APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1989, p. 2832–2836
0099-2240/89/112832-05$02.00/0

Vol. 55, No. 11

Microbial Utilization of Naturally Occurring Hydrocarbons at the Guaymas Basin Hydrothermal Vent Site†

DENNIS A. BAZYLINSKI,* CARL O. WIRSEN, AND HOLGER W. JANNASCH
Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Received 3 July 1989/Accepted 21 August 1989

The Guaymas Basin (Gulf of California; depth, 2,000 m) is a site of hydrothermal activity in which petroliferous material is formed by thermal alteration of deposited planktonic and terrestrial organic matter. We investigated certain components of these naturally occurring hydrocarbons as potential carbon sources for a specific microflora at these deep-sea vent sites. Respiratory conversion of [1-^{14}C]hexadecane and [1(4,5,8)-^{14}C]naphthalene to $^{14}CO_2$ was observed at 4°C and 25°C, and some was observed at 55°C, but none was observed at 80°C. Bacterial isolates were capable of growing on both substrates as the sole carbon source. All isolates were aerobic and mesophilic with respect to growth on hydrocarbons but also grew at low temperatures (4 to 5°C). These results correlate well with previous geochemical analyses, indicating microbial hydrocarbon degradation, and show that at least some of the thermally produced hydrocarbons at Guaymas Basin are significant carbon sources to vent microbiota.

The Guaymas Basin is one of a series of deep semi-closed basins in the Gulf of California (2). It consists of two rift valleys, the Northern and Southern Troughs, which are separated by a 20-km transform fault area (21). The site is tectonically active, which results in high heat flow locally exceeding 1.2 W m^{-2} (4, 8). The surface waters are very productive (2) and are responsible for a rapid sediment accumulation rate of >1 m per 1,000 years (3). The average thickness of the sediment is about 100 m (22) but is up to 400 m (20). Hydrothermal fluids discharge through both chimneys at 270 to 325°C and through porous sediments at about 50°C (1, 9, 10). The flow is impeded not only by the sediment but also by subsurface dikes and sills which interrupt the hydrothermal circulation (5).

Compared with other known hydrothermal vent sites, the Guaymas Basin is unique in that the organic matter in the accumulating hemipelagic sediment is pyrolyzed under high temperature conditions to petroleumlike products (18–21). These products consist of gasoline-range aliphatic and aromatic hydrocarbons and predominantly residual polar asphaltic material (21). The composition of this material includes polynuclear aromatic hydrocarbons and small amounts of olefins and indicates a thermal origin and rapid quenching by hydrothermal removal prior to condensation at the seabed (20, 21). Petroleums are thought to undergo microbial degradation at precipitated mineral mound surfaces or unconsolidated sediments (1, 20) as well as at the sediment water interface (1). Geochemical analysis of extracted petroleums suggest this to be the case (1, 20). Hydrocarbon constituents derived primarily from photosynthate are suggested to be equal to or quantitatively more important to vent microbiota than chemosynthetically derived organic carbon is (20).

As part of our overall effort to study the microbial ecology of hydrothermal vent areas, we examined the potential for microbial hydrocarbon utilization at Guaymas Basin. In addition, this area afforded us the chance to investigate microbial processes at a natural source of oil in the deep sea.

MATERIALS AND METHODS

Sample collection. Surface tube cores (30 to 40 cm) were taken with the use of DSV *ALVIN* during August 1985 (dives 1611 and 1615) and February 1988 (dives 1966 to 1968). The area sampled was in the Southern Trough in the vicinity of hydrothermal patches (Fig. 1). Water depth at the site was approximately 2,020 m. Cores from the above dives were quickly sectioned on board and stored at 3 to 4°C. Temperature profiles in sediments were measured in situ with a 1-m-long thermocouple probe. Core or samples numbers are identical with ALVIN dive numbers, with a and b added if more than one core per dive was used.

