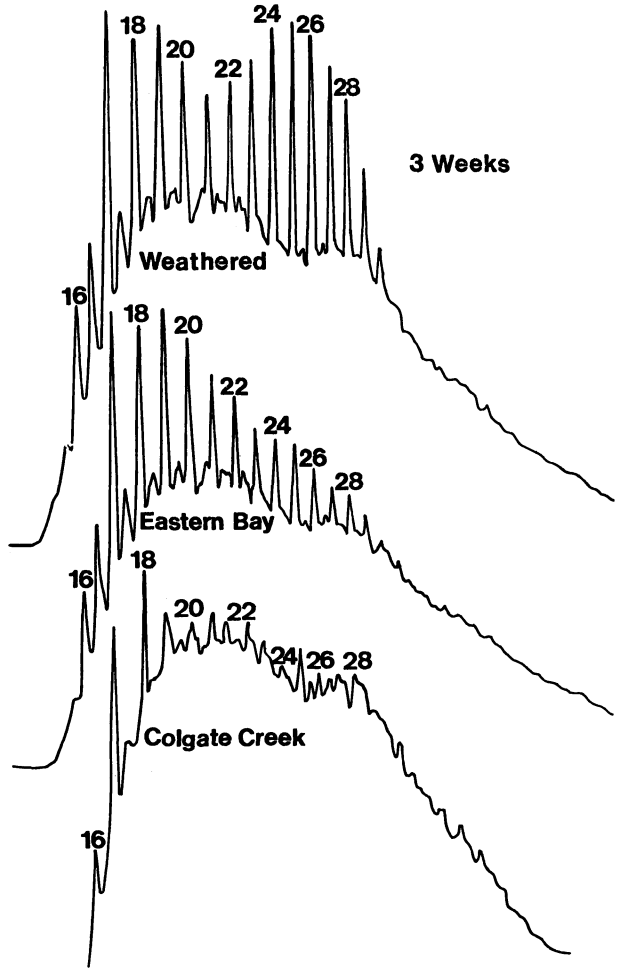

Figure 4. GLC tracings of saturated hydrocarbons of South Louisiana crude oil subjected to weathering and degradation by Eastern Bay and Colgate Creek sediment bacteria (at 3 weeks).

Bay and Colgate Creek microorganisms, but the amount of degradation was significantly greater for the Colgate Creek sediment samples.

As was anticipated, the saturate fraction of the oil was found to be most susceptible to weathering and degradation (figure 6). After three weeks, ca. 40% of the saturate fraction was lost via degradation, compared with 25% lost by weathering. Gas-liquid chromatography (GLC) was used to examine this fraction and the GLC results revealed qualitative changes occurred in the alkanes of the saturate fraction (figure 7). Compared with the weathered sample, which resembled the control, the Eastern Bay sample showed that degradation of the *n*-alkanes occurred, especially the longer chain-length *n*-alkanes. Essentially all of the alkanes, except C_{17} and C_{18} (pristane and phytane), were degraded by Colgate Creek sediment bacteria. At six weeks, there were considerable differences noted, since weathering of the saturate fraction resulted in 40% degradation. Degradation by sediment bacteria from Eastern Bay was 45% and by Colgate Creek bacteria, 60% of the control analysis of the saturate fraction of the South Louisiana crude oil at seven weeks revealed further alteration of the GLC patterns for the samples (figure 8). Long-chain alkanes were no longer clearly detectable in weathered samples, whereas little alteration was noted in the Eastern Bay sample and complete removal of the alkanes, as well as significant removal of material under the envelope, was noted for the Colgate Creek sample (figure 8).

Aromatics in the weathered sample oscillated between 65% and 6% above and below the control with a steady increase observed at four to seven weeks to 34% above the control (figure 9). During the first two weeks of growth, aromatics exposed to biodegradation by Eastern Bay microorganisms remained at the initial concentration, whereas aromatics originally present in the South Louisiana crude oil subjected to biodegradation by Colgate Creek microorganisms decreased 20%. At seven weeks, the concentration of aromatics in

