3-1-2010

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

Hong Xiang Guan

Guangzhou Institute of Geochemistry Chinese Academy of Sciences

Dong Feng

Guangzhou Institute of Geochemistry Chinese Academy of Sciences

Neng You Wu

Guangzhou Institute of Geochemistry Chinese Academy of Sciences

Duo Fu Chen

Guangzhou Institute of Geochemistry Chinese Academy of Sciences

H. H. Roberts

Louisiana State University

See next page for additional authors

Follow this and additional works at: https://digitalcommons.lsu.edu/geo_pubs

Recommended Citation

Guan, H., Feng, D., Wu, N., Chen, D., Roberts, H., & Wu, D. (2010). Fatty-acids and their $\delta^{13}\text{C}$ characteristics of seep carbonates from the northern continental slope of Gulf of Mexico. *Chinese Science Bulletin*, 55 (8), 730-735. <https://doi.org/10.1007/s11434-009-0478-7>

This Article is brought to you for free and open access by the Department of Geology and Geophysics at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.

Authors

Hong Xiang Guan, Dong Feng, Neng You Wu, Duo Fu Chen, H. H. Roberts, and Dai Dai Wu

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

GUAN HongXiang^{1,2,3}, FENG Dong¹, WU NengYou^{1,2}, CHEN DuoFu^{1,2*}, ROBERTS H H⁴ & WU DaiDai²

¹ CAS Key Laboratory of Marginal Sea Geology, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China;

² CAS Key Lab of Renewable Energy and Gas Hydrate, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, China;

³ Graduate University of Chinese Academy of Sciences, Beijing 100049, China;

⁴ Coastal Studies Institute, Louisiana State University, Baton Rouge, LA 70803, USA

Received April 9, 2009; accepted June 18, 2009; published online July 27, 2009

Here we reported the fatty-acids and their $\delta^{13}\text{C}$ 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 (*isolateiso*). The low $\delta^{13}\text{C}$ values (–39.99‰ to –32.36‰) of *n*- $\text{C}_{12:0}$, *n*- $\text{C}_{13:0}$, *i*- $\text{C}_{14:0}$ and *n*- $\text{C}_{14:0}$ suggest that they may relate to the chemosynthetic communities at seep sites. The unsaturated fatty acids *n*- $\text{C}_{18:2}$ and $\text{C}_{18:1}\Delta^9$ have the same $\delta^{13}\text{C}$ values, they may originate from the *Beggiatoa*/*Thioploca*. Unlike other fatty acids, the terminally branched fatty acids (*isolateiso*) show lower $\delta^{13}\text{C}$ 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

Citation: Guan H X, Feng D, Wu N Y, et al. Fatty-acids and their $\delta^{13}\text{C}$ characteristics of seep carbonates from the northern continental slope of Gulf of Mexico. Chinese Sci Bull, 2010, 55: 730–735, doi: 10.1007/s11434-009-0478-7

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]. Authi-

genic 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

* Corresponding author (email: cdf@gig.ac.cn)

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 $\delta^{13}\text{C}$ characteristics of seep carbonates collected from upper and lower continental slope of the GOM. The similarities and differences of the fatty acids and their $\delta^{13}\text{C}$ 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; $26^{\circ}21' \text{ N}/94^{\circ}31' \text{ W}$) at the lower continental slope of GOM by manned submersible *Alvin* in 1990, where water depth is $\sim 2200 \text{ m}$. 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 $\delta^{13}\text{C}$ 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; $27^{\circ}46' \text{ N}; 91^{\circ}30' \text{ W}$), where water depth is $\sim 540 \text{ m}$ (Figure 1), and the average bottom water temperature is $\sim 7^{\circ}\text{C}$. Serpulid wormtubes, Lucinid-vesicomyidare calm shells are common in sample GC-F [4,22,25–27]. This sample mainly consists of aragonite (97.1 wt%), and their $\delta^{13}\text{C}$ 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 fraction (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-CH₃OH 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 splitless 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 ($\delta^{13}\text{C}$ 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 C₁₂ to C₂₈ and C₁₂ to C₂₄ series compounds, respectively. Unsaturated fatty acids C_{14:1}Δ⁷, C_{16:1}Δ⁷, C_{18:1}Δ⁹, and C_{18:2} were detected in both samples. These two compounds are maximized at C₁₆ fatty acid, followed by C_{18:1}Δ⁹, *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 $\delta^{13}\text{C}$ values of the *n*-fatty acids are from -32.36‰ to -27.64‰, while the $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of *n*-fatty acids range from -26.52‰ to -39.99‰, while the $\delta^{13}\text{C}$ value of the unsaturated fatty acid C_{18:1} is -31.04‰.