Potential for microbial utilization of hydrocarbons. Potential for hydrocarbon utilization by microorganisms in sediment was determined by conversion of ^{14}C-radiolabeled compounds to $^{14}CO_2$. Used as model aliphatic and aromatic substrates, respectively, were [1-^{14}C]hexadecane, with a specific activity of 45 mCi $mmol^{-1}$ (ICN Radiochemicals, Irvine, Calif.), and [1(4,5,8)-^{14}C]naphthalene, with a specific activity of 4.5 mCi $mmol^{-1}$ (Amersham Corp., Arlington Heights, Ill.). The naphthalene was dissolved in hexane prior to use. One microcurie of substrate in hexane (~10 μl) was injected onto a small piece of sterile Whatman filter paper in a sterile 10-ml funnel-top ampoule (Wheaton Scientific, Millville, N.J.). A stream of nitrogen gas was passed through the vial for several seconds in order to evaporate and drive off the hexane. This procedure did not result in significant losses of substrate due to volatilization. Then, 5 ml of a 1:1 sediment:artificial seawater (ASW) (11) slurry was injected into the ampoule, which was then flame sealed.

The slurry was a homogenate of the top 25 to 30 cm of sediment. Replicate ampoules were incubated at 4, 25, 55, and 80°C for 8 to 9 weeks. Controls with autoclaved sediment slurry were harvested at each time point. At specific times, ampoules were cooled to 1°C in an ice bath at least 1 h prior to and during the $^{14}CO_2$ collection. This cooling greatly reduced volatilization of the radiolabeled hydrocarbons and carryover during collections. The respired $^{14}CO_2$ was collected by bubbling for 30 min with N_2 by a modification of the method of Wirsen and Jannasch (24). The $^{14}CO_2$ was trapped in two vials containing 10 ml of 0.6 N NaOH. Subsamples from these base traps were added to Aquassure

* Corresponding author.
† Contribution no. 7125 of the Woods Hole Oceanographic Institution.