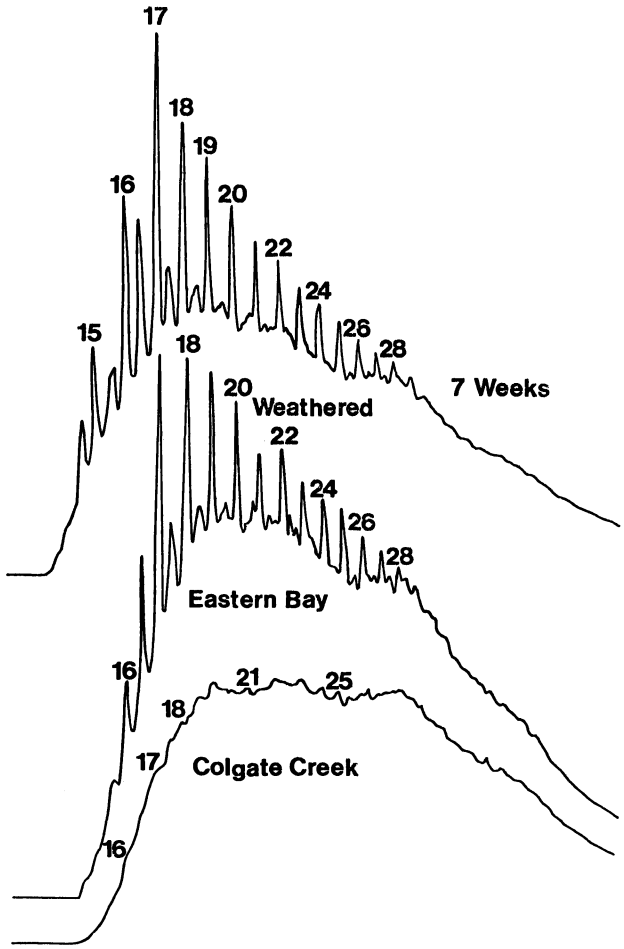

Figure 5. GLC tracings of saturated hydrocarbons of South Louisiana crude oil subjected to weathering and degradation by Eastern Bay and Colgate Creek sediment bacteria (at 7 weeks).

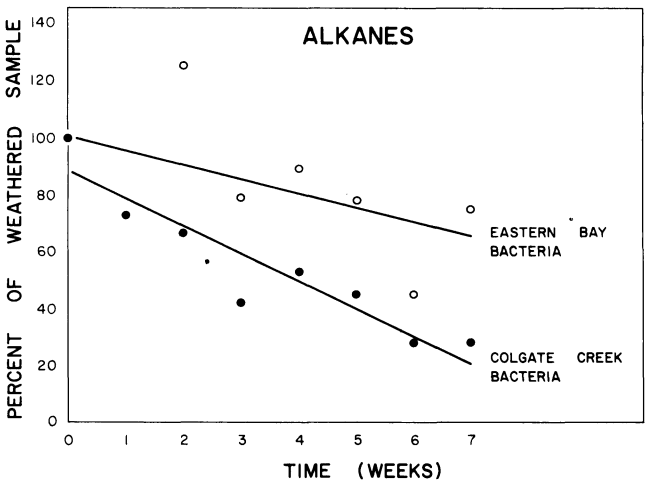

Figure 6. Alkane degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

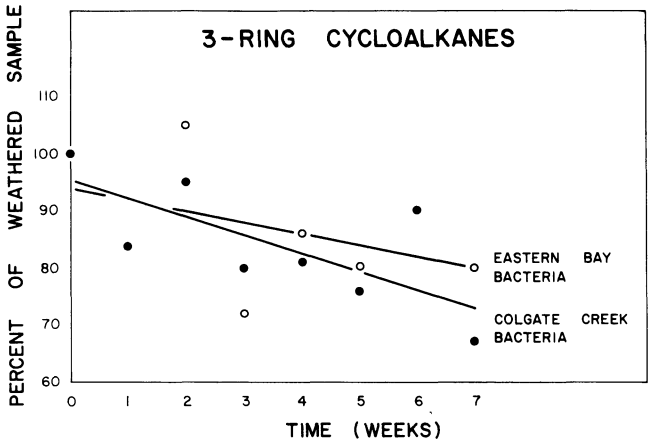

Figure 7. 3-Ring cycloalkane degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

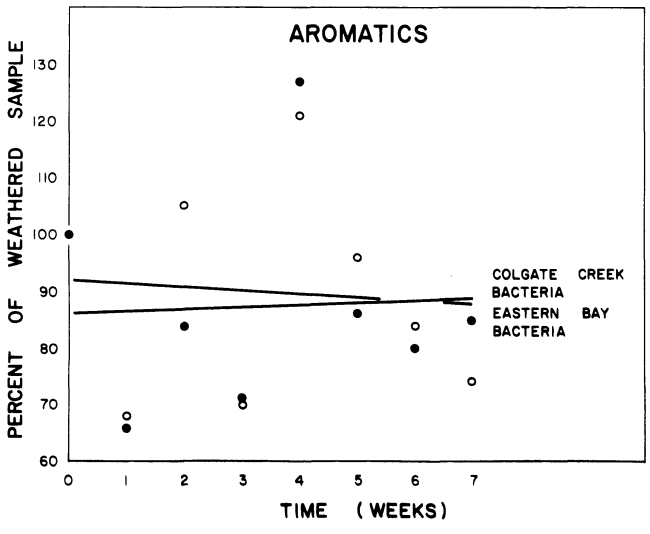

Figure 8. Aromatic degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

Eastern Bay and Colgate Creek cultures was approximately the same.

