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Bacterial incorporation of relict carbon in the hydrothermal environment of Guaymas Basin

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Abstract—Radiocarbon analyses of bulk carbon and individual organic compounds are presented for the hydrothermal environment of the Rebecca's Roost vent in the southern trough of the Guaymas Basin hydrothermal field. The $\Delta^{14}C$ values of CO_2 and CH_4in the hottest hydrothermal fluids (317°C) are nearly "radiocarbon dead" (−944‰ and −923‰, respectively). In contrast, the $\Delta^{14}C$ values of sediments and individual fatty acids (−418‰ to −227‰) obtained from a bacterial mat located south of the vent site are similar to values previously reported for hydrothermal petroleum in this environment and are more depleted in ^{14}C than overlying waters. Hydrothermal fluids moving through the sediments appear to supply ^{14}C of intermediate age to the bacteria. This carbon may take the form of, or may be supplied by processes similar to, the generation of hydrothermal petroleum. Although the bacterial mat visibly was dominated by *Beggiatoa* spp., such mats are known to include numerous other species. Individual compound data show that preaged carbon is being consumed by the integrated bacterial assemblage. Values of $\delta^{13}C$ and $\Delta^{14}C$ indicate that petroleum-derived carbon is incorporated directly into fresh bacterial biomass. Subsequently, some of this newly synthesized material also is consumed by heterotrophs, as eukaryotic sterols from the same sample also have ^{14}C-depleted values ($\Delta^{14}C = -136‰$ to −110‰). Therefore, the entire system may operate as a complex consortium to transform relict carbon back into biomass. Bacterial consumption of relict carbon occurs despite the ample supply of fresh carbon delivered from the productive, overlying water column. *Copyright © 2005 Elsevier Ltd*

1. INTRODUCTION

The hydrothermal environment of Guaymas Basin is located in an area of high sediment loading that has been the subject of numerous studies for more than 25 yrs. High biologic productivity in the overlying surface waters results in deposition of abundant organic-rich sediments with total organic carbon (TOC) concentrations up to 4% by weight. Petroleum is actively produced within the basin sediments as the result of magmatic heating of the thick, organic-rich sediment cover (Lonsdale et al., 1980; Simoneit, 1985; Gieskes et al., 1988; Simoneit et al., 1992). The measured radiocarbon ages are not infinite (^{14}C-"dead"), indicating that some of this petroleum originates from recently-deposited sediments (Peter et al., 1991; Simoneit and Kvenvolden, 1994). The estimated sedimentation rate of Guaymas Basin sediments is between 1 and 5 m/Ka (Curray et al., 1979; Gieskes et al., 1982), which places the average depth of petroleum generation within the upper ~10–50 m of sediment to yield these relatively young ^{14}C ages (Peter et al., 1991). Compositionally, hydrothermal petroleum is similar to crude oil produced in slowly subsiding sedimentary basins (Simoneit and Schoell, 1995). The eventual fate of the petroleum generated in Guaymas Basin is thought to be vertical migration within low-velocity pore fluids and discharge at the sediment-water interface, in addition to venting through high-temperature vents or "smokers" (Lonsdale and Becker, 1985; Von Damm et al., 1985; Gieskes et al., 1988). During transport, some of the carbon may be available as a metabolic substrate for transformation into biomass and labile biologic products.

Bacterial mats are a common feature found at sediment-water interfaces. They occur as cohesive structures that are <1 to several millimeters in thickness and appear white or yellow in color (e.g., Nelson et al., 1989; Gundersen et al., 1992; McHatton et al., 1996). The mats are dominated visually by the filamentous sulfur bacteria, *Beggiatoa* and *Thioploca* spp., but these macroscopic species also hide considerable microbial diversity representing other groups of prokaryotes and micro-eukaryotes (e.g., Larkin et al., 1994; Edgcomb et al., 2002; Dhillon et al., 2003).

The filamentous sulfur bacteria are chemolithotrophs that oxidize reduced sulfur using O_2 or NO_3^- (McHatton et al., 1996). They live as autotrophs by fixing CO_2 (Nelson et al., 1989; Hagen and Nelson, 1996; McHatton et al., 1996) or as mixotrophs by inducing simultaneous chemoorganotrophy (Nelson and Castenholz, 1981; Hagen and Nelson, 1996). Typical locations hosting such mat systems include hydrothermal sediments (Jannasch and Wirsen, 1979; Nelson et al., 1989; Gundersen et al., 1992), cold petroleum seeps (McHatton et al., 1996; Larkin et al., 1994), and coastal margins under the O_2-minimum zone (Gallardo, 1977; Williams and Reimers, 1983). The bacterial mats of Guaymas Basin have been studied with respect to their filament morphology (Nelson et al., 1989) and participation in redox chemistry and boundary zone processes (Gundersen et al., 1992). Little is known about their system-specific role in carbon metabolism, but an association with petroleum seeps has been observed previously (Karl et al., 1988; Larkin et al., 1994). In Guaymas Basin, the petroleum-rich sediment layer immediately underlying the mat filaments

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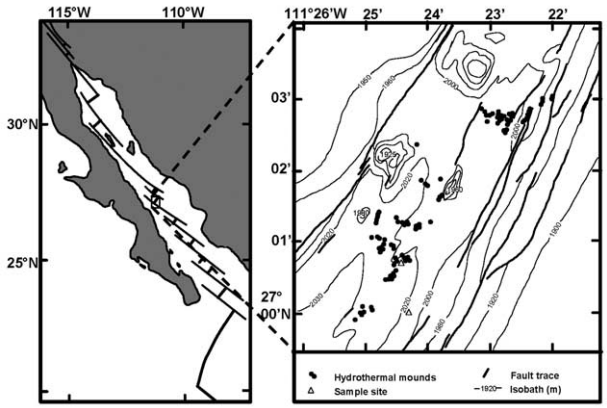

Fig. 1. Location of sampling sites in Guaymas Basin (adapted from Peter et al., 1991).

also has been studied and shows considerable microbial diversity (Edgcomb et al., 2002; Teske et al., 2002; Dhillon et al., 2003).