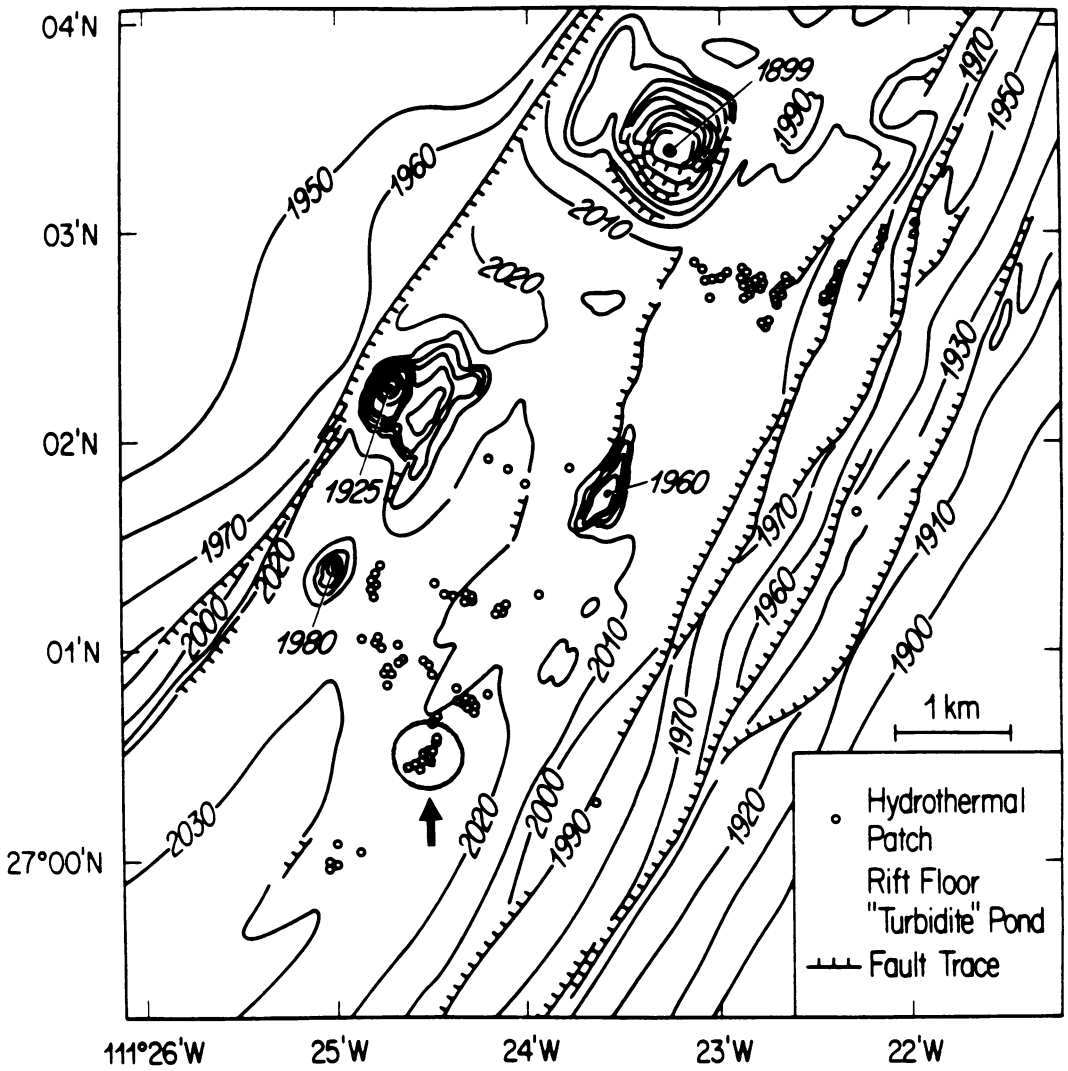

FIG. 1. Chart of dive location in the Gulf of California. Larger circle (marked with arrow) indicates sampling site. Smaller circles indicate hydrothermal vents. Depth contours are in meters. [Adapted from *Nature* (*London*) (21) with permission of the publisher.]

(Dupont, NEN Research Products, Boston, Mass.) scintillation fluid and counted in a Minaxiβ Tri-Carb 4000 series liquid scintillation counter (United Technologies Packard, Sterling, Va.).

Enrichment cultures. Enrichments for aerobic hydrocarbon-utilizing bacteria were done in ASW medium containing the following: NH_4Cl, 0.5 g liter^{-1}; KH_2PO_4, 0.2 g liter^{-1}; Wolfe mineral elixir (25) modified by the addition of 0.4 g of $Na_2MoO_4 \cdot 2H_2O$ liter^{-1}, 5 ml; and vitamin solution, 5 ml. The vitamin solution contained (in milligrams liter^{-1}): niacin, 10; calcium pantothenate, 10; *p*-aminobenzoic acid, 10; thiamine, 10; riboflavin, 10; pyridoxine, 10; cobalamin, 10; thioctic (α-lipoic) acid, 10; folic acid, 4; and biotin, 4 (this solution was filter sterilized and stored in the dark at 4°C).

For aerobic enrichments, the medium was adjusted to pH 7.4 and filter sterilized. Hexadecane and naphthalene were filter sterilized separately (solvent-resistant FP Vericel; porosity, 0.2 μm; Gelman Sciences, Inc., Ann Arbor, Mich.), the latter after dissolution in hexane which was subsequently evaporated under a stream of N_2. Both carbon sources were added to the liquid ASW medium at 0.1%.

Purified agar (2.0%; Difco Laboratories, Detroit, Mich.) was added to liquid ASW medium for streak plates. Drops of hexadecane or crystals of naphthalene as carbon sources were placed in the covers of inverted petri dishes.

For anaerobic enrichments, the Hungate system was used throughout. The medium was not filter sterilized but autoclaved and cooled under O_2-free N_2, after which anaerobic stock solutions of KH_2PO_4, vitamins, and carbon sources were added. All anaerobic media contained 0.2 ml of 0.2% resazurin liter^{-1}. For nitrate-reducing and denitrifying bacteria, 5 mM KNO_3 was included. For sulfur-reducing anaerobes, 1.0% (wt/vol) of elemental sulfur was included. The medium of Widdel and Pfennig (23) as modified by Widdel (F. Widdel, Ph.D. thesis, University of Göttingen, Göttingen, Federal Republic of Germany, 1980) was used for sulfate-reducing hydrocarbon-utilizing bacteria except that the growth-stimulating factors were omitted. All media were incubated at 25, 55, and 80°C.

