



Original Paper

Carbon isotopes of asphaltene-occluded hydrocarbons: A proxy for bitumen vein source identification in northwest Sichuan Basin, China



Peng Fang^{a,b,*}, Jia Wu^{b,c,**}, Xue-Min Xu^a, Bin Shen^a, Wei-Lin Sun^a, Xiao Jin^d,
Feng Chen^e, Ning-Ning Zhong^b, Ming-Hui Zhou^f

^aNational Research Center for Geoanalysis, Beijing, 100037, China

^bCollege of Geoscience, China University of Petroleum (Beijing), Beijing, 102249, China

^cState Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, 510640, Guangdong, China

^dKey Laboratory of Coupling

O

A B S T R A C T

Article history:

Received 10 February 2025

Received in revised form

6 October 2025

Accepted 11 November 2025

Available online 20 November 2025

Edited by Xi Zhang and Jie Hao

Keywords:

Bitumen veins

Asphaltenes

Adsorbed/occluded hydrocarbons

Carbon isotopes

Organic matter source

Sichuan Basin

The Lower Cambrian bitumen veins in northwestern Sichuan Basin originated from Precambrian paleo-oil reservoirs, yet the severe alterations of bitumen and high maturity of potential source rocks challenge conventional oil-source correlation. In this study, we focus on analyzing the stability of carbon isotopic compositions in asphaltene-occluded hydrocarbons to reveal their preservation of original organic signatures. Asphaltenes from the solid bitumen were investigated through gold tube closed-system thermal simulation experiments (300–400 °C). Free saturated hydrocarbons in raw samples exhibit ¹³C-enrichment ($\delta^{13}\text{C} = -31.3$ ‰) due to biodegradation, while thermal simulation experiments reveal progressive ¹³C-depletion of free saturated hydrocarbons ($\Delta^{13}\text{C} = -4.7$ ‰) caused by kinetic isotope effects during thermal cracking. In contrast, asphaltene-occluded saturated hydrocarbons maintain exceptional ¹³C stability (-28.5 ± 0.2 ‰) across the same stages, demonstrating complete protection by macromolecular shielding, and adsorbed hydrocarbons display intermediate behavior with moderate ¹³C shifts, indicating partial shielding at asphaltene surfaces. Biomarker distributions (*C*₂₇-dominant steranes, *n*-alkane bimodality, and *n*-alk-(1)-enes) in occluded hydrocarbons probably preserve original signatures unaffected by secondary alterations. The combined ¹³C signature (within the -26 to -31 ‰ range characteristic of shallow-water Doushantuo Formation) and biomarker evidence (consistent with Precambrian eukaryotic algal contribution) in occluded hydrocarbons conclusively identify the Ediacaran Doushantuo Formation as the bitumen source. These results demonstrate that asphaltene nanoaggregates effectively shield occluded components from secondary alterations and thermal maturation to a certain extent, providing reliable proxies for Precambrian oil-source correlation in altered reservoirs.

© 2025 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

* Corresponding author.

** Corresponding author.

E-mail addresses: fangpeng@mail.cgs.gov.cn (P. Fang), jia.wu@cup.edu.cn (J. Wu).

Peer review under the responsibility of China University of Petroleum (Beijing).

1. Introduction

The outcropping of bitumen veins in the northwestern Sichuan Basin of China is thought to be derived from Precambrian paleo-oil reservoir, which is probably the critical evidence of primary oil and gas resources in the Meso-Neoproterozoic (Wang and Han, 2011). Accurately revealing the source of these bitumen veins is significant for understanding the enrichment of organic matter and oil and gas resource potential of Meso-Neoproterozoic strata in Sichuan Basin. However, the formation of bitumen veins through complex tectonic uplift and subsequent secondary alterations,

including biodegradation, water washing, and oxidation, has substantially modified the original geochemical signatures in the bitumen (Fang et al., 2021). Moreover, the high thermal maturity of regional source rocks (Liang et al., 2009) has caused convergence in conventional biomarker distributions (e.g. steranes and terpanes), rendering standard oil-source correlation methods ineffective. Consequently, correlating the bitumen with the potential source rock represents a significant challenge.