Compound-specific ^{14}C dating (Eglinton et al., 1996, 1997) has numerous applications, including the identification of carbon substrates utilized by microorganisms in the environment (Pearson et al., 2001). Here we show that a microbial mat community in Guaymas Basin can utilize the upward flux of petroleum-derived carbon to generate biomass. These bacteria appear to exploit the sediment-water interface for carbon substrates in addition to oxidizing reduced sulfur for energy. The natural ^{14}C concentration of bulk carbon pools and of individual biomarkers extracted from a *Beggiatoa* mat shows that the bacterial biomass has a ^{14}C signature more depleted than the surrounding water or sediment. The ^{14}C concentration and $\delta^{13}C$ values of fresh biomass are similar to the isotopic composition of hydrothermal petroleum, consistent with significant heterotrophic consumption of this carbon source.

2. METHODS

2.1. Samples

Hydrothermal fluid samples were collected in 1998 from a 317°C vent located on a large sulfide structure known as Rebecca's Roost (27° 00.67′ N, −111° 24.42′ W; depth 1989 m). The *Beggiatoa* mat, surface sediment and bottom water samples were collected on the flank at 27° 00.02′ N, −111° 24.3′ W; depth 2015 m (Fig. 1). All sampling was accomplished with the *Jason* ROV during a cruise aboard the R/V Atlantis.

Bottom water was obtained in a 1L Go-Flo bottle, at a depth 1 m above the sea floor. The sample was preserved using $HgCl_2$ and stored at room temperature; DIC was extracted as CO_2 and isotopic measurements were made at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) Facility, Woods Hole, MA. Hydrothermal vent fluid from Rebecca's Roost was collected in a gas-tight, titanium sampler. The sample was acidified and total gases were extracted on a vacuum line. The resulting CO_2 concentration represents total dissolved inorganic carbon (DIC); CO_2 was separated from CH_4 cryogenically (Isotech Laboratories, Champaign, IL, USA) and the CH_4 subsequently converted to CO_2 by combustion.

The *Beggiatoa* mat plus entrained surface sediment was collected using a push core sampler. The upper 0–1 cm of mat plus sediment was sampled immediately upon shipboard recovery; the sample was divided into upper (mat-rich) and lower (sediment-rich) fractions and stored at −20°C until processing.

2.2. Lipid Extraction and Purification

The mat-rich sample (8.12 gdw) was extracted for 72 hours in 93:7 DCM/MeOH using a Soxhlet apparatus. The resulting total lipid extract (TLE; lipid concentration 0.0129 gC/gdw) was transesterified using 95:5 MeOH:HCl, and fatty acid methyl ester (FAME) and sterol fractions were separated by silica-gel flash chromatography as described in Pearson et al. (2001). Sterols were acetylated in 2:1 pyridine/acetic anhydride (Alltech, Lot # 11689, $\delta^{13}C = -27.1‰$, $\Delta^{14}C = -997‰$). The contribution of derivative carbon to the $\Delta^{14}C$ values subsequently was removed by mass balance.

Individual compounds were purified for radiocarbon analysis using preparative capillary gas chromatography (Eglinton et al., 1996, 1997). A HP 5890 series II GC, equipped with HP 7673 auto-injector, Gerstel CIS-3 cooled injection system, and Gerstel preparative trapping device (PTD) was fitted with a SGE BPX-5 (95%-dimethyl-5%-phenyl-polysiloxane; 60m × 0.53 mm i.d. × 0.5 μm film thickness) capillary column. Individual compounds were collected in a series of seven U-tube traps; six were programmed to collect compounds of interest, while the seventh received the unseparated material.

GC temperature programs were: for the FAME sample (in CH_2Cl_2), 40°C (1 min), 20°C/min to 160°C, 4°C/min to 320°C (28 min); and for sterols (in CH_2Cl_2), 40°C (1 min), 20°C/min to 270°C, 2.5°C/min to 320°C (25 min). The PTD was operated at 320°C and the U-tube traps at room temperature. The compounds condensed in the U-tubes as solids. FAMEs smaller than C_{20} were collected with the U-tube traps chilled to 0°C to prevent volatile losses.

Column bleed was removed from pure compounds by eluting the recovered material with 10% ethyl acetate/hexane through SiO_2-gel columns (prepared in Pasteur pipettes, then combusted at 450°C, 8 h; all solvents, Fisher GC Resolv). Finally, solvent was evaporated under ultra-high-purity N_2.

2.3. Radiocarbon Analysis by AMS

Water samples were processed as stated above. All other samples were sealed in evacuated quartz tubes with 100 mg CuO and combusted to CO_2 (850°C, 5 h). The CO_2 was reduced to graphite over cobalt catalyst. ^{14}C-AMS analysis was performed using special methods necessary for the accurate determination of $\Delta^{14}C$ in samples containing <0.5 mgC (Pearson et al., 1998; von Reden et al., 1998). For our samples, the reported $\Delta^{14}C = 1000[f_m e^{(1950-x)\lambda} - 1]$ (Stuiver and Polach, 1977), where $\lambda = 1/8267$ (yr^{-1}), f_m = fraction modern ^{14}C, corrected for isotopic fractionation using $\delta^{13}C$, and "x" equals the year of collection (1998).

3. RESULTS AND DISCUSSION

3.1. High-Temperature Hydrothermal Fluids

3.1.1. Stable isotopes

Extensive interaction of hydrothermal fluids with organic-rich sediments that overlie the tectonic spreading center at Guaymas Basin has resulted in high concentrations of dissolved carbon compounds. Dissolved CH_4 and total dissolved inorganic carbon (DIC) concentrations in hydrothermal fluids at Rebecca's Roost are 54 and 50 mmol/kg fluid, respectively (Seewald et al., 1998). Relative to the ^{13}C content of DIC (−9.4‰), the dissolved CH_4 is significantly more ^{13}C-depleted (−43.8‰; Table 1). Dissolved CO_2 and CH_4 in vent fluids from Rebecca's Roost may be derived from the hydrothermal degradation of organic matter, dissolution of diagenetic carbonates, microbial activity, and/or magmatic degassing (Welhan and Lupton, 1987; Welhan, 1988). Attainment of carbon isotopic equilibrium between CO_2 and CH_4 at Rebecca's Roost is not expected due to sluggish reaction kinetics for carbon isotope exchange at temperatures near 317°C (Giggenbach, 1982). Indeed, if it is assumed that DIC = $CO_{2(aq)}$ at the

Table 1. Carbon isotopic data for bulk samples and individual compounds from Guaymas Basin.