Terminal branched odd-number fatty acids (*iso/an- tei- so*-C_{15:0}) were also detected in sample AC-E. These acids show the lowest $\delta^{13}\text{C}$ values from -63.95‰ to -50.48‰. The branched odd-number fatty acids *isolateiso*-C_{13:0}, -C_{15:0} and -C_{17:0} detected in sample GC-F have relatively high $\delta^{13}\text{C}$ 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 (<C₂₀) in seep carbonates samples from both sites. The $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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	$\delta^{13}\text{C}$ (PDB ‰)	
		AC-E	GC-F
1	<i>i</i> -C _{12:0}	—	—39.99
2	<i>n</i> -C _{12:0}	—	—48.62
3	<i>i</i> -C _{13:0}	—	—45.47
4	<i>ai</i> -C _{13:0}	—	—36.32
5	<i>n</i> -C _{13:0}	—36.6	—33.71
6	<i>i</i> -C _{14:0}	—	—
7	C _{14:1} Δ ⁷	—32.36	—36.12
8	<i>n</i> -C _{14:0}	—63.95	—45.30
9	<i>i</i> -C _{15:0}	—50.48	—47.64
10	<i>ai</i> -C _{15:0}	—27.64	—30.00
11	<i>n</i> -C _{15:0}	—27.64	—
12	<i>i</i> -C _{16:0}	—19.97	—
13	C _{16:1} Δ ⁷	—29.15	—30.61
14	<i>n</i> -C _{16:0}	—	—47.12
15	<i>i</i> -C _{17:0}	—	—44.17
16	<i>ai</i> -C _{17:0}	—27.85	—31.11
17	<i>n</i> -C _{17:0}	—25.48	—28.04
18	<i>n</i> -C _{18:2}	—25.48	—28.04
19	C _{18:1} Δ ⁹	—28.99	—31.04
20	<i>n</i> -C _{18:0}	—	—
21	<i>n</i> -C _{19:0}	—	—
22	<i>n</i> -C _{20:0}	—	—
23	<i>n</i> -C _{21:0}	—	—
24	<i>n</i> -C _{22:0}	—	—
25	<i>n</i> -C _{23:0}	—	—
26	<i>n</i> -C _{24:0}	—	—
27	<i>n</i> -C _{25:0}	—	—
28	<i>n</i> -C _{26:0}	—	—
29	<i>n</i> -C _{27:0}	—	—
30	<i>n</i> -C _{28:0}	—	—

range of $\pm 2.0\text{‰}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of

these fatty acids are lower in both samples. The $\delta^{13}\text{C}$ values of soft tissues of mussels are $-43.2\text{‰}\pm 4.1\text{‰}$ [34], while the $\delta^{13}\text{C}$ values of soft tissues of tubeworm are $-45.6\text{‰}\pm 5.2\text{‰}$ [35] in seep environments of the northern continental slope of GOM. The $\delta^{13}\text{C}$ values of soft tissues of *Bathymodiolus childressi* are $-38.9\text{‰}\pm 1.2\text{‰}$ in seep site of GC 185 [36]. The $\delta^{13}\text{C}$ 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}Δ⁷, C_{16:1}Δ⁷, C_{18:1}Δ⁹ and C_{18:2} were also detected. The $\delta^{13}\text{C}$ values of *n*-C_{18:2} and C_{18:1}Δ⁹ is -28.04‰ in sample AC-E. The $\delta^{13}\text{C}$ values of *n*-C_{18:2} and C_{18:1}Δ⁹ 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}Δ⁹ 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}Δ⁹ might be originated from *Beggiatoa/Thioploca*.

Unlike other fatty acids, the $\delta^{13}\text{C}$ value of unsaturated fatty acids C_{16:1}Δ⁷ is relatively 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‰ to -19.10‰) [32,40]. In addition, high content of C_{16:1}Δ⁷ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of *isolateiso*-C_{13:0}, -C_{15:0} and -C_{17:0} are from -48.62‰ to -44.17‰ in sample GC-F. The $\delta^{13}\text{C}$ 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.

We appreciate the constructive comments provided by the reviewers that improved the manuscript. The seep carbonates were obtained on the cruises sponsored by the Minerals Management Service (MMS). Profs. Xu Shiping and Jia Rongfen (Both GIGCAS) helped with the analyses of the samples. This is contribution No. IS-1084 from GIGCAS. This work was supported by the National Basic Research Program of China (Grant No. 2009CB219508), National Natural Science Foundation of China (Grant Nos. 40725011 and U0733003), the State Key Laboratory of Organic Geochemistry (Grant No. OGL-200811), and Knowledge Innovation Program of Chinese Academy of Sciences (Grant No. KGCX2-YW-805).

