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Fatty-acids and their $\delta^{13}C$ characteristics of seep carbonates from the northern continental slope of Gulf of Mexico

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Here we reported the fatty-acids and their $\delta^{13}C$ values in seep carbonates collected from Green Canyon lease block 185 (GC 185; Sample GC-F) at upper continental slope (water depth: ~540 m), and Alaminos Canyon lease block 645 (GC 645; Sample AC-E) at lower continental slope (water depth: ~2200 m) of the Gulf of Mexico. More than thirty kinds of fatty acids were detected in both samples. These fatty acids are maximized at C_{16}. There is a clear even-over-odd carbon number predominance in carbon number range. The fatty acids are mainly composed of *n*-fatty acids, *iso-/anteiso*-fatty acids and terminally branched odd-numbered fatty acids (*iso/anteiso*). The low $\delta^{13}C$ values (–39.99‰ to –32.36‰) of *n*-$C_{12:0}$, *n*-$C_{13:0}$, *i*-$C_{14:0}$ and *n*-$C_{14:0}$ suggest that they may relate to the chemosynthetic communities at seep sites. The unsaturated fatty acids *n*-$C_{18:2}$ and $C_{18:1}\triangle^{9}$ have the same $\delta^{13}C$ values, they may originate from the *Beggiatoa/Thioploca*. Unlike other fatty acids, the terminally branched fatty acids (*iso/anteiso*) show lower $\delta^{13}C$ values (as low as −63.95‰) suggesting a possible relationship to sulfate reducing bacteria, which is common during anaerobic oxidation of methane at seep sites.

fatty acids, carbon isotope of individual lipid, sulfate reducing bacteria, anaerobic oxidation of methane, seep carbonate, Gulf of Mexico

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The Gulf of Mexico (GOM) salt basin is a prolific petroleum province, the opening of the basin commenced in the Late Triassic-Middle Jurassic as a consequence of crustal rifting, thick salt was deposited in the basin during rifting, and ongoing salt deformation and active faults provide efficient conduits for fluid migration from the deep subsurface petroleum system into the shallow sediments, that controlled the cold seep occurrence [1−3]. Recent research indicated at least hundreds of ongoing fluid-gas seep systems at the full-depth range of the continental slope [4,5]. Authigenic carbonates, gas hydrates, and chemosynthetic communities are common at seep sites [4,6–12]. Carbonate formed at seep sites as a result of microbial oxidation of hydrocarbon (mainly methane). Methane is oxidized with sulfate as terminal electron acceptor during the anaerobic oxidation of methane (AOM). AOM is mediated by a syntrophic consortium of methanotrophic archaea and sulfate-reducing bacteria [13−17]. Thus, it is plausible that methane-oxidizing archaea and sulfate-reducing bacteria could be preserved as lipid biomarkers in seep carbonates as has been suggested.

The studies of gas hydrates, authigenic carbonates and

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chemosynthetic communities from the northern continental slope of the Gulf of Mexico can be found in numerous literatures [4,5,10,14,15,18–23]. However, few seep sites in deep water (greater than 2 km) had been visited and sampled in the past due to the cost of the work. In addition, detailed studies focused on the biomarkers of seep carbonates are not as abundant as these taking a pure inorganic approach. This paper focuses on the study of fatty acids and their δ^{13}C characteristics of seep carbonates collected from upper and lower continental slope of the GOM. The similarities and differences of the fatty acids and thire δ^{13}C features from the upper and lower continental slopes were then examined.

1 Sampling and analyses

1.1 Sampling

The seep carbonates were collected at the upper and the lower continental slope of GOM (Figure 1). Sample AC-E was collected from Alaminos Canyon lease block 645 (AC 645; 26°21′ N/94°31′ W) at the lower continental slope of GOM by manned submersible *Alvin* in 1990, where water depth is ~2200 m. This site displays abundant chemosynthetic communities composed of tubeworm clusters, mussel beds with scattered clams, and bacterial mats. Bioclasts and carbonate cements are the main component, and pores are also common in this sample (Figure 2). Former studies revealed that the carbonate is mainly composed of aragonite (up to 98 wt%) and shows low δ^{13}C values (–31.3‰—–23.4‰) [24]. Sample GC-F was collected in 1997 by manned submersible Johnson Sea Leak (JSL) from the Green Canyon 185 (GC 185, Bush Hill; 27°46'N; 91°30'W), where water depth is ~540 m (Figure 1), and the average bottom water temperature is ~7°C. Serpulid wormtubes, Lucinid-vesycomyidare calm shells are common in sample GC-F [4,22,25–27]. This sample mainly consists of aragonite (97.1 wt%), and their δ^{13}C values are –29.4‰—–15.1‰ [27].