	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)[a]	
Water			Concentration
Surface water DIC (23 m)[b]	1.8 ± 0.1	+62 ± 4	2.01 mmol/kg
Bottom water DIC (2015 m)[c]	−0.6 ± 0.1	−228 ± 6	2.34 mmol/kg
Hydrothermal Vent Fluid DIC	−9.4 ± 0.1	−944 ± 1	50 mmol/kg[f]
Hydrothermal Vent Fluid CH_4	−43.8 ± 0.1	−923 ± 1	54 mmol/kg[f]
Bulk Bacterial Mat			
TOC-upper mat layer	−22.1 ± 0.1	−207 ± 5	
TOC-underlying mat/sediment	−21.6 ± 0.1	−197 ± 10	
Mat-TLE	−25.7 ± 0.1	−578 ± 3	
Mat-Sterols			
C_{27+28} mixed	−25.2 ± 0.5	−110 ± 13	
$C_{29}\Delta^5$ + 5Δ-C_{29}	−26.6 ± 0.3	−136 ± 31	
Mat-Fatty Acids			GC Peak
iso-$C_{15:0}$	−29.4 ± 0.3		a
anteiso-$C_{15:0}$	−26.3 ± 0.4	−418 ± 10[d]	a
$C_{16:1}$	−18.3 ± 0.3	−363 ± 12	b
$C_{16:0}$	−24.5 ± 0.2	−227 ± 10	c
$C_{18:1}$	−20.5 ± 0.7	−369 ± 10	d
C_{22}	−25.2 ± 0.1		e
C_{24}	−25.0 ± 0.2		e
C_{26}	−25.3 ± 0.4	−347 ± 15[e]	e

[a] $\Delta^{14}C$ values by definition are normalized to a constant value of isotopic fractionation equivalent to $\delta^{13}C = -25‰$ (Stuiver and Polach, 1977). When used in mass-balance mixing equations, no further correction needs to be made to account for isotopic fractionation during biosynthesis.
[b] Isotopes and concentration from WOCE track P18N, Station 190, 22.0°N, −110.0°W.
[c] Isotopes, this work; concentration from WOCE track P18N, Station 190, 22.0°N, −110.0°W.
[d] Combined *i, a*-$C_{15:0}$ into one sample for $\Delta^{14}C$.
[e] Combined $C_{22,24,26}$ into one sample for $\Delta^{14}C$.
[f] Concentration data from Seewald et al. (1998).

relatively low in situ pH of these fluids (Seewald et al., 1990), isotopic disequilbrium is indicated by the observed difference in $\delta_{13}C$ values for CO_2 and CH_4. The measured difference between CO_2 and CH_4 ($\Delta_{CO2-CH4}$) is 34.4‰, which is considerably larger than the difference expected if the system were at equilibrium at 317°C ($\Delta_{CO2-CH4(Equil)} = 20‰$; Richet et al., 1977).

Biogenic methane formed in sedimentary environments is characterized by a large range of $\delta^{13}C$ values that vary from −42‰ to −105‰ as a function of community structure, metabolic pathway, and environmental variables such as temperature (Whiticar, 1999). The high end of this range overlaps with the isotopic signature of thermogenic methane formed from the abiogenic alteration of sedimentary organic carbon (typically −20‰ to −50‰). A significant portion of the methane at Rebecca's Roost is likely to be derived from thermal maturation of sediments, given the abundant evidence for active petroleum generation in the Guaymas Basin (e.g., Simoneit et al., 1992) and the presence of thermogenic C_{2+} hydrocarbons in vent fluids (Welhan and Lupton, 1987). Laboratory experiments, however, have demonstrated that the carbon isotopic composition of CH_4 generated at 325 to 400 °C during hydrothermal alteration of Guaymas Basin sediment varies from −20‰ to −23‰ (Seewald et al., 1994). The CH_4 at Rebecca's Roost is ^{13}C-depleted relative to these values, which suggests that high-temperature alteration of sediments in deep reaction zones is not the primary source of CH_4 in the venting fluids. The more depleted $\delta^{13}C$ value of −43.8‰ in the vent fluids is similar either to the maximum expected value for biogenic CH_4, or to the thermogenic formation of CH_4 at intermediate temperatures. The fractionation between CO_2 and CH_4 ($\Delta_{CO2-CH4} = 34.4‰$) is equivalent to an average formation temperature of ~180°C (Richet et al., 1977), assuming no further equilibration has occurred and assuming a homogeneous source temperature. However, because life is restricted to temperatures below ~120°C, any microbial contribution of biogenic methane to the hydrothermal fluids would only occur at temperatures <120°C rather than 180°C. Both of these interpretations suggest that the primary source for the CH_4 in the vent fluids is from relatively shallow sediments, possibly entrained into pore fluids in lower temperature recharge zones (Fig. 2). Therefore, although the apparent average temperature of CH_4 formation is ~180°C, this likely represents a mixture of sources (thermogenic and biogenic) across a broad range of intermediate temperatures. Regardless, the depth of CH_4 formation is constrained by the ^{14}C data (below), which indicate that such methanogenic zones also must be deep enough that ^{14}C concentrations have decayed nearly to zero (Fig. 2).

The stable carbon isotopic composition of DIC ($\delta^{13}C$ = −9.4 ‰) at Rebecca's Roost is similar to the range of values (−7.9‰ to −10.7‰) observed during the hydrothermal sediment alteration experiments of Seewald et al. (1994). During these experiments, release of CO_2 to solution was attributed to the dissolution of ^{13}C-enriched diagenetic carbonates (~0%) and to CO_2 generated during the hydrothermal alteration of sedimentary organic matter (−5‰ to −18‰). Similar sediment alteration processes probably contribute the majority of the new CO_2 to the vent fluids at Guaymas Basin. However, the ^{13}C content of DIC at Rebecca's Roost is also similar to mantle-derived CO_2 (−5‰ to −10‰; Welhan, 1988; Shanks,