Numerous solid bitumen veins are exposed in the Kuangshanliang area, northwestern Sichuan Basin (Huang and Wang, 2008). Currently, the source of these bitumen veins remains controversial between two candidate formations: Ediacaran Doushantuo and Lower Cambrian Qiongzhusi formations. Wang and Han (2011) explained the main geological origin of the bitumen veins as a consequence of tectonic movements that disrupted paleo-oil reservoirs with a large amount of liquid petroleum. The presence of dolomitic and siliceous inclusions from the Precambrian period in the bitumen veins led to the conclusion that the black shale of the Doushantuo Formation was the source of the bitumen (Han et al., 2022; Wang and Han, 2011). Furthermore, the Re-Os isotope dating of solid bitumen indicated ages between 572 and 559 million years ago (Wang et al., 2016a), thereby supporting the hypothesis that the bitumen originated from the source rocks of the Doushantuo Formation. On the other hand, the analysis of biomarkers (e.g., sterane distributions) in the bitumen and the potential source rocks by catalytic hydrolysis indicates that the bitumen was derived from the Qiongzhusi Formation (Fang et al., 2022c). Nevertheless, the composition of the substances released by catalytic hydrolysis may not be representative of the original one due to alterations of the biomarker distributions at high temperatures (Fang et al., 2022a). Moreover, the generally low signal-to-noise ratio of compounds in biomarker chromatograms of bitumens or seep oils subjected to catalytic hydrolysis (Fang et al., 2022c) also impairs the reliability of biomarker interpretation. As a result, the source of bitumen remains an open question.

Essentially, identifying the comparable original geochemical information in both bitumen and its potential source rock simultaneously is key to resolving the dispute. It has been found that the carbon isotope values (^{13}C) of kerogen in the bitumen change ($\pm 0.3\text{‰}$) during the hydrolysis process.

The carbon isotope values (^{13}C) of the two potential source rocks (Doushantuo and Qiongzhusi formations) are likely to retain their original characteristics. However, surface oxidation can significantly alter the carbon isotopes of bitumen (Liang et al., 2020), thereby precluding the direct tracing of oil sources from the isotopic composition of bitumen. Encouragingly, it has been found that the macromolecular structures of asphaltenes can trap (adsorb or occlude) some native small molecules, protecting them from biodegradation and thermal alteration (Cheng et al., 2016, 2017; Fang et al., 2022a; Kashirtsev, 2018; Liao et al., 2006b; Snowdon et al., 2016; Zhao