For growth temperature studies, pure cultures were grown in a polythermostat on the hydrocarbon source on which they were isolated and on filter-sterilized Marine Broth 2216 (Difco).

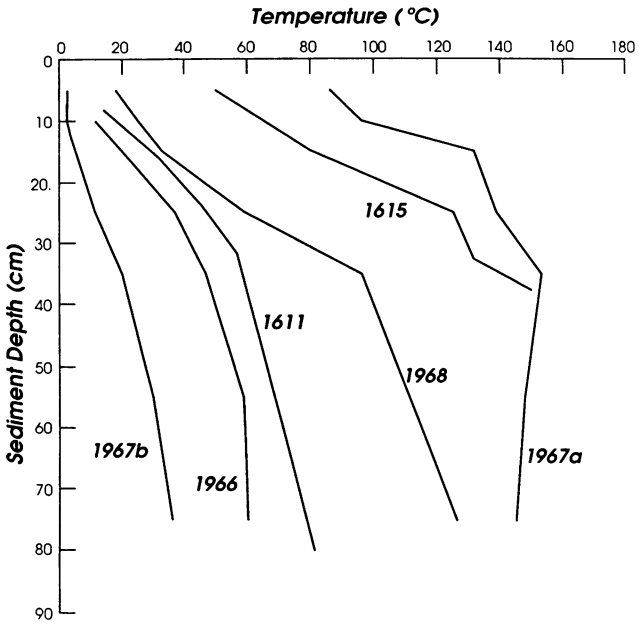

FIG. 2. Temperature profiles of sediments at the Guaymas Basin hydrothermal vent site. Numbers refer to specific ALVIN dive and core numbers.

RESULTS

In situ conditions. Temperature in sediments was extremely heterogeneous with respect to both vertical and horizontal profiles (Fig. 2). Ambient temperature of the bottom water is about 2.8°C. Sediments contained high amounts of H_2S but appear to be oxic at the surface. In places, the oxic top sediment was several centimeters deep (J. F. Grassle, personal communication). Massive mats of *Beggiatoa* spp., the gliding S^{2-}-oxidizing microaerophile (15), are common at the sediment surface. All cores collected had a petroliferous odor, although differing in degree. Chemical heterogeneity of hydrocarbon components over small vertical scales (tens of centimeters) in sediment cores and large horizontal scales (tens of meters) throughout the basin has been documented (1, 7), and sediments have been shown to contain both hexadecane and naphthalene (1, 7, 10, 21).

Potential for the microbial utilization of hydrocarbons. The percent respiratory conversion of [1-^{14}C]hexadecane and [1(4,5,8)-^{14}C]naphthalene to $^{14}CO_2$ in core sediment slurries is shown in Tables 1 and 2, respectively. Generally, counts from autoclaved controls rarely exceeded 0.03% of the total. The control counts are presumably from a small amount of unavoidable volatilization during collection. However, we believe that values ≥1.0% represent actual utilization of the substrate. Increases in percent conversion usually increased with incubation time (Fig. 3).

The temperature at which maximal conversion occurred varied from core to core. Two core slurries (cores 1615 and 1966) showed little or no conversion of hexadecane at any temperature tested. Three cores (1611, 1615, and 1966) showed little or no conversion of naphthalene at any temperature tested. Little or no conversion of either compound was observed at 80°C in any core slurry.

Enrichment Cultures. (i) Hexadecane. Positive microbial enrichments on hexadecane were obtained from all mesophilic (25°C) aerobic incubations and from all cores with the exception of that from dive 1615. From these a number of isolates were obtained that utilized hexadecane as the sole carbon and energy source. All isolates were mesophilic and had optima >20°C and <45°C but could grow at 4 to 5°C (Table 3).

(ii) Naphthalene. As with hexadecane, positive enrichments on naphthalene were only obtained from aerobic and mesophilic incubations and from all cores except those from dives 1611 and 1615. Isolates obtained from these enrichments were mesophilic but also grew at 4 to 5°C (Table 3; F. Goetz and H. W. Jannasch, personal communication). No positive enrichments were obtained under anaerobic or thermophilic conditions for either hydrocarbon.