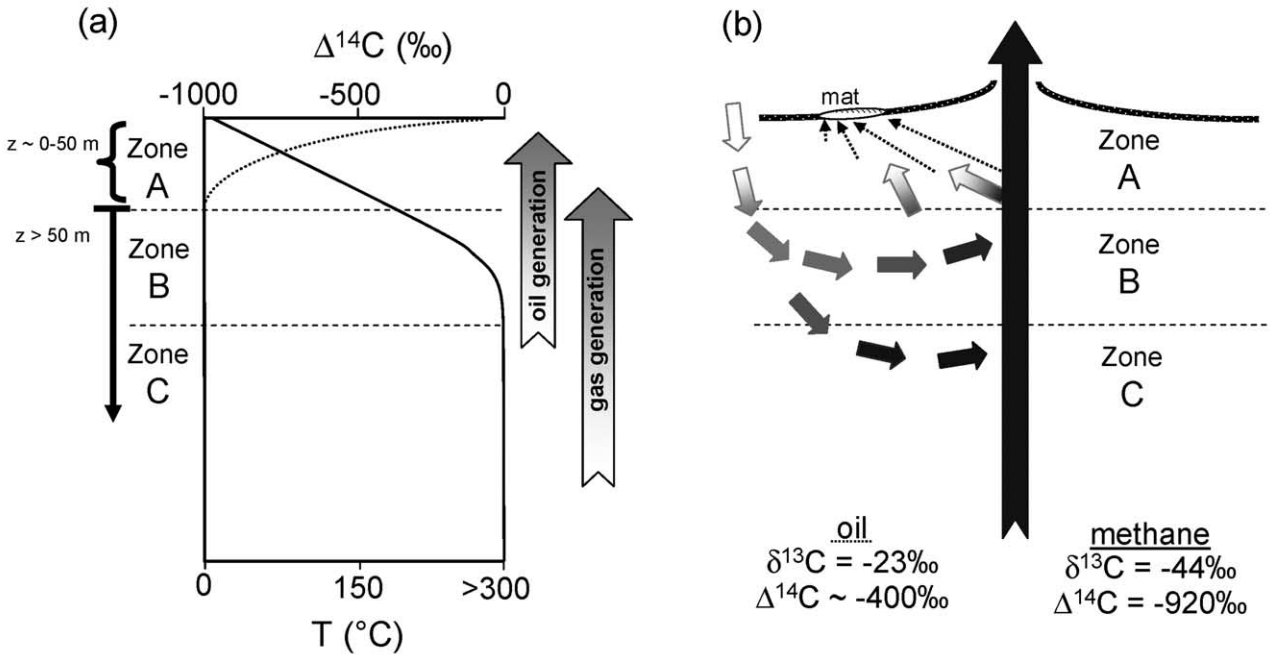

Fig. 2. Idealized diagram of the presumed petroleum carbon cycle dynamics in Guaymas Basin and the influence of this carbon on the microbial mat community sampled for this study. **(a)** Depletion of ^{14}C in sedimentary organic matter (dashed line) occurs in zone A, before 50m depth in the sediments; in zone A/B, biogenic CH_4 may be formed at T <120°C; in zone B, thermogenic but isotopically light CH_4 is formed at temperatures <300°C; in zone C, hydrothermal fluids are heated to >300°C and entrain CH_4 during hydrothermal flow and venting. **(b)** Cartoon of possible CH_4 and oil generation dynamics. Shading of wide arrows is proportional to the concentration of CH_4, which does appear to reach (or alternatively, not be consumed by) the bacterial mat. Dashed arrows show formation of oil in shallow, ^{14}C -modern sediments residing with in the oil window (~50 to 175 °C), as well as emanating from the hot hydrothermal fluid.

1995). Accordingly, the isotopic composition of CO_2 at Guaymas Basin does not preclude an additional component of CO_2 derived from the degassing of basaltic magmas, although this phenomenon typically is more common in unsedimented ridge-crest hydrothermal systems (Lilley et al., 2003; Seewald, 2003).

3.1.2. Radiocarbon

The DIC and dissolved CH_4 in vent fluids at Rebecca's Roost are very depleted in radiocarbon (Table 1). The $\Delta^{14}C$ value of −944‰ for DIC is equivalent to 94% radiocarbon "dead" material (i.e., "dead" $\Delta^{14}C = -1000‰$); the difference between the values for DIC and CH_4 ($\Delta^{14}C = -923‰$) is relatively insignificant. The concentrations of these dissolved species in the hydrothermal fluid are enriched relative to their seawater source (Table 1). Since there is insignificant CH_4 in seawater, all of the dissolved CH_4 in the hydrothermal fluid must be generated in the subsurface environment, below the depth of most ^{14}C decay. Similarly, comparison of the DIC concentrations between deep seawater and hydrothermal fluid indicates that the preaged sources account for the increase in DIC concentration from 2.3 mmol/kg to 50 mmol/kg. Isotopic mass balance yields a $\Delta^{14}C$ value of −982‰ for the hydrothermal DIC endmember. This indicates that >98% of the new dissolved carbon is derived from radiocarbon-dead substances, again consistent with a source from deep sediments and/or magmatic processes.

Estimates of sedimentation rate in Guaymas Basin approach 5 m/kyr (Curray et al., 1979; Gieskes et al., 1982). Given these rates, radiocarbon concentrations in sedimentary organic matter should decay to $\Delta^{14}C$ values approaching −1000‰ (radiocarbon-"dead") by 10–50m depth within the sediments. The ^{14}C data for hot fluids from Rebecca's Roost suggest that the major zone of chemical alteration responsible for the release of CO_2 and CH_4 into hydrothermal fluids lies deeper than 10–50 m below sea floor. When combined with the evidence from $\delta^{13}C$ measurements, the data suggest that the CO_2 and CH_4 in the hydrothermal fluid originate from at least this depth (Fig. 2), but at temperatures cooler than 180°C (based on $\delta^{13}C$ of CH_4).

3.1.1. The origin of hydrothermal petroleum

Simoneit and Kvenvolden (1994) measured a mean radiocarbon age of 4690 yrs (range 3200–6600 yrs) for petroleum samples obtained from the southern trough of Guaymas Basin. Their data imply that in this location, oil formation is a modern process that incorporates young carbon from recently deposited sediments. Our data show that similarly shallow sediments do not appear to supply CH_4 to the hydrothermal fluid, since CH_4 in the hot fluid is exclusively ^{14}C-"dead" (Fig. 2). The radiocarbon ages for petroleum suggest it is produced on average between 10 and 30 m depth, and certainly shallower than the depth of production of CH_4. Active petroleum generation reflects the presence of elevated temperatures in these shallow sedimentary horizons (the oil window typically falls between