1.2 Experiments

For lipid biomarkers analysis, carbonates were crushed into small pieces and dried, and then soxhlet was extracted for 72 h by a mixture of dichloromethane and methanol. Remaining carbonate pieces were dried and 10% HCl was slowly poured on to dissolve the remaining carbonate pieces. To avoid transesterification reactions, no HCl was added after 80% carbonate had been dissolved. Lipids were extracted repeatedly from HCl dissolved solution by dichloromethane, and then put together with the soxhlet-extracted organic matter. The organic extracts were separated by column chromatography into fractions: (1) hydrocarbons (*n*-hexane); (2) aromatic (*n*-hexane/dichloromethane, 6∶4; v∶v); and (3) polar frac tion (methanol) [19, 20,28–31].

The HCl-dissolved residue was freeze-dried, the residue and polar fraction were subsequently saponified in 6% KOH in methanol, lay 12 h and then organic matters were extracted by *n*-hexane. The extracts were separated by column chromatography into ketones (*n*-hexane/dichloromethane, 3∶1; v∶v) and alcohols (dichloromethane/ketone, 9∶1; v∶v). Getting fatty acids by stripping the remain solutions. Fatty acids methyl esters (FAMEs) were prepared from free fatty acids by subjecting the dried fatty acid fraction to saturated

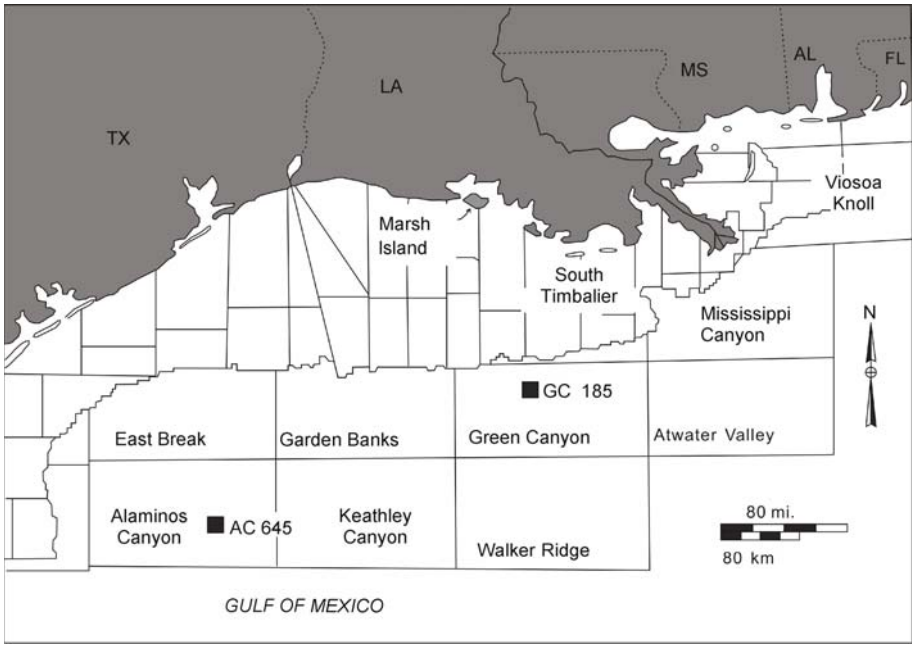

Figure 1 Location of the study sites (after Feng et al., 2009 [27]).

Figure 2 Morphology of seep carbonate samples. (a) Sample AC-E collected from an active cold seep of AC 645 on the lower continental slope of GOM, where water depth is ~2200 m; (b) sample GC-F collected from the Bush Hill gas seep and hydrate site of GC 185 on the upper continental slope of GOM, where water depth is ~540 m. All bar scale = 1 cm.

HCL-CH_3OH solution and heated at 80°C for 2 h, and then FAMEs were examined by GC-MS, GC/IRM.

1.3 Apparatus analyses

The gas chromatography and mass spectrometry analysis (GC-MS) were performed with HP 6890 series II Gas Chromatography interfaced with PlatformII mass selective detector using electron impact ion source (70 eV). The GC was equipped with a DB-5MS fused silica capillary column (30 m×0.25 mm i.d.×0.25 μm film thickness). The injector temperature of GC was 290°C and the oven temperature was initially held at 80°C for 5 min, and then programmed in a heating rate of 3°C/min to 290°C and maintained for 20 min. Helium was the carrier gas at a flow rate of 1 mL/min.