TABLE 1. Maximum percent conversion of [1-^{14}C]hexadecane to $^{14}CO_2$ in core sediment slurries over an 8- to 9-week period

Core no.	% Conversion at incubation temp (°C):			
	4	25	55	80
1611	7.5	2.3	0.04	0.06
1615	0.01	0.09	0.03	0.19
1966	0.23	0.58	0.38	0.30
1967a	21.9	26.0	1.7	0.69
1967b	0.77	1.1	50.9	0.58
1968	1.1	31.5	5.83	0.28
Controls[a]				
1	0.01	0.04	0.01	0.02
2	0.01	0.01	0.26	0.03

[a] Autoclaved sediment.

DISCUSSION

The Guaymas Basin is a unique area in which a continuing naturally occurring oil seep offers an opportunity to study its effects at a deep-sea site. Petroleum formation, migration, and evolution occur here instantaneously in geologic terms (7). Geochemical data on petroleum composition suggest that hydrocarbon utilization by microorganisms is also an important process here (1, 20), although direct demonstration of this is lacking.

In a recent publication, we reported in detail on chemical analyses and the physical parameters of sediments from cores of ALVIN dives 1611 and 1615 (1). Analysis of core 1611 showed geochemical evidence of aliphatic hydrocarbon utilization as indicated by the low nC_{17}/pristane and nC_{18}/phytane ratios and the lack of *n*-alkanes in the surface sections of the core (0 to 20 cm) as compared with deeper sections (1). In contrast, results from core 1615 showed no

TABLE 2. Maximum conversion of [1(4,5,8)-^{14}C]naphthalene to $^{14}CO_2$ in core sediment slurries over an 8- to 9-week period

Core no.	% Conversion at incubation temp (°C):			
	4	25	55	80
1611	0.03	0.05	0.07	0.02
1615	0.02	0.04	0.02	0.05
1966	1.0	0.93	0.09	1.0
1967a	23.3	10.8	0.20	0.25
1967b	57.3	34.2	79.5	0.16
1968	0.89	0.10	3.6	0.62
Controls[a]				
1	0.01	0.02	0.03	0.03
2	0.03	0.06	0.10	0.08

[a] Autoclaved sediment.

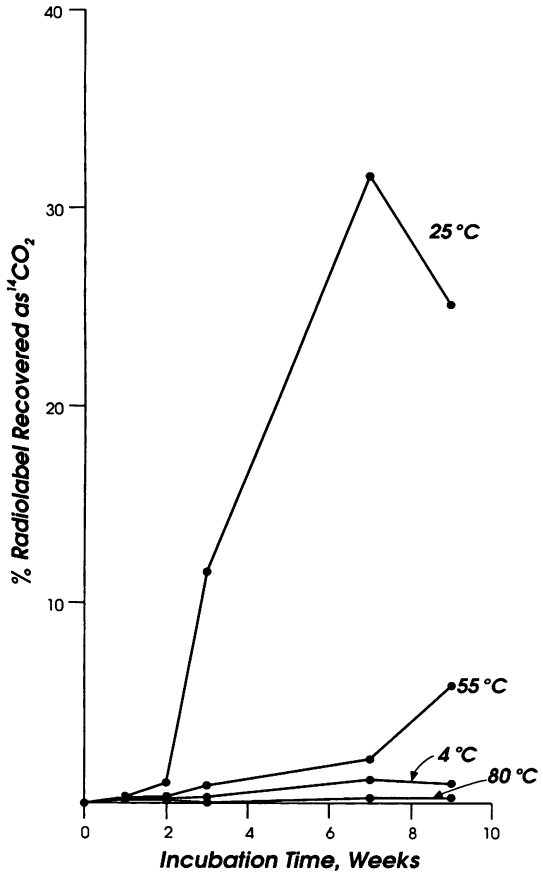

FIG. 3. Percent recovery of [1-^{14}C]hexadecane as respired $^{14}CO_2$ in sediment slurries from core 1968 at four temperatures.

indication of aliphatic hydrocarbon utilization on the basis of these criteria. There was no evidence of aromatic hydrocarbon utilization in either core. Conversion of [1-^{14}C]hexadecane to $^{14}CO_2$ was observed in slurries of sediment (4 and 25°C) from core 1611 but not core 1615, and conversion of radiolabeled naphthalene to $^{14}CO_2$ was not observed in slurries of either core. Furthermore, microorganisms that utilized hexadecane as a sole carbon and energy source were isolated from core 1611 but not core 1615 at 4 and 25°C.