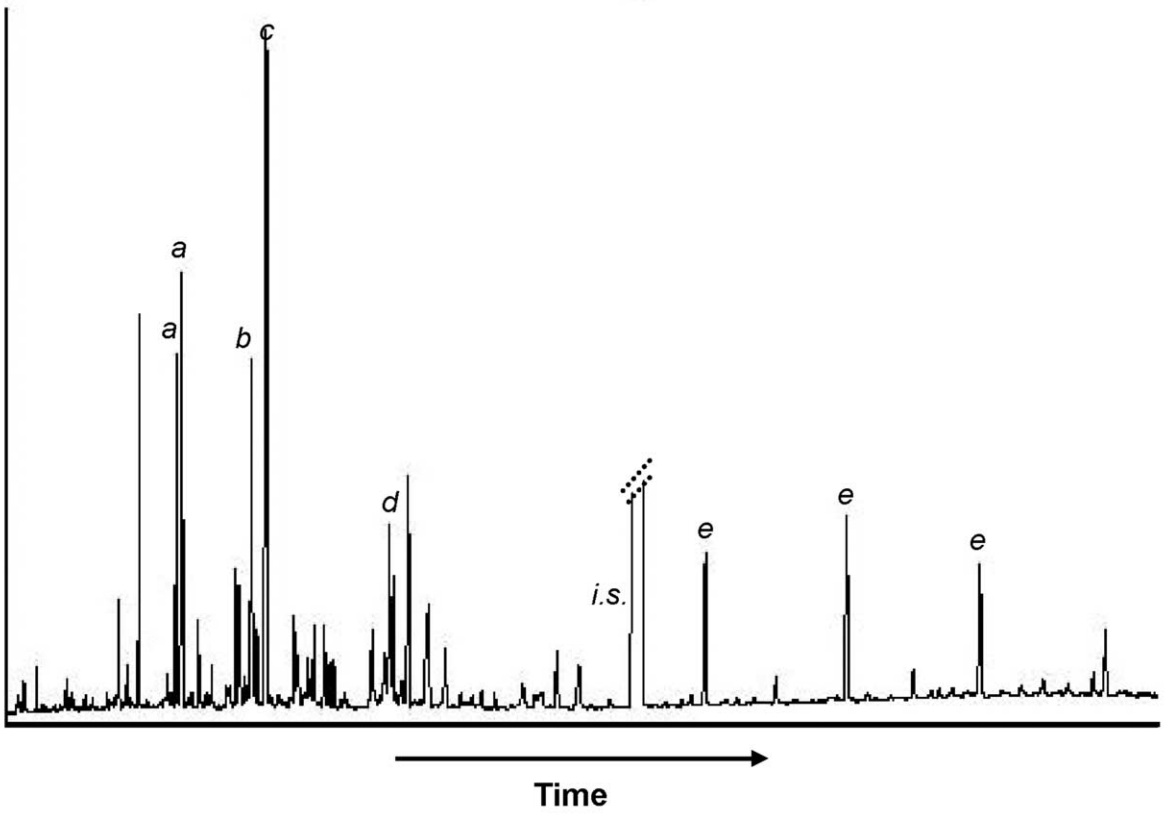

Fig. 3. GC chromatogram of fatty acids (as FAMEs) obtained from bacterial mat; compounds *a*, *b*, *c*, *d*, *e*, identified in Table 1; *i.s.*, internal standard.

~50 to 175°C). However, it has also been suggested that petroleum forms rapidly in Guaymas Basin sediments at temperatures up to 315°C (Simoneit, 1985; Simoneit et al.,1992), similar to the temperature of the hottest hydrothermal fluids. If production of oil were associated with these deep, hot sedimentary horizons, the oil would be ^{14}C-dead. Therefore, to yield petroleum in the surficial sediments that has an age in the range of 3200–6000 yrs, there must be formation of oil in very shallow sedimentary horizons, although additional, deeper sources of "dead" oil can't be ruled out. It also is possible that formation of petroleum is heterogeneously distributed and localized to the boundaries of hot, hydrothermal fluid seeps. Such "patchy" localization of hydrothermal petroleum formation and seepage could help to influence the uneven distribution of benthic microbial mats.

3.2. Bulk Bacterial Mat and Sediments

Radiocarbon concentrations (Δ^{14}C values) for TOC from the bacterial mat and sediments (−207‰, −197‰) are similar to bottom water DIC (−228‰; Table 1). However, the TOC and bacterial mat are expected to contain ^{14}C integrated from a mixture of sources, including surface-water primary production (~ +50 to + 100‰, a typical range of values for "postbomb" surface waters), chemoautotrophy at the sediment-water interface (−228‰), and terrestrial organic matter representing a broad range of ^{14}C ages and Δ^{14}C values. More significantly, the ^{14}C data show that the bacterial mat and surface sediment located at a distance from the extremely ^{14}C-depleted hydrothermal fluid do not exhibit similarly negative Δ^{14}C values. Biologic fixation of CO_2 or CH_4 from channeled, hot-temperature sources of vent fluids does not contribute to a large fraction of biomass in the mat or to the TOC.

In addition, the stable isotope compositions of the bacterial mat and TOC indicate that their biomass carbon is not significantly derived from CH_4. The δ^{13}C values of these samples are near −22‰, which is consistent with bulk marine primary production and heterotrophy. In contrast, bacterial mats that incorporate carbon from CH_4 display more depleted δ^{13}C signatures (Paull et al., 1992). This suggests that if CH_4 does migrate in hydrothermal fluids to reach the surface sediments in this environment, it is not utilized as a quantitatively significant carbon substrate within the microbial mat. Alternatively, CH_4 within the sedimentary pore fluids may be consumed at depth in this location (Teske et al., 2002) and does not reach the bacterial mat at the sediment-water interface (Fig. 2).

3.3. Individual Compounds

Individual compounds were purified from the total lipid extract (TLE) of the *Beggiatoa* mat sample. An example chromatogram for fatty acids (as their corresponding methyl esters, FAMEs) shows compounds selected for isolation and analysis (Fig. 3). Data for individual compounds are shown in Table 1.

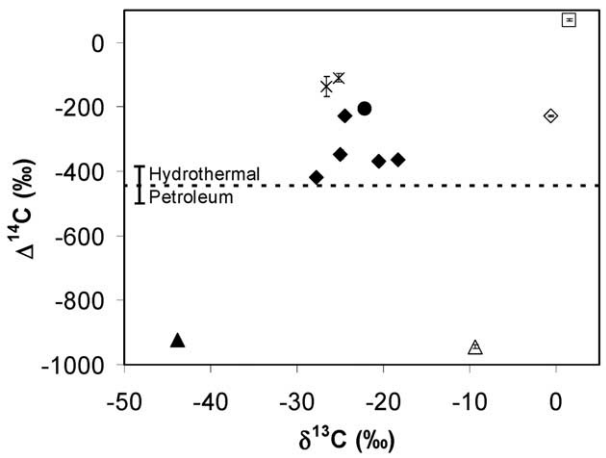

Fig. 4. Isotopic composition of organic and inorganic carbon pools in Guaymas Basin. Open square, surface water DIC; open diamond, bottom water DIC; filled circle, bacterial mat TOC; filled diamonds, individual FAMEs; open triangle, hydrothermal fluid DIC; filled triangle, hydrothermal fluid CH_4; cross, individual sterols. When not visible, measurement error is smaller than the size of the symbol.