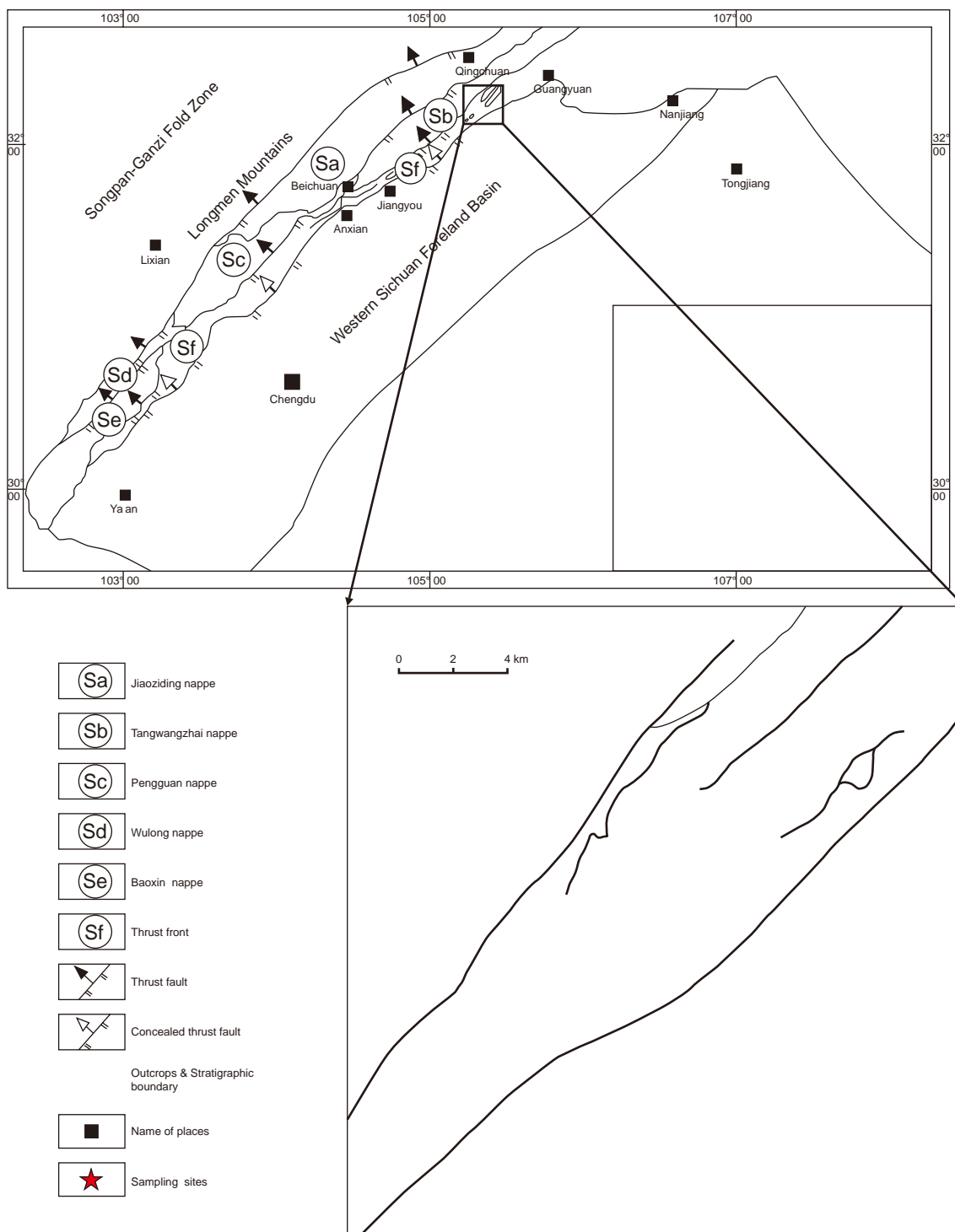


Fig. 1. Tectonic sketch map of the Kuangshanliang area of the Sichuan Basin (modified after Wu et al., 2012).

concentrated to isolate the asphaltene-adsorbed maltenes. At the same time, the purified asphaltenes were collected.

2.2.2. Thermal maturation experiments

Gold-tube experiments were conducted in an ST-120-II apparatus under rigorously controlled conditions (Landais et al., 1989; Michels and Landais, 1994; Monthieux et al., 1985; Wu et al., 2020). The length, the inner diameter, and the wall thickness of the gold tube were 60, 5.5, and 0.25 mm, respectively. Each tube

was first heated to approximately 800 °C with a butane-air torch for 3 min to remove all residual organic materials on its wall, then welded on one end using a PUK U4 microscope argonarc welder (Wu et al., 2022). Each gold tube was filled with approximately 30 mg of asphaltenes and 100 mg of deionized water. The other end of gold tube was weld after the sample was loaded. The thermal maturation experiments were held at temperatures of 300, 350 and 400 °C (±1 °C precision) under a constant pressure of 30 MPa (±0.1 MPa precision). The application of confining pressure

in the range of 20–50 MPa is a standard practice in pyrolysis studies to simulate the confined geo-pressure conditions typical of the oil window (Li et al., 2022; Michels and Landais, 1994; Wu et al., 2021). For the purpose of this study, which is to investigate the fundamental behavior and relative stability of different hydrocarbon occurrences (free, asphaltene-adsorbed/occluded) under controlled, geologically realistic conditions, this established experimental parameter is robust and appropriate. All reactors were heated to the target temperature within 1 h, and held at this temperature for 24 h. The corresponding calculated equivalent vitrinite reflectance values (using the Easy% R_o calculation scheme) (Sweeney and Burnham, 1990) for 300, 350 and 400 °C were 0.64%, 0.92% and 1.49%, respectively. These Easy% R_o values simulate maturation stages spanning the early oil window ($R_o = 0.6\%–1.0\%$) to the peak oil window ($R_o = 1.0\%–1.5\%$).