The alkanes were the most susceptible of the hydrocarbons in the saturate fraction to microbial degradation (figures 10 and 11). However, 1- to 6-ring cycloalkanes were degraded, for example the three-ring cycloalkanes in figure 11. Eastern Bay sediment bacteria were less capable of degrading components of the saturate fraction than were the Colgate Creek sediment bacteria. Weathering had the least effect on the saturates.

The hydrocarbons comprising the aromatic fraction, exemplified by the tetraaromatics (figure 12), were degraded only very slightly with the monoaromatics most susceptible to degradation. An increase in the aromatic nucleus was associated with decreased susceptibility to degradation, as was the presence of sulfur in the aromatic nucleus.

In conclusion, degradation of the total crude oil fractions and hydrocarbons was found to be significantly greater for Colgate Creek sediment microorganisms than for the Eastern Bay sediment microorganisms. Clearly, degradation accomplished by the microorganisms introduced with the inoculum was greater than from weathering. Degradation of the major hydrocarbon components of South Louisiana crude oil did not show a continuous increase with time except in the case of total saturates and alkanes degraded by

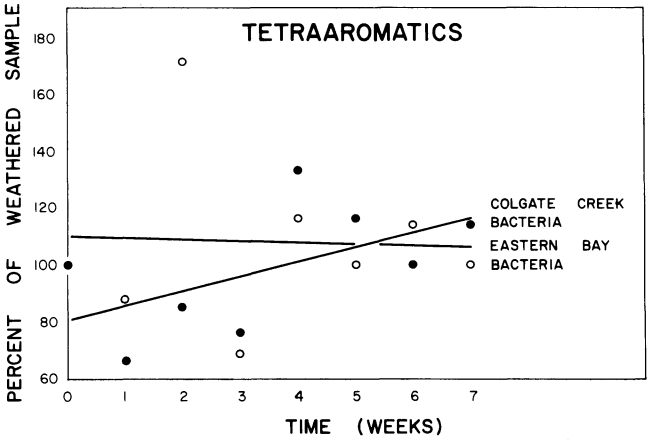

Figure 9. Tetraaromatic degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

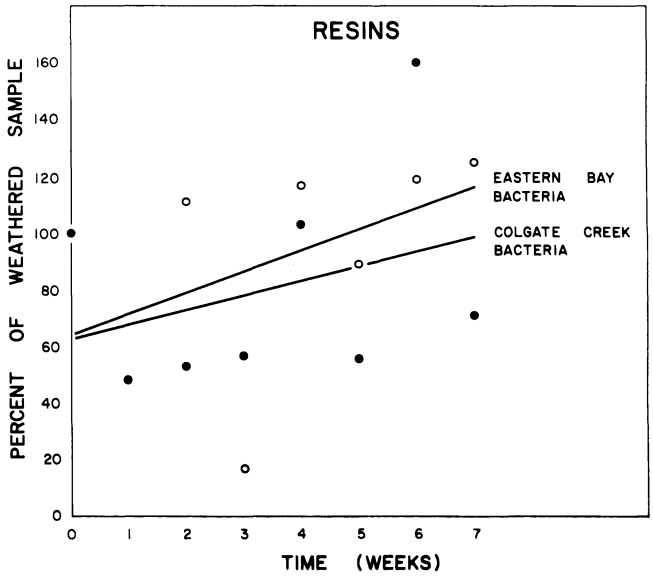

Figure 10. Resin degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

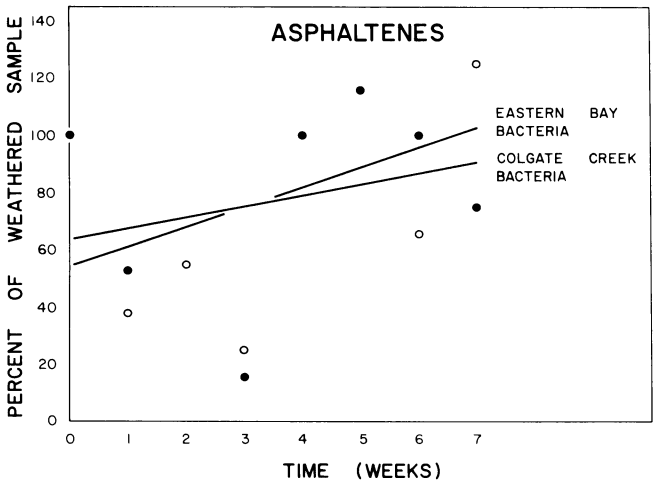

Figure 11. Asphaltene degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

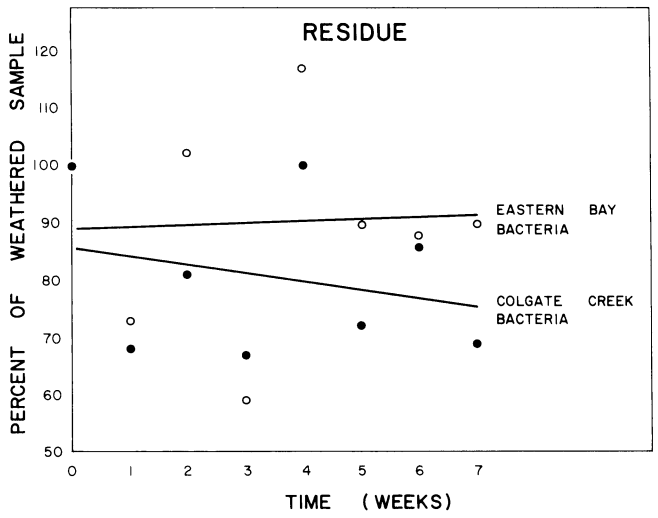

Figure 12. Residue degradation by sediment bacteria from Eastern Bay (○) and Colgate Creek (●).

Colgate Creek sediment bacteria. Biodegradation carried out in situ, thus, is affected markedly by such factors as prior exposure to oil and the nature of the autochthonous flora. Enrichment for petroleum degrading microorganisms does occur in nature but, even under optimum conditions, total and complete biodegradation does not occur except, if at all, over an extremely long time period.