The $\Delta^{14}C$ value measured for the whole TLE was significantly more negative than the value measured for TOC; this is consistent with the presence of ^{14}C-depleted hydrothermal petroleum as part of the total lipid composition of the sample. The $\Delta^{14}C$ values obtained for the individual fatty acids derived from biomass also were ^{14}C-depleted: typical $\Delta^{14}C$ values for individual lipids are more negative than for the TOC and are similar to the value for the TLE.

3.3.1. $\Delta^{14}C$ data for fatty acids

The major fatty acids of the filamentous sulfur bacteria, *Beggiatoa* and *Thioploca* spp., are $C_{16:1}$, $C_{16:0}$, and $C_{18:1}$ (McCaffrey, 1989). The $\Delta^{14}C$ values obtained for purified samples of $C_{16:1}$ and $C_{18:1}$ fatty acids obtained from the Guaymas Basin bacterial mat sample were identical within measurement errors (−363‰ and −369‰). These compounds reflect the biomass of *Beggiatoa* within the mat, but also include contributions from other microbial species within the sample. Marine strains of *Beggiatoa* have been shown in laboratory and field studies to obtain their carbon through autotrophic fixation of CO_2, coupled to oxidation of S^{2-} by O_2 or NO_3^- to provide energy (Hagen and Nelson, 1996; McHatton et al., 1996). Here, the measured $\Delta^{14}C$ values for the $C_{16:1}$ and $C_{18:1}$ fatty acids are more negative than can be explained by carbon fixation derived solely from bottom water DIC ($\Delta^{14}C = -228$‰), despite the fact that these filaments grew on the sediment surface, exposed to overlying bottom waters. The $\Delta^{14}C$ values for the other fatty acids, with the exception of $C_{16:0}$, also contained similarly depleted levels of ^{14}C (range −418‰ to −347‰; Fig. 4).

All of these values (except $\Delta^{14}C$ of $C_{16:0}$) are very similar to previous reports of the radiocarbon age of hydrothermal petroleum in Guaymas Basin. An average $\Delta^{14}C$ value of -431 ± 55‰ was reported for samples of whole oils from the Southern Trough (Simoneit and Kvendvolden, 1994)[1]. Using $\Delta^{14}C = -431 \pm 55$‰ as the petroleum endmember and −228‰ as the bottom water DIC endmember, and assuming that these are the primary sources of carbon to the bacterial population that produces C_{15}, $C_{16:1}$, $C_{18:1}$, and $C_{22+24+26}$ fatty acids, the mass balance equation:

$$\Delta^{14}C_{fatty\_acid} = f_{petroleum}\,\Delta^{14}C_{petroleum} + \left(1 - f_{petroleum}\right)\Delta^{14}C_{DIC} \quad (1)$$

suggests that between 46 and 100% of the bacterial biomass could derive from the flux of petroleum (average 72%).

The one outlier is the $C_{16:0}$ fatty acid, which at $\Delta^{14}C = -227$‰ more closely resembles average TOC and bottom-water DIC than it resembles the petroleum. This compound is not solely derived from bacteria; it is also a major membrane lipid of eukaryotes. Here it probably integrates three sources: bacterial biomass, the biomass of micro-eukaryotes within the bacterial mat, and the input of phytodetritus from the overlying water column.

3.3.2. $\delta^{13}C$ data for fatty acids

None of the compound-specific $\delta^{13}C$ data indicate that methanotrophy is a major contributor to biomass in this bacterial mat. Values for individual compounds range between −29.4‰ and −18.3‰; the average is −24.3‰, a typical value found in marine samples. In contrast, the lipids from methanotrophs would be expected to have $\delta^{13}C$ values similar to those observed for archaeal, anaerobic methanotrophy (~ −100‰ to −60‰; e.g., Hinrichs et al., 1999) or bacterial, aerobic methanotrophy (~ −70‰ to −40‰; e.g., Elvert et al., 2000; Thiel et al., 2001; Werne et al., 2002). Bacterial communities associated with methanotrophy in deeper Guaymas Basin sediments taken from another location showed considerable isotopic depletion ($\delta^{13}C$ values < −40‰; Teske et al., 2002), in contrast to what is observed here.

The overall similarity between the compound-specific $\Delta^{14}C$ data and the radiocarbon ages reported for petroleum suggest that heterotrophic consumption of petroleum-derived carbon is an important process within the bacterial mat. In addition, CO_2 derived from the respiration of petroleum or from entrainment of hydrothermal CO_2 into pore-waters also could represent additional sources of preaged DIC. Autotrophic fixation of this DIC into biomass also would produce depleted $\Delta^{14}C$ signatures. However, the $\delta^{13}C$ data for individual fatty acids suggest that this scenario is less significant quantitatively than the direct consumption of petroleum-derived carbon. Both the respiration of petroleum and the entrainment of hydrothermal CO_2 would effectively decrease the ^{13}C concentration of the pore-water DIC to a $\delta^{13}C$ value closer to the isotopic composition of Guaymas Basin oils (−23.9‰ to −22.3‰; Simoneit and Schoell, 1995). Hydrothermally-derived CO_2 (−9.4‰) entrained in the pore waters would not be as light isotopically, but still would contrast with the overlying bottom water (−0.6‰) and surface water (+1.8‰) of the Gulf of California (Table 1.). The extent to which bacterial biomass is formed by the autotro-

[1] Back-calculated from reported ages using $1/\lambda = 8033$ yr (Libby half-life).

phic fixation of DIC from isotopically-light pore waters can be estimated from the $\delta^{13}C$ values of the fatty acids.