The solid bitumens in the Kuangshanliang area originated from paleo-reservoir destruction (Wang et al., 2014). Despite the high-over maturity of marine source rocks in the Sichuan Basin Palaeozoic and older strata (Liang et al., 2009), these bitumens were isolated from the source rock thermal regime during reservoir leakage (Wang et al., 2014), preserving a lower thermal maturity (equivalent $R_o \sim 0.6\%$) (Liang et al., 2020). In addition, the 300–400 °C range is suitable to simulate hydrocarbon generation from low-maturity bitumen, avoiding unrealistic over cracking (450 °C), such as aromatic ring cracking (Lorant and Behar, 2002). Therefore, the thermal simulation of the sample with the set maturity range conforms to the general law of its natural evolution, and is also the forward modeling of the possible process in the study area.

2.2.3. Post-treatment of the product

The reactors were allowed to cool down at the end of the experiment, and the gold tubes were removed for subsequent analysis. After opening the gold tubes, the product was extracted with petroleum ether to collect the free maltenes. Then the

asphaltenes were extracted with DCM. Subsequently, the asphaltene-adsorbed maltenes were isolated by the above purification treatment (Wu et al., 2020). The occluded hydrocarbons within purified asphaltenes were isolated using a nondestructive dispersive solid-phase extraction (DSPE) method, following an optimized protocol from our prior study (Fang et al., 2022b). The detailed step-by-step DSPE procedure is described in the Supplementary Materials. In brief, the method involves dispersing asphaltene clusters in dilute DCM and utilizing silica gel to physically adsorb polar macromolecules and thus separate the occluded maltenes without chemical alteration (Fig. 2). This method was chosen over oxidative degradation or pyrolysis due to its ability to effectively release occluded hydrocarbons while avoiding the introduction of oxidative damage or thermal cracking effects (Fang et al., 2022b; Snowdon et al., 2016).

The maltenes (both from the raw sample and the post-pyrolysis product) in the three different fractions (free, asphaltene-adsorbed, occluded) were separated into compound groups by liquid–solid chromatography using a silica gel–alumina column. The saturated hydrocarbons were eluted with 30 mL petroleum ether. A mixture (20 mL) of DCM/petroleum ether (volume ratio 2/1) was used to elute the aromatic hydrocarbons. Finally, the polar components (resins) were eluted with 20 mL of a DCM/methanol mixture (volume ratio 9/1) (Zhu et al., 2022). The detailed experimental flow chart is shown in Fig. 3.

2.3. Analysis

2.3.1. Isotopic analysis of organic carbon

The ^{13}C analysis of fractions was conducted using a gas chromatography–isotope ratio mass spectrometry (FLASH HT EAMAT 253 IRMS) system following the Chinese petroleum industry standard SY/T 5238–2019 (Analysis method for carbon and oxygen isotope of organic matter and carbonate), with all values