- 1 Weimer P, Rowan M G, McBride B C, et al. Evaluating the petroleum systems of the Northern Deep Gulf of Mexico through integrated basin analysis: An overview. AAPG Bull, 1998, 82: 865–877
- 2 Milkov A V, Sassen R. Estimate of gas hydrate resource, northwestern Gulf of Mexico continental slope. Mar Geol, 2001, 179: 71–83
- 3 Sassen R, Joye S, Sweet S T, et al. Thermogenic gas hydrates and hydrocarbon gases in complex chemosynthetic communities, Gulf of Mexico continental slope. Org Geochem, 1999, 30: 485–497

- 4 Roberts H H, Aharon P. Hydrocarbon-derived carbonate build ups of the northern Gulf of Mexico continental slope: a review of submersible investigations. Geo-Mar Lett, 1994, 14: 135–148
- 5 Roberts H H, Fisher C R, Bernard B, et al. ALVIN explores the deep northern Gulf of Mexico slope. EOS, 2007, 88: 341–342
- 6 Chen D F, Cathles L M, Roberts H H. The geochemical signatures of variable gas venting at gas hydrate sites. Mar Pet Geol, 2004, 21: 317–326
- 7 MacDonald I R, Boland G S, Baker J S, et al. Gulf of Mexico chemosynthetic communities II: Spatial distribution of seep organisms and hydrocarbons at Bush Hill. Mar Biol, 1989, 101: 235–247
- 8 Nelson D C, Fisher C R. Chemoautotrophic and methanotrophic endosymbiotic bacteria at deep-sea vents and seeps. In: Karl D M, ed. The Microbiology of Deep-sea Hydrothermal Vents. New York: CRC Press, 1995. 125–167
- 9 Zhang C L, Li Y, Wall J D, et al. Lipid and carbon isotopic evidence of methane-oxidizing and sulfate-reducing bacteria in association with gas hydrates from the Gulf of Mexico. Geology, 2002, 30: 239–242
- 10 Zhang C L, Pancost R D, Sassen R, et al. Archaeal lipid biomarkers and isotopic evidence of anaerobic methane oxidation associated with gas hydrates in the Gulf of Mexico. Org Geochem, 2003, 34: 827–836
- 11 Lanoil B D, Sassen R, LaDuc M T, et al. Bacteria and Archaea physically associated with Gulf of Mexico gas hydrates. Appl Environ Microb, 2001, 67: 5143–5153
- 12 Mills H J, Hodges C, Wilson K, et al. Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. FEMS Microbiol Ecol, 2003, 46: 39–52
- 13 Nauhaus K, Treude T, Boetius A, et al. Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. Environ Microbiol, 2005, 7: 98–106
- 14 Pancost R D, Chopmans E, Sinninghe J S. Archaeal lipids in Mediterranean Cold Seeps: Molecular proxies for anaerobic methane oxidation. Geochim Cosmochim Acta, 2001, 65: 1611–1627
- 15 Boetius A, Ravensschlag K, Schubert C J, et al. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature, 2000, 407: 623–626
- 16 Chen D F, Chen X P, Chen G Q. Geology and geochemistry of cold seepage and venting-related carbonates (in Chinese). Acta Sediment Sin, 2002, 20: 34–40
- 17 Chen Z, Yan W, Chen M H, et al. Discovery of seep carbonate nodules as new evidence for gas venting on the northern continental slope of South China Sea. Chinese Sci Bull, 2006, 51: 1228–1237
- 18 Hinrichs K U, Hayes J M, Sylva S P, et al. Methane-consuming archae bacteria in marine sediments. Nature, 1999, 398: 802–805
- 19 Birgel D, Thiel V, Hinrichs K U, et al. Lipid biomarker patterns of