The most negative $\delta^{13}C$ values measured for the individual fatty acids were those for *iso*- and *anteiso*-$C_{15:0}$ (−29.4‰ and −26.3‰), slightly depleted relative to the average value for all fatty acids (−24.3‰). In comparison, Pearson et al. (2001) measured $\delta^{13}C$ values for *iso*- and *anteiso*-$C_{15:0}$ fatty acids for a similar, bacterial mat-covered surface sediment from the Santa Monica Basin (−23.2‰ and −24.1‰, respectively). The average $\delta^{13}C$ value for all fatty acids in Santa Monica Basin was −24.6‰. These two systems are not different with respect to the average isotopic composition of their fatty acids, suggesting there is no preferential incorporation of ^{13}C-depleted pore-water DIC into biomass in the Guaymas Basin sample. Values near ~ −24‰ also are typical for the $\delta^{13}C$ values of total lipids of marine primary and secondary (heterotrophic) production as measured in water-column particulate matter (Wang et al., 1998), physically distant from any influence from pore-water DIC. Therefore, the $\delta^{13}C$ data measured here for fatty acids are not consistent with autotrophic fixation of CO_2 obtained from ^{13}C-depleted pore-water DIC. The most depleted values were measured here for the C_{15} fatty acids ($\delta^{13}C$ = −29.4, −26.3‰), reflecting the largest potential contribution from a pore-water source. Assuming that CO_2 derived from respired petroleum ($\delta^{13}C$ ~ −23‰) represents the pore-water (PW) endmember, that the other endmember is bottom water (BW) DIC, and that the isotopic fractionation between fatty acids and DIC is 24‰ ($\varepsilon_{\text{DIC-fatty acid}} = 24‰$),

$$\delta^{13}C_{C15} = f_{PW\_DIC}\left(\delta^{13}C_{petroleum\_CO2} - \varepsilon\right) + \left(1 - f_{PW\_DIC}\right)\left(\delta^{13}C_{BW\_DIC} - \varepsilon\right) \quad (2)$$

The microbial species producing C_{15} fatty acids could derive only 8%–21% of their biomass from autotrophic fixation of petroleum-derived CO_2, with the remaining 79%–92% coming directly from heterotrophy. In summary, the entire microbial consortium appears to be involved in the assimilation of pre-aged carbon, and the $\delta^{13}C$ values suggest that the primary metabolism appears to be heterotrophy.

3.3.3. Metabolism of filamentous sulfur bacteria

Chemolithotrophic growth of *Beggiatoa* first was suggested by Winogradsky (1887), but the metabolism of organic compounds in addition to autotrophy has been debated ever since. Here, the ^{14}C-depleted values obtained for lipids from the bacterial mat suggest the *Beggiatoa* and/or other species associated with the mat are heterotrophic. Hagen and Nelson (1996) previously investigated the potential for chemoheterotrophy in two strains of marine *Beggiatoa*, MS-81-6 and MS-81-1c. Strain MS-81-1c was dependent on fixation of CO_2 but was able to accumulate up to 20% of its cellular carbon from acetate. Strain MS-81-6 also was able to utilize propionate, oxaloacetate, pyruvate, lactate, fumarate, malate, and succinate in addition to CO_2 and acetate. Martens (1990) found large concentrations of acetate and propionate in Guaymas Basin sediments (0.66 and 14.8 mg/L), within the range of concentrations used in the experiments of Hagen and Nelson (1996). Heterotrophy, especially the metabolism of low molecular weight acids, may help support the *Beggiatoa* population in bacterial mats of Guaymas Basin.

Freshwater strains of *Beggiatoa* grow as chemoorganotrophs, oxidizing organic carbon for energy. They generally do not derive significant energy from the oxidation of reduced sulfur species (Strohl and Larkin, 1978; Nelson, 1992; Hagen and Nelson, 1997). Sulfide oxidation in marine systems, however, provides energy for chemolithotrophic growth of *Beggiatoa*, and the location of bacterial mats is well correlated with the presence of S^{2-}, active hydrocarbon seeps, and/or CH_4 at the seafloor (e.g., Gundersen et al., 1992; Larkin et al., 1994). Regardless of the inorganic or organic carbon source, the overall conclusion remains the same: if the $\Delta^{14}C$ values measured here reflect the carbon source to the *Beggiatoa*, this community derives a high percentage of its carbon (in addition to energy) from the flux of preaged material out of the sediments, rather than from the water column.

3.3.4. Evidence for a complex metabolic consortium

Biodiversity studies (Edgcomb et al., 2002; Dhillon et al., 2003) and our biomarker analyses demonstrate that not all of the biomass in the mat is derived from *Beggiatoa*. Sterols and *iso*- and *anteiso*- fatty acids indicate the presence of eukaryotes and other bacteria, respectively. McHatton et al. (1996) estimate that 99% of the total bacterial biovolume in Guaymas Basin mats is contributed by *Beggiatoa*, the largest and most visible species. However, fluorescence microscopy has shown previously that such bacterial filaments frequently are colonized by smaller rods and cocci (Fukui et al., 1999). A simple geometrical calculation suggests that the contribution of lipids from these smaller cells can be significant. The lipid concentrations of *Beggiatoa* and other cells should be proportional to the surface area ratio of open cylinders (analogous to infinitely long filaments) to spheres (small cocci). A cross-section of a cylinder with 50 μm radius and 1 μm length covered with sufficient 1-μm diameter cocci to produce a volume:volume ratio of 99:1 would equate to less than a monolayer of cells covering the *Beggiatoa* filament; and yet the surface area ratio of the cylinder to the cocci would be 40:60. In other words, sixty percent of the lipids extracted from a sample of bacterial mat actually could derive from non-filamentous, smaller bacteria such as aerobic heterotrophs, anaerobic heterotrophs such as denitrifying bacteria, or other sulfide oxidizers.

Rueter et al. (1994) demonstrated the oxidation of hydrocarbons in crude oil by sulfate-reducing bacteria using enrichment cultures obtained from Guaymas Basin sediments. Similar results were obtained by Marchand et al. (1994), indicating the petroleum is biodegradable. High rates of sulfate reduction also have been observed in Guaymas Basin sediments (Jorgensen et al., 1990; Elsgaard et al., 1994). The highest rates were observed in samples at 50 to 60 °C, consistent with sediment depths >2 cm. This suggests a system in which sulfate reducers could be important oxidizers of hydrocarbons; the resulting organic acids and S^{2-} may help fuel the activity of *Beggiatoa* and other facultative chemoorganotrophs. Our $\Delta^{14}C$ data are consistent with this scenario: the lowest $\Delta^{14}C$ value was observed for the C_{15} fatty acids (−418‰). These compounds are known products of δ-proteobacteria, including sulfate-reducers of the genus *Desulfovibrio* (Kaneda, 1991). Other metabolic

groups also are known to degrade petroleum in Guaymas Basin; this includes aerobic, heterotrophic organisms that degrade aromatic hydrocarbons (Goetz and Jannasch, 1993).