TABLE 3. Growth optima of hexadecane- (H) and naphthalene- (N) utilizing isolates obtained from sediments and water of the Guaymas Basin hydrothermal vent site

Strain	Morphology	Growth temp range (°C)	Optimum growth temp (°C)
H1	Gram-negative motile rod	4–40	31
H2	Gram-negative motile curved rod	4–44	32
H3	Gram-positive nonmotile rod	4–34	29
H4	Similar to H1	4–40	28
H5	Similar to H1	4–40	31
H6	Gram-negative pleomorphic motile rod	4–43	31–40
H7	Similar to H6	4–46	35–38
H8	Gram-negative nonmotile rod	8–43	ND[a]
H9	Gram-negative nonmotile rod	8–46	40
N1	Gram-negative motile rod	5–37	27–31

[a] ND, Not determined.

Enrichments for naphthalene-utilizing microorganisms were negative from both cores. Thus, the geochemical data correlate well both with the potential of hydrocarbon utilization as measured by conversion of substrate in sediment slurries and the presence of the appropriate microorganisms detected in enrichment cultures and isolation. We are currently analyzing the petroleums extracted from sediments of cores from ALVIN dives 1966 to 1968 taken on our recent cruise.

Sediments from most cores used in the present study showed the conversion of radiolabeled hexadecane and naphthalene to $^{14}CO_2$. This potential hydrocarbon utilization coincided well with the presence of the appropriate microorganisms as demonstrated by positive enrichment cultures. Subsequently, isolates could be obtained that utilized the test compounds as sole sources of carbon. These observations suggest that hydrocarbons are indeed microbially significant carbon and energy sources in Guaymas Basin sediments.

Other results of this study are more difficult to interpret. The production of $^{14}CO_2$ from hexadecane and naphthalene was observed in several experiments at 55°C, but enrichment cultures at this temperature were negative. The most probable explanation is that the enrichment medium was unsuitable, e.g., missing a necessary growth factor. On the other hand, hexadecane and naphthalene were not necessarily the major carbon sources available in the isotope slurry experiments in contrast to the enrichments. The moderately thermophilic organisms may only cometabolize these substrates. We did not examine the role of cometabolism in this study, but preliminary results with other substrates at mesophilic temperatures (e.g., dibenzothiophene, phenanthrene) suggest that cometabolizing organisms are present (D. A. Bazylinski, F. Goetz, and H. W. Jannasch, unpublished results). It is doubtful that a microbial, extremely thermophilic utilization of hydrocarbons occurs, since all 80°C incubations were negative despite the fact that other bacterial isolates from Guaymas Basin sediment fermented yeast extract or produced methane from H_2 and CO_2 at an optimal temperature of 85 to 95°C (6, 27; H. W. Jannasch, *in* D. L. Wise, ed., *Bioprocessing and Biotreatment of Coal*, in press). Moderately thermophilic alkane-oxidizing bacteria, however, have been isolated from freshwater and shallow marine (littoral sediments) environments (12, 16, 17, 26).

Aerobic mesophiles capable of growth at low temperatures were the major type of organism isolated from sediments and overlying water of the Guaymas Basin vents. They appear to be the dominant hydrocarbon-utilizing organisms and to play the most significant role in hydrocarbon degradation at this site. This is not surprising, since many of the sediments have moderate and low temperatures, particularly near the surface where much of the oil driven upward by the high heat condenses because of the cold (~2.8°C) overlying ambient seawater (20, 21). The upward flow of pore water also appears to drive some of the more soluble oil components into the overlying water (1). This is particularly true of some of the aromatic compounds which are generally more soluble than alkanes and cycloalkanes of the molecular weight ranges of C_{10}-C_{30} compounds (13). Here the low temperature and available oxygen would facilitate degradation by psychrophilic aerobic bacteria.