REFERENCES

1. Jobson, A.; Cook, F.D.; and Westlake, D.W.S. 1972. Microbial utilization of crude oil. *Appl. Microbiol.* 23: 1082-89.
2. Miget, R.J.; Oppenheimer, C.H.; Kator, H.I.; and LaRock, P.A. 1969. Microbial degradation of normal paraffin hydrocarbons in crude oil. In API/FWPCA *Conference on Prevention and Control of Oil Spills,* pp. 327-31. Washington, D.C.: American Petroleum Institute.
3. Walker, J.D., and Colwell, R.R. 1973. Microbial ecology of petroleum utilization in Chesapeake Bay. In API/EPA/USCG *Conference on Prevention and Control of Oil Spills*, pp. 685-90. Washington, D.C.: American Petroleum Institute.
4. Walker, J.D.; Colwell, R.R.; Hamming, M.C.; and Ford, H.T. 1974. Extraction of petroleum hydrocarbons from oil-contaminated sediments. *Bull. Environ. Contam. Toxicol.* 13 (in press).
5. Walker, J.D.; Colwell, R.R.; and Petrakis, L. 1975. Use of computerized low-resolution mass spectrometry to analyze biodegradation of petroleum. *Can. J. Microbiol.* Submitted.
6. Walker, J.D.; Colwell, R.R.; and Petrakis, L. 1975. Chesapeake Bay sediment bacteria and the biodegradation of petroleum. *Nature*. To be submitted.
7. Walker, J.D.; Colwell, R.R.; and Petrakis, L. 1975. Petroleum degradation by bacteria from Chesapeake Bay sediment: Fate of fractions of petroleum subject to degradation by fresh, frozen and enriched cultures. *J. Gen. Microbiol.* To be submitted.
8. Walker, J.D.; Colwell, R.R.; and Petrakis, L. 1975. Petroleum degradation by bacteria from Chesapeake Bay sediment: Fate of saturated hydrocarbons of petroleum subject to degradation by fresh, frozen, and enriched cultures. *J. Gen. Microbiol.* To be submitted.
9. Walker, J.D.; Colwell, R.R.; and Petrakis, L. 1975. Petroleum degradation by bacteria from Chesapeake Bay sediment: Fate of aromatic hydrocarbons of petroleum subject to degradation by fresh, frozen, and enriched cultures. *J. Gen. Microbiol.* To be submitted.