Therefore, based on the available data, it is very difficult to conclude what fraction of the biomass studied here is derived from filamentous sulfur bacteria. The chromatogram in Figure 3 shows that the C_{15} fatty acids, which are not major constituents of *Beggiatoa* (McCaffrey et al., 1989), are present as a significant fraction of the total compositional mixture. Future work employing the use of nucleic acids as natural isotopic "biomarker molecules" (MacGregor et al., 2002; VanMooy et al., 2004; Pearson et al., 2004); or the use of intact, phylogenetically distinct polar lipids (Sturt et al., 2004) may illuminate the roles of specific microbial groups or species and may allow quantitation of carbon fluxes between these groups.

In summary, the isotopic data for fatty acids can be interpreted as representative of the entire, active biologic community. This bacterial mat may be similar to other syntrophic mat systems that have highly complex chemical and energetic interactions (e.g., Hoehler et al., 2001). In this Guaymas Basin sample, the ^{14}C-isotopic mass balance suggests that 46%–100% of total microbial community carbon ultimately could be derived from petroleum; and 79%–92% of this consumption appears to be heterotrophic. The majority of all the fatty acid $\Delta^{14}C$ values are close in isotopic composition to the hydrothermal petroleum, indicating this is a benthic-supported microbial community that is not significantly dependent on phytoplanktonic detritus raining from surface waters. This appears to occur despite the abundant, and presumably labile, organic matter supplied from the productive surface ocean in this environment, although the lateral heterogeneity and relative importance of these processes remains under-investigated.

3.3.5. *Mobilization of preaged carbon into the active carbon cycle*

Two sterol fractions were purified from the *Beggiatoa* mat sample for ^{14}C analysis. Sterols are products primarily of eukaryotes (Volkman, 2003); in particular, the C_{29} sterols isolated here for $\Delta^{14}C$ measurement have never been found definitively in bacteria (Volkman 2003; Pearson et al., 2003). Therefore, these compounds must reflect a mixture of sources representing both direct inputs from phytoplanktonic detritus and in situ biosynthesis by Guaymas Basin heterotrophs such as foraminifera, radiolarians, and other protozoa (Edgcomb et al., 2002).

If the deep-water heterotrophs feed on both the decaying remains of fresh phytoplankton, as well as on the bacterial biomass within the *Beggiatoa* mat, the average $\Delta^{14}C$ value (−123‰) for the sterols produced in situ will reflect a simple mass balance of the two organic carbon sources. Using $\Delta^{14}C$ for the fresh, phytoplanktonic endmember (+50‰ to + 100‰) and the average $\Delta^{14}C$ value for carbon derived from bacterial biomass (C_{15}, $C_{16:1}$ and $C_{18:1}$ average = −383‰) in the mass balance equation:

$$\Delta^{14}C_{sterol} = f_{plankton}\,\Delta^{14}C_{DIC\_surfacewater} + \left(1 - f_{plankton}\right)\Delta^{14}C_{bacterial\_mat} \quad (3)$$

the percentage of the sterols derived from modern, phytoplanktonic carbon is 54%–60%, while the remaining 40%–46% could be derived from consumption of the "preaged" biomass from the bacterial mat.

The sterol data suggest that the metabolism within the mat community also includes transfer of re-worked petroleum carbon to higher trophic levels. Eventually this carbon is consumed by eukaryotes (sterol producers) living within the system, providing a source of preaged carbon up the food chain. The negative $\Delta^{14}C$ values measured here for sterols are more depleted than could be derived solely from the settling of phytoplanktonic detritus from overlying surface waters, which would have positive (postbomb) $\Delta^{14}C$ values. This is consistent with previous observations of the grazing of microbial mats by macroscopic eukaryotes (Stein, 1984) and with the presence of high eukaryotic microbial diversity in Guaymas Basin samples (Edgcomb et al., 2002).

This process represents a re-working of aged carbon in a "reverse" geological direction. Recalcitrant, buried sedimentary organic carbon is heated, transformed into petroleum, and transported by hydrothermal circulation. Mobilization of oil in hydrothermal fluids provides a carbon substrate to support the microbial mat population at the sediment-water interface. The resulting labile bacterial biomass becomes an organic-rich substrate for eukaryotes and other heterotrophic bacteria. The eventual death, decay and remineralization of this heterotrophic biomass may thus serve as an important means to mobilize radiocarbon-aged organic matter and return it to the water column in the form of preaged dissolved organic carbon (DOC). This has implications for a contribution of aged DOC to oceanic bottom waters in the form of biolabile compounds rather than as recalcitrant bio- or geopolymeric material.

4. SUMMARY AND CONCLUSIONS

In summary, the ^{14}C and ^{13}C isotope distributions in the deep Guaymas Basin at Rebecca's Roost suggest that the *Beggiatoa*-dominated bacterial mat sampled here participates in the remineralization of hydrothermal petroleum. This petroleum previously has been shown to have relatively modern $\Delta^{14}C$ values (Peter et al., 1991; Simoneit and Kvenvolden, 1994), and the total lipid extract we measured here has a similar age. These relatively modern values are distinct from the $\Delta^{14}C$ values that we measured for CO_2 and CH_4 venting in hot (317°C) hydrothermal fluids (formed in zones B and C, Fig. 2). This indicates that petroleum supplied to the sediment-water interface and to the bacterial mat (zone A, Fig. 2) does not derive directly from the hot hydrothermal fluid; these processes appear to be separated spatially.

The $\Delta^{14}C$ and $\delta^{13}C$ values for total lipids and for individual fatty acids in the *Beggiatoa* mat record the uptake of "preaged" carbon by biomass; this must occur either directly through the metabolism of petroleum-derived carbon, or indirectly, through the assimilation of ^{14}C-depleted CO_2; the compound-specific $\delta^{13}C$ values favor the former option. All of the bacterial fatty acid $\Delta^{14}C$ values we measured (compounds C_{15}, $C_{16:1}$, $C_{18:1}$) were within the range of values previously reported for hydrothermal petroleum, indicating that 100% of the carbon could derive directly from these oils. More conservatively, using the full range of $\Delta^{14}C$ values reported by Simoneit and Kvenvolden (1994), mass balance suggests that 46%–100% of the

carbon in the bacteria derives from the oil. Some of this carbon is transferred to eukaryotes that produce sterols. Together, this system appears to remobilize "preaged" carbon back into the benthic food chain.

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