GC/IRMS analyses on fatty acids were performed on a British-made Isoprime Gas Chromatography-isotopic ratio mass spectrometry. JW-DB-5 fused silica capillary column (60 m×0.25 mm i.d.×0.25 μm film thickness) was used. The injector was set at sliptless mode and 290°C, helium was used as carrier gas. The oven temperature was initially set at 80°C and programmed to 290°C in a heating rate of 3°C/min. The isotopic error of parallel analysis is less than 0.5‰. Carbon isotope ratios are given as δ-values (δ^{13}C in ‰) relative to the V-PDB-standard and have been corrected for the addition of carbon during preparation of FAMEs [31,32].

2 Results

More than thirty kinds of fatty-acids were detected from samples AC-E and GC-F. These fatty acids are mainly composed of *n*-fatty acids and *iso-/anteiso*-fatty acids, which are dominated by low molecular weight fatty acids with a few numbers of high molecular weight fatty acids (Table 1, Figures 3 and 4).

The fatty acids in sample AC-E and sample GC-F mainly consist of C_{12} to C_{28} and C_{12} to C_{24} series compounds, respectively. Unsaturated fatty acids $C_{14:1}\triangle^7$, $C_{16:1}\triangle^7$, $C_{18:1}\triangle^9$, and $C_{18:2}$ were detected in both samples. These two compounds are maximized at C_{16} fatty acid, followed by $C_{18:1}\triangle^9$, *n*-$C_{14:0}$ to *n*-$C_{18:0}$ in sample AC-E, and *n*-$C_{14:0}$, *ai*-$C_{15:0}$ to *n*-$C_{18:0}$ in sample GC-F. In sample AC-E, the δ^{13}C values of the *n*-fatty acids are from −32.36‰ to −27.64‰, while the δ^{13}C values of the unsaturated fatty acids *n*-$C_{16:1}$ and *n*-$C_{18:1}$ are −19.97‰ and −25.48‰, respectively. In sample GC-F, the δ^{13}C values of *n*-fatty acids range from −26.52‰ to −39.99‰, while the δ^{13}C value of the unsaturated fatty acid $C_{18:1}$ is −31.04‰.

Terminal branched odd-number fatty acids (*iso*/*an*- *teiso*-$C_{15:0}$) were also detected in sample AC-E. These acids show the lowest δ^{13}C values from −63.95‰ to −50.48‰. The branched odd-number fatty acids *iso*/*anteiso*-$C_{13:0}$, -$C_{15:0}$ and -$C_{17:0}$ detected in sample GC-F have relatively high δ^{13}C values ranging from −48.62‰ to −44.17‰.

3 Discussion and conclusion

The ongoing salt deformation and active faults provide efficient conduits for fluid migration from the deep petroleum system into the shallow sediments or even sea water. The hydrocarbons migrated to the seafloor surface and support abundant chemosynthetic communities, e.g. tubeworm clusters and bivalve shells. Authigenic carbonates formed during the microbial activities, hence, specific fatty acids closely related to the seep communities should be preserved in these seep carbonates.

The fatty acids are mainly composed of low molecular weight fatty acids (<C_{20}) in seep carbonates samples from both sites. The δ^{13}C values of *n*-$C_{15:0}$, *i*-$C_{16:0}$, *n*-$C_{16:0}$, *n*-$C_{17:0}$, and *n*-$C_{18:0}$ are from −28.99‰ to −27.64‰ in sample AC-E, and from −31.11‰ to −30‰ in sample GC-F. The difference in δ^{13}C values between these fatty acids are within the

Table 1 Major fatty acids and their carbon isotopes of seep carbonates from the Gulf of Mexico