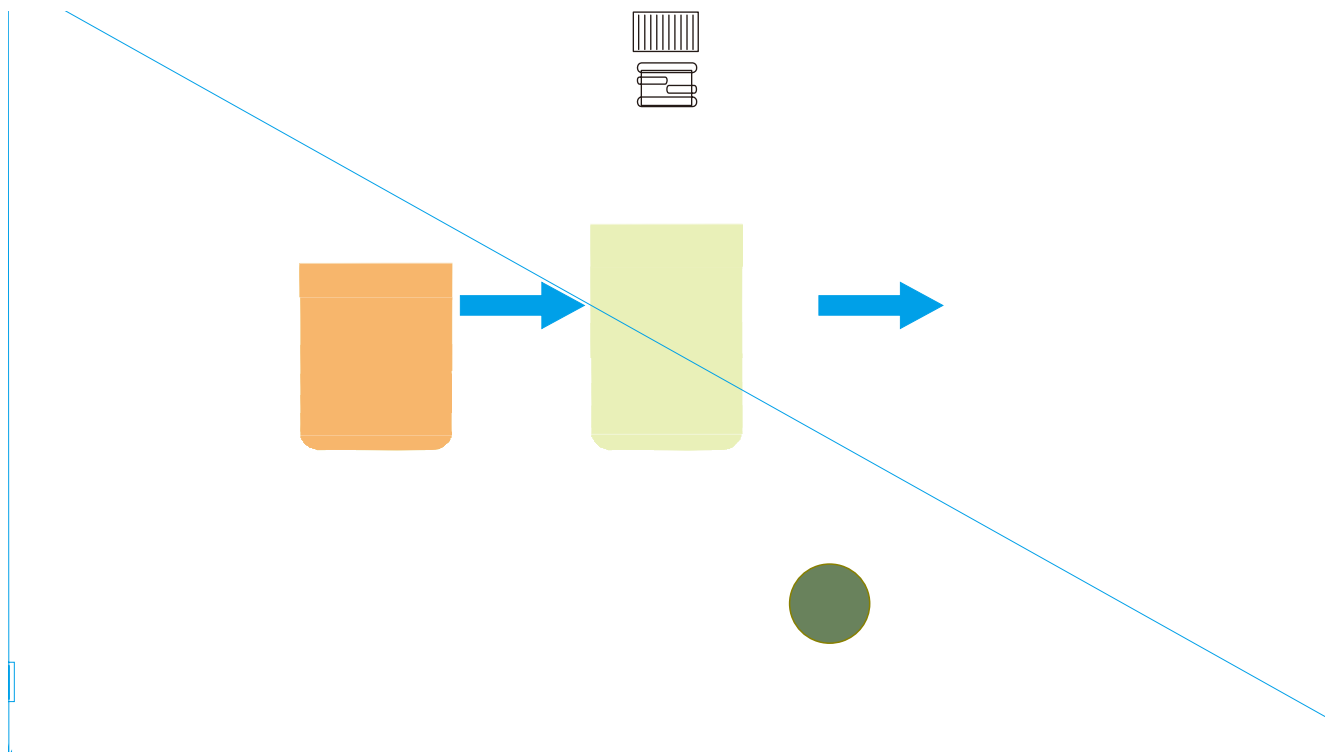


Fig. 2. Schematic diagram of dispersive solid-phase extraction method.

reported in per mil (‰) relative to the Vienna PDB standard. The analytical conditions were optimized to ensure complete combustion and precise measurement. Helium was used as the carrier gas with a flow rate of 100 mL/min. High purity oxygen with flow rate of 250 mL/min was used as combustion improver. The oxidation reactor was filled with chromium oxide, copper, and silver-containing cobalt oxide and run at a temperature of 980 °C. Standard samples are used for daily calibration of the instrument, including GBW04408 ($^{13}\text{C} = -36.9$ ‰), IAEA-600 Caffeine ($^{13}\text{C} = -27.771$ ‰), USGS62 Caffeine ($^{13}\text{C} = -14.79$ ‰), and USGS24 Graphite ($^{13}\text{C} = -16.05$ ‰). Standard samples were analyzed in parallel with samples to validate accuracy, and laboratory crude oil internal standard ($^{13}\text{C} = -27$ ‰)

invariance (-28.5 ± 0.2 ‰), demonstrating asphaltene protection of original signatures. (3) Biomarker parameters: based on the reanalysis of biomarker data, a series of potential biomarker parameters that can be used for oil source correlation are re-counted (Table 3). The variation law of most parameters with temperature is the same as that of ^{13}C value, showing the characteristics of stable occluded hydrocarbon parameters and obvious fluctuation of free/adsorbed hydrocarbon parameters (Table 3).