Some mesophilic isolates were obtained from hot cores (e.g., ALVIN dive 1967a) with probe measurements of 85 to 153°C (Fig. 2), temperatures at which these organisms cannot be expected to survive. However, the heterogeneity of the sediment on a centimeter basis is such that integrating probe measurements may easily miss special temperature

variations. Furthermore, the sampling of sediment cores poses the inherent problem of introducing surface contaminants that survive during the quick cooling of the sample while the core is removed from the hot sediment.

Why certain samples from this site show no evidence of biodegradation (1, 7, 20), e.g., core 1615, is also unclear. Toxicity of certain oil components (13) and physical access to hydrocarbon deposits (20) as well as temperature must play significant roles in hydrocarbon utilization at Guaymas Basin. It is also possible that some samples may contain oil of recent occurrence (1) in which a significant microbial population has not yet been established.

An important observation at the site is the massive *Beggiatoa* mats covering large areas of the petroliferous Guaymas Basin sediments (15). The development of these aerobic, chemolithotropic, sulfide-oxidizing microbial mats may be supported by a substratum population which, by oxidizing hydrocarbons, supplies low-level amounts of specific organic compounds to the mats. In addition, this substratum population may also help to establish an oxygen gradient, a condition that appears to be required by *Beggiatoa* spp. (14). Vice versa, it can be expected that the competition for oxygen by *Beggiatoa* spp. acts as a limiting factor of hydrocarbon utilization at the Guaymas Basin vent site.

ACKNOWLEDGMENTS

We thank Frederick Goetz and John W. Farrington for valuable discussions and suggestions and David M. Karl for water samples. This research was supported by National Science Foundation grant OCE86-581 and Office of Naval Research contact N00014-86-K-0481.

D.A.B. was partially supported by a grant from EXXON Research and Engineering Company.