Number	Fatty acids	δ^{13}C (PDB ‰)	
		AC-E	GC-F
		—	—
1	*i*-$C_{12:0}$	—	–39.99
2	*n*-$C_{12:0}$	—	–48.62
3	*i*-$C_{13:0}$	—	–45.47
4	*ai*-$C_{13:0}$	—	–36.32
5	*n*-$C_{13:0}$	–36.6	–33.71
6	*i*-$C_{14:0}$	—	—
7	$C_{14:1}\triangle^7$	–32.36	–36.12
8	*n*-$C_{14:0}$	–63.95	–45.30
9	*i*-$C_{15:0}$	–50.48	–47.64
10	*ai*-$C_{15:0}$	–27.64	–30.00
11	*n*-$C_{15:0}$	–27.64	—
12	*i*-$C_{16:0}$	–19.97	—
13	$C_{16:1}\triangle^7$	–29.15	–30.61
14	*n*-$C_{16:0}$	—	–47.12
15	*i*-$C_{17:0}$	—	–44.17
16	*ai*-$C_{17:0}$	–27.85	–31.11
17	*n*-$C_{17:0}$	–25.48	–28.04
18	*n*-$C_{18:2}$	–25.48	–28.04
19	$C_{18:1}\triangle^9$	–28.99	–31.04
20	*n*-$C_{18:0}$	—	—
21	*n*-$C_{19:0}$	—	—
22	*n*-$C_{20:0}$	—	—
23	*n*-$C_{21:0}$	—	—
24	*n*-$C_{22:0}$	—	—
25	*n*-$C_{23:0}$	—	—
26	*n*-$C_{24:0}$	—	—
27	*n*-$C_{25:0}$	—	—
28	*n*-$C_{26:0}$	—	—
29	*n*-$C_{27:0}$	—	—
30	*n*-$C_{28:0}$	—	—

range of ±2.0‰ in the same sample, suggesting that these fatty acids are originated from bacteria or planktons in the same or similar ecological environments [32,33].

The δ^{13}C values of *iso*-saturated fatty acids *i*-$C_{14:0}$ and normal saturated fatty acids *n*-$C_{14:0}$ are from –36.6‰ to –32.36‰ in sample AC-E, while the δ^{13}C values of *iso*-saturated fatty acids *i*-$C_{14:0}$ and normal saturated fatty acids *n*-$C_{12:0}$, *n*-$C_{13:0}$ and *n*-$C_{14:0}$ range from –39.99‰ to –33.71‰ in sample GC-F. It is obvious that the δ^{13}C of these fatty acids are lower in both samples. The δ^{13}C values of soft tissues of mussels are –43.2%±4.1‰ [34], while the δ^{13}C values of soft tissues of tubeworm are –45.6%±5.2‰ [35] in seep environments of the northern continental slope of GOM. The δ^{13}C values of soft tissues of *Bathymodiolus childressi* are –38.9%±1.2‰ in seep site of GC 185 [36]. The δ^{13}C values of those megafaunas are much lower than the megafaunas in normal sea environments, suggesting that the energy of megafaunas is mainly from chemosynthetic communities (e.g. methanotrophs) at seep sites [34]. Recent research indicated that bivalves and tubeworms at seep sites are closely related to the microbes, and the different species of *bathymodiolus* mussels harbor either methanotrophic, thiotrophic, or both types of symbionts. Vestimentiferan tubeworms lack a digestive tract as adults and rely on internal, sulfide-oxidizing bacteria for their nutrition [37]. Thus, the *n*-$C_{12:0}$, *n*-$C_{13:0}$, *n*-$C_{14:0}$ and *i*-$C_{14:0}$ detected in our samples most probably originated from megafaunas at seep sites.

Unsaturated fatty acids $C_{14:1}\triangle^7$, $C_{16:1}\triangle^7$, $C_{18:1}\triangle^9$ and $C_{18:2}$ were also detected. The δ^{13}C values of *n*-$C_{18:2}$ and $_{18:1}\triangle^9$ is –28.04‰ in sample AC-E. The δ^{13}C values of *n*-$C_{18:2}$ and $C_{18:1}\triangle^9$ is –25.48‰ in sample GC-F. It is suggested that the origination and biosynthetic process of these fatty acids are similar [32]. The *n*-$C_{18:2}$ and $C_{18:1}\triangle^9$ are frequently detected in *Beggiatoa/Thioploca* [38], and these two compounds are also common in marine plankton especially in diatom [39]. Our carbonate samples were collected at seep sites and lack plankton fossils. Thus, the fatty acids *n*-$C_{18:2}$ and $C_{18:1}\triangle^9$ might be originated from *Beggiatoa/Thioploca*.

Unlike other fatty acids, the δ^{13}C value of unsaturated fatty acids $C_{16:1}\triangle^7$ is relatirely high (–19.97‰) in sample AC-E. However, it coincides with that of organic matter in modern sediments of middle-to-low latitude sea (–23.10‰ —–19.10‰) [32,40]. In addition, high content of $C_{16:1}\triangle^7$ is common in marine microalgae [41].