4. Discussion

4.1. Heterogeneous ^{13}C distributions of different components from the bitumen

In contrast to isotopic trends for components in crude oil (Stahl, 1978), the ^{13}C value of free saturated hydrocarbons separated from the original sample was significantly higher (enriched in ^{13}C) than that of aromatic and polar components (Fig. 6). In general, with increasing polarity, crude oil components are more enriched in ^{13}C , possibly resulting in the ^{13}C value of polar components being 3–4 ‰ higher than that of non-polar components (Stahl, 1977). However, different geochemical histories of crude oil may result in heterogeneous isotope distributions (Chung et al., 1981). Biodegradation of non-polar hydrocarbons in crude oil preferentially consumes isotopically lighter species without significantly

changing the carbon isotopic composition of the aromatic components (Stahl, 1980). This leads to relative enrichment of ^{13}C in the residual saturated hydrocarbons and relative enrichment of ^{12}C in the resulting asphaltenes in biodegradation-associated bitumen. Previous biomarker analyses of the solid bitumen samples of the study area clearly indicated that the solid bitumen has undergone severe biodegradation (Fang et al., 2021, 2022c; Wang et al., 2014). The pronounced UCM in the free hydrocarbon TIC and the characteristic biomarker patterns (e.g., depleted *n*-alkanes; short-chain sterane dominance) further confirm moderate-to-severe biodegradation in the raw sample (Fig. 4). Therefore, it is probable that biodegradation caused the free saturated hydrocarbons from solid bitumen to be enriched in ^{13}C .

The adsorbed and occluded saturated hydrocarbons that extracted from

carbon isotopic compositions of free hydrocarbons, i.e., those related to the thermal maturation of organic matter, are also affected by biodegradation. It can thus be concluded that the carbon isotopic composition of asphaltene-occluded hydrocarbons is not a result of biodegradation. Rather, it may be related to the original source of organic matter.

During the diagenetic process, organic matter undergoes complex biogeochemical reactions, leading to the formation of kerogen (Bushnev and Burdel'Naya, 2009). In this process, certain non-thermogenic, original saturated hydrocarbons (biomarkers) derived from organisms become occluded within the macromolecular structure of kerogen. When kerogen undergoes thermal degradation to generate substantial amounts of asphaltene, these occluded saturated hydrocarbons are subsequently inherited into the asphaltene structure (Cheng et al., 2017). Therefore, the asphaltene-occluded saturated hydrocarbons may retain the biogeochemical influences experienced during diagenesis. Nevertheless, since the precursor kerogen also underwent the same biogeochemical processes during its formation, the carbon isotopic compositions of the occluded saturated hydrocarbons and kerogen should theoretically remain comparable. However, the aromatic and polar compounds occluded in asphaltene do not possess direct biological relevance. These components are more likely derived from the thermal degradation of kerogen and subsequently occluded into the macromolecular structure alongside original saturated hydrocarbons in localized regions of the kerogen matrix. This results in the carbon isotopic compositions of the occluded aromatics and polar compounds being influenced by primary thermal cracking, leading to lower ^{13}C values (Fig. 6) due to kinetic fractionation effects. It can be inferred that although different hydrocarbon components occluded in asphaltene structure are protected during late-stage thermal evolution, only the occluded saturated hydrocarbons can maximally preserve the original characteristics of the organic matter.

It is expected that differences in $\delta^{13}\text{C}$ values of asphaltene-occluded hydrocarbons and kerogen are related to the original source of organic matter.

n-alkane series

Fig. 5. Distributions of biomarkers in asphaltene thermal simulation products (Thev Bcu a(YPq ua p h:Y",

Table 3
Selected biomarker parameters of the free and asphaltene-adsorbed/occluded fractions.