LITERATURE CITED

1. **Bazylinski, D. A., J. W. Farrington, and H. W. Jannasch.** 1988. Hydrocarbons in surface sediments from a Guaymas Basin hydrothermal vent site. Org. Geochem. **12**:547–558.
2. **Byrne, J. V., and K. O. Emery.** 1960. Sediments in the Gulf of California. Geol. Soc. Am. Bull. **71**:983–1010.
3. **Curray, J. R., D. G. Moore, J. E. Aquayo, M.-P. Aubry, G. Einsele, D. J. Fornari, J. Gieskes, J. C. Guerrero, M. Kastner, K. Kelts, Y. Matoba, A. Molina-Cruz, J. Niemitz, J. Rueda, A. D. Saunders, B. R. T. Simoneit, and V. Vacquier.** 1979. Leg 64 seeks evidence on the development of basins. Geotimes **24**:18–20.
4. **Curray, J. R., D. G. Moore, J. E. Aquayo, M.-P. Aubry, G. Einsele, D. J. Fornari, J. Gieskes, J. C. Guerrero, M. Kastner, K. Kelts, Y. Matoba, A. Molina-Cruz, J. Niemitz, J. Rueda, A. D. Saunders, B. R. T. Simoneit, and V. Vacquier.** 1982. Initial reports of the deep sea drilling project, vol. 64, parts I and II. U.S. Government Printing Office, Washington, D.C.
5. **Einsele, G., J. M. Gieskes, J. R. Curray, D. G. Moore, J. E. Aguayo, M.-P., Aubry, D. J. Fornari, J. C. Guerrero, M. Kastner, K. Kelts, M. Lyle, Y. Matoba, A. Molina-Cruz, J. Niemitz, J. Rueda, A. D. Saunders, H. Schrader, B. R. T. Simoneit, and V. Vacquier.** 1980. Intrusion of basaltic sills into highly porous sediments and resulting hydrothermal activity. Nature (London) **283**:441–445.
6. **Jones, W. J., C. E. Stugard, and H. W. Jannasch.** 1989. Comparison of thermophilic methanogens from submarine hydrothermal vents. Arch. Microbiol. **151**:314–318.
7. **Kawka, O. E., and B. R. T. Simoneit.** 1987. Survey of hydrothermally-generated petroleums from the Guaymas Basin spreading center. Org. Geochem. **11**:311–328.
8. **Lawver, L. A., D. L. Williams, and R. P. von Herzen.** 1975. A major geothermal anomaly in the Gulf of California. Nature (London) **257**:23–28.
9. **Lonsdale, P. J., and K. Becker.** 1985. Hydrothermal plumes, hot springs, and conductive heat flow in the Southern Trough of Guaymas Basin. Earth Planet. Sci. Lett. **73**:211–225.
10. **Lonsdale, P. J., J. L. Bischoff, V. M. Burns, M. Kastner, and R. E. Sweeney.** 1980. A high-temperature thermal deposit on the seabed at a Gulf of California spreading center. Earth Planet. Sci. Lett. **49**:8–20.
11. **Lyman, J., and R. H. Fleming.** 1940. Composition of seawater, J. Mar. Res. **3**:134–146.
12. **Merkel, G. J., W. H. Underwood, and J. J. Perry.** 1978. Isolation of thermophilic bacteria capable of growth solely in long-chain hydrocarbons. FEMS Microbiol. Lett. **3**:81–83.
13. **National Research Council.** 1985. Oil in the sea, fates, and effects. National Research Council, National Academy Press, Washington, D.C.
14. **Nelson, D. C., and H. W. Jannasch.** 1983. Chemoautotrophic growth of a marine *Beggiatoa* in sulfide-gradient cultures. Arch. Microbiol. **136**:262–269.
15. **Nelson, D. C., C. O. Wirsen, and H. W. Jannasch.** 1989. Characterization of large, autotrophic *Beggiatoa* spp. abundant at hydrothermal vents of the Guaymas Basin. Appl. Environ. Microbiol. **55**:2909–2917.
16. **Perry, J. J.** 1985. Isolation and characterization of thermophilic hydrocarbon-utilizing bacteria. Adv. Aquat. Microbiol. **3**:109–139.
17. **Phillips, W. E., and J. J. Perry.** 1976. *Thermomicrobium fosteri* sp. nov., a hydrocarbon-utilizing obligate thermophile. Int. J. Syst. Bacteriol. **26**:220–225.
18. **Simoneit, B. R. T.** 1983. Effects of hydrothermal activity on sedimentary organic matter: Guaymas Basin, Gulf of California—petroleum genesis and protokerogen degradation, p. 451–471. *In* P. A. Rona, K. Boström, L. Laubier, and K. L. Smith, Jr. (ed.), Hydrothermal processes at seafloor spreading centers. Plenum Publishing Corp., New York.
19. **Simoneit, B. R. T.** 1984. Hydrothermal effects on organic matter—high vs. low temperature components. Org. Geochem. **6**:857–864.
20. **Simoneit, B. R. T.** 1985. Hydrothermal petroleum: composition and utility as a biogenic carbon source. Biol. Bull. Soc. Wash. **6**:49–56.
21. **Simoneit, B. R. T., and P. F. Lonsdale.** 1982. Hydrothermal petroleum in mineralized mounds at the seabed of Guaymas Basin. Nature (London) **295**:198–202.
22. **Simoneit, B. R. T., M. A. Mazurek, S. Brenner, P. T. Crisp, and I. R. Kaplan.** 1979. Organic geochemistry of recent sediments from Guaymas Basin, Gulf of California. Deep-Sea Res. **26A**:879–891.
23. **Widdel, F., and N. Pfennig.** 1977. A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. Arch. Microbiol. **112**:119–122.
24. **Wirsen, C. O., and H. W. Jannasch.** 1986. Microbial transformations in deep-sea sediments: free vehicle studies. Mar. Biol. **91**:277–284.
25. **Wolin, E. A., M. J. Wolin, and R. S. Wolfe.** 1963. Formation of methane by bacterial extracts. J. Biol. Chem. **238**:2882–2886.
26. **Zarilla, K. A., and J. J. Perry.** 1984. *Thermoleophilum album* gen. nov. and sp. nov., a bacterium obligate for thermophily and *n*-alkane substrates. Arch. Microbiol. **137**:286–290.
27. **Zhao, H., A. G. Wood, F. Widdel, and M. P. Bryant.** 1988. An extremely thermophilic *Methanococcus* from a deep sea hydrothermal vent and its plasmid. Arch. Microbiol. **150**:178–183.