In addition to the above fatty acids, both samples contain terminally branched odd-numbered fatty acids *iso*/*an*-

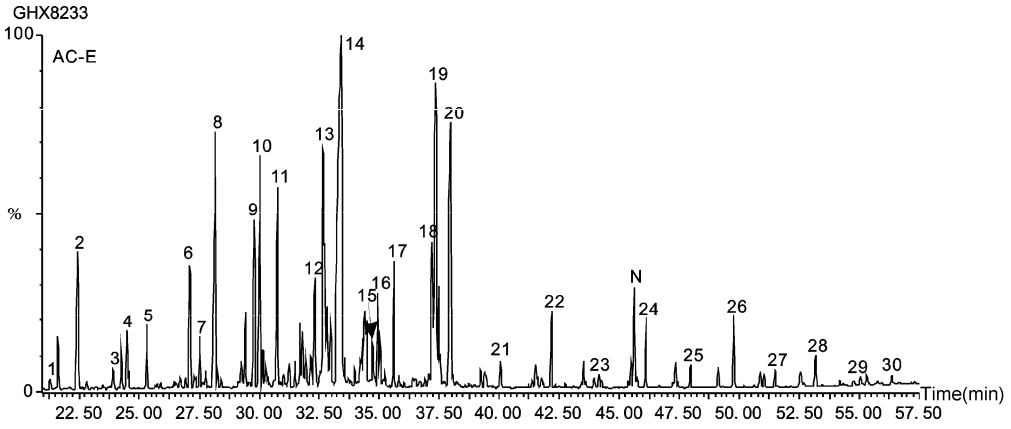

Figure 3 Major fatty acids and their carbon isotopic compositions in sample AC-E from AC 645 of the Gulf of Mexico. The numbers above each peak are represent the correspond fatty acids in Table 1. The "N" in Figure 3 denotes an unknown lipid.

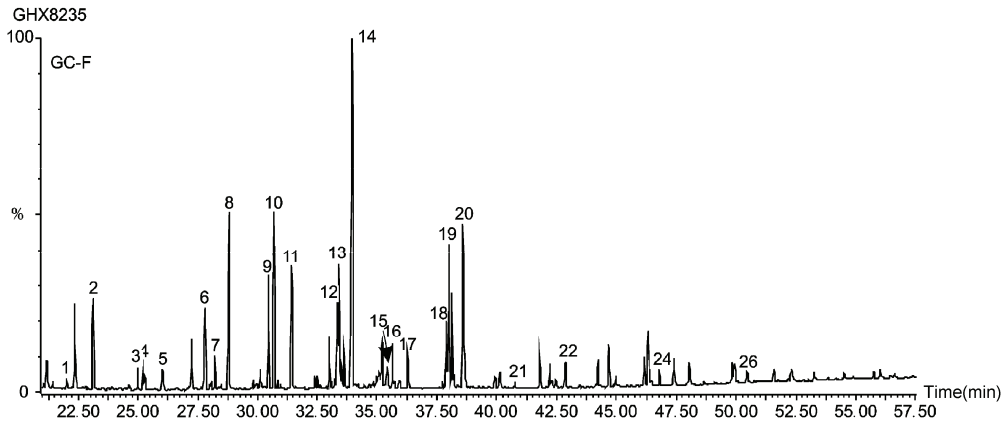

Figure 4 Major fatty acids and their carbon isotopic compositions in sample GC-F from GC 185 of the Gulf of Mexico. The numbers above each peak represent the corresponding fatty acids in Table 1.

teiso-$C_{13:0}$, -$C_{15:0}$ and -$C_{17:0}$. The $\delta^{13}C$ values of *i*-$C_{15:0}$ and *ai*-$C_{15:0}$ fatty acids show extremely depleted ^{13}C values ranging from −63.95‰ to −50.48‰ in sample AC-E, while the $\delta^{13}C$ values of *iso*/*anteiso*-$C_{13:0}$, -$C_{15:0}$ and -$C_{17:0}$ are from −48.62‰ to −44.17‰ in sample GC-F. The $\delta^{13}C$ values of terminally branched odd-numbered fatty acids are lower than any known carbon sources, e.g. seep carbonates (−31.3‰—−15.1‰), seep hydrocarbons (−28‰—−26‰) and seep methane (−44.1—−46.7‰) around the GC area [22,24,27], which suggested that isotope fractionation occurred during the formation of terminally branched odd-numbered fatty acids. Recent study suggested that ^{13}C depleted terminally branched odd-numbered fatty acids in marine sediments and bacterial mats are originated from sulfate reducing bacteria at seep sites [15,18,20,21,42–44]. Thus, these ^{13}C depleted terminally branched saturated fatty acids in both two samples are most probably originate from sulfate reducing bacteria during the process of the anaerobic oxidation of methane.

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