- methane-seep microbialites from the Mesozoic convergent margin of California. *Org Geochem*, 2006, 37: 1289–1302
- 20 Pape T, Blumenberg M, Seifer R, et al. Lipid geochemistry of methane-seep-related Black Sea carbonates. *Palaeogeogr Palaeoclimatol Palaeoecol*, 2005, 227: 31–47
 - 21 Bouloubassi I, Aloisi G, Pancost R D, et al. Archaeal and bacterial lipids in authigenic carbonate crusts from eastern Mediterranean mud volcanoes. *Org Geochem*, 2006, 37: 484–500
 - 22 Sassen R, Roberts H H, Carney R, et al. Free hydrocarbon gas, gas hydrate, and authigenic minerals in chemosynthetic communities of the northern Gulf of Mexico continental slope: relation to microbial processes. *Chem Geol*, 2004, 205: 195–217
 - 23 Birgel D, Peckmann J. Aerobic methanotrophy at ancient marine methane seeps: A synthesis. *Organic Geochemistry. Org Geochem*, 2008, 39: 1659–1667
 - 24 Feng D, Chen D F, Qi L, et al. Petrographic and geochemical characterization of seep carbonate from Alaminos Canyon, Gulf of Mexico. *Chinese Sci Bull*, 2008, 53: 1716–1724
 - 25 MacDonald I R, Guinasso N L, Sassen R, et al. Gas hydrate that breaches the sea floor on the continental slope of the Gulf of Mexico. *Geology*, 1994, 22: 699–702
 - 26 Tryon M D, Brown K M. Fluid and chemical cycling at Bush Hill: Implications for gas and hydrate-rich environments. *Geochem Geophys Geosyst*, 2004, 5: 12004–12004
 - 27 Feng D, Chen D F, Roberts H H. Petrographic and geochemical characterization of seep carbonate from Bush Hill (GC 185) gas vent and hydrate site of the Gulf of Mexico. *Mar Pet Geol*, 2009, 26: 1190–1198
 - 28 Thiela V, Peckmann J, Oliver S, et al. A new straight-chain hydrocarbon biomarker associated with anaerobic methane cycling. *Org Geochem*, 2001, 32: 1019–1023
 - 29 Guan H X, Chen D F, Song Z G. Biomarkers and bacterial processes in the sediment at gas seep site (in Chinese). *Mar Geol Quat Geol*, 2007, 27: 75–83
 - 30 Song Z G, Wang C P. The biomarkers of 2,6,10,15,19-pentamethylcosenes and their carbon isotopic composition in the sediments from the Gulf of Mexico. *Chinese Sci Bull*, 2006, 51: 1736–1740
 - 31 Song Z G, Wang L, Liu J F, et al. Characterizing organic matter in marine sediments associated with gas hydrate and oil seepage from the Gulf of Mexico. *Geofluids*, 2008, 8: 293–300.
 - 32 Duan Y, Song J M, Zhang H. Carbon isotopic studies of individual lipids in organisms from the Nansha sea area, China. *Sci China Ser D-Earth Sci*, 2004, 47: 593–598
 - 33 Duan Y, Xu Y Q. Study on organic geochemistry of fatty acids in recent sediments from NanSa sea area (in Chinese), *Mar Geol Quat Geol*, 1996, 16: 23–31
 - 34 Aharon P. Carbon and oxygen isotope tracers of submarine hydrocarbon emissions: Northern Gulf of Mexico. *Isr J Earth Sci*, 1994, 43: 153–164
 - 35 Macavoy S E, Carney R S, Morgan E, et al. Stable isotope variation among the mussel *bathymodiolus childressi* and associated heterotrophic fauna at four cold-seep communities in the Gulf of Mexico. *J Shellfish Res*, 2008, 27: 147–151
 - 36 Paull C K, Chanton J P, Neumann A C, et al. Indicators of Methane-Derived Carbonates and Chemosynthetic Organic Carbon Deposits: Examples From the Florida Escarpment. *Palaos*, 1992, 7: 361–379
 - 37 Cordes E E, Bergquist D C, Fisher C R. Macro-ecology of Gulf of Mexico cold seeps. *Ann Rev Mar Sci*, 2009, 1: 143–168
 - 38 Zhang C L, Huang Z Y, Cantu J, et al. Lipid biomarkers and carbon isotope signatures of a Microbial (Beggiatoa) Mat associated with gas hydrates in the Gulf of Mexico. *Appl Environ Microbiol*, 2005, 71: 2106–2112
 - 39 Duan Y, Song J M, Cui M Z, et al. Organic geochemical studies of sinking particulate material in China sea area. *Sci China Ser D-Earth Sci*, 1998, 41: 208–214
 - 40 Hunt J M. The significance of carbon isotope variations in marine sediments. In: *Advances in Organic Geochemistry*. Oxford: Pergamon, 1970. 27–35
 - 41 Dong L H, You J T, Lin Q Q, et al. Comparison of Fatty Acids and Freshwater Composition in Marine microalgae (in Chinese). *J Tropical Subtropical Bot*, 2004, 12: 226–232
 - 42 Wang J S, Suess E. Indicators of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of gas hydrate-associated sediments. *Chin Sci Bull*, 2002, 47: 1659–1663
 - 43 Bart E, Dongen V, Helen M T, et al. Well preserved Palaeogene and Cretaceous biomarkers from the Kilwa area, Tanzania. *Org Geochem*, 2006, 37: 539–557
 - 44 Hopmans E C, Schouten S, Pancost R D, et al. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun Mass Spectrom*, 2000, 14: 585–589