Parameter	State	Raw	300 °C	350 °C	400 °C	Range Min ~ Max	Δ (Max–Min)	¹³ C correlation
Tri/Pentacyclic terpane	Free	1.24	0.52	0.83	1.40	0.52–1.40	0.88	Strong
	Adsorbed	0.91	0.49	0.39	0.42	0.39–0.91	0.52	
	Occluded	0.69	0.54	0.57	0.54	0.54–0.69	0.15	
Ts/Tm	Free	0.45	0.27	0.05	0.06	0.05–0.45	0.40	Strong
	Adsorbed	1.08	0.65	0.61	0.54	0.54–1.08	0.54	
	Occluded	1.01	0.89	0.90	0.79	0.79–1.01	0.22	
C ₂₉ Ts/C ₂₉ H	Free	0.25	0.13	0.06	0.33	0.06–0.33	0.27	Strong
	Adsorbed	0.27	0.29	0.22	0.23	0.22–0.29	0.07	
	Occluded	0.32	0.37	0.37	0.33	0.32–0.37	0.05	
C ₃₁ H 22S/(22S + 22R)	Free	0.57	0.55	0.53	0.52	0.52–0.57	0.05	Weak
	Adsorbed	0.54	0.56	0.54	0.55	0.54–0.56	0.02	
	Occluded	0.54	0.56	0.54	0.54	0.54–0.56	0.02	
C ₂₁ P/C ₂₂ P	Free	2.30	1.49	2.25	3.78	1.49–3.78	2.29	Strong
	Adsorbed	1.52	1.57	1.78	2.85	1.52–2.85	1.33	
	Occluded	1.37	1.39	1.58	1.72	1.37–1.72	0.35	
C ₂₁ ~ C ₂₂ P/C ₂₇ ~ C ₂₉ S	Free	1.14	0.13	0.20	0.48	0.13–1.14	1.01	Strong
	Adsorbed	0.06	0.07	0.08	0.12	0.06–0.12	0.06	
	Occluded	0.08	0.05	0.08	0.06	0.05–0.08	0.03	
C ₂₇ S/C ₂₇ ~ C ₂₉ S	Free	0.22	0.27	0.20	0.24	0.20–0.27	0.07	Weak
	Adsorbed	0.37	0.32	0.29	0.33	0.29–0.37	0.08	
	Occluded	0.32	0.36	0.31	0.34	0.31–0.36	0.05	
C ₂₈ S/C ₂₇ ~ C ₂₉ S	Free	0.21	0.19	0.18	0.23	0.18–0.23	0.05	Weak
	Adsorbed	0.30	0.29	0.28	0.29	0.28–0.30	0.02	
	Occluded	0.34	0.26	0.28	0.30	0.26–0.34	0.08	
C ₂₉ S/C ₂₇ ~ C ₂₉ S	Free	0.57	0.54	0.62	0.52	0.52–0.62	0.10	Weak
	Adsorbed	0.33	0.39	0.43	0.38	0.33–0.43	0.10	
	Occluded	0.34	0.38	0.41	0.36	0.34–0.41	0.07	
C ₂₉ S $\delta\delta\delta$ 20S/(20S + 20R)	Free	0.54	0.34	0.46	0.58	0.34–0.58	0.24	Moderate
	Adsorbed	0.45	0.46	0.46	0.47	0.45–0.47	0.02	
	Occluded	0.48	0.46	0.47	0.49	0.46–0.49	0.03	
C ₂₉ S $\phi\beta\beta$ ($\delta\delta\delta + \phi\beta\beta$)	Free	0.53	0.43	0.50	0.55	0.43–0.55	0.12	Moderate
	Adsorbed	0.38	0.40	0.42	0.41	0.38–0.42	0.04	
	Occluded	0.42	0.40	0.42	0.45	0.40–0.45	0.05	

Notes: “Raw” refers to the sample that have not been subjected to thermal simulation experiments. “¹³C Correlation” refers to the degree of consistency with the change of ¹³C values (Table 1). The original biomarker data comes from our previous study (Fang et al., 2022a), where the specific meaning of parameter abbreviation can be found. Δ = Maximum value–minimum value across all samples (raw and thermal simulation temperatures of 300–400 °C), representing the total fluctuation range of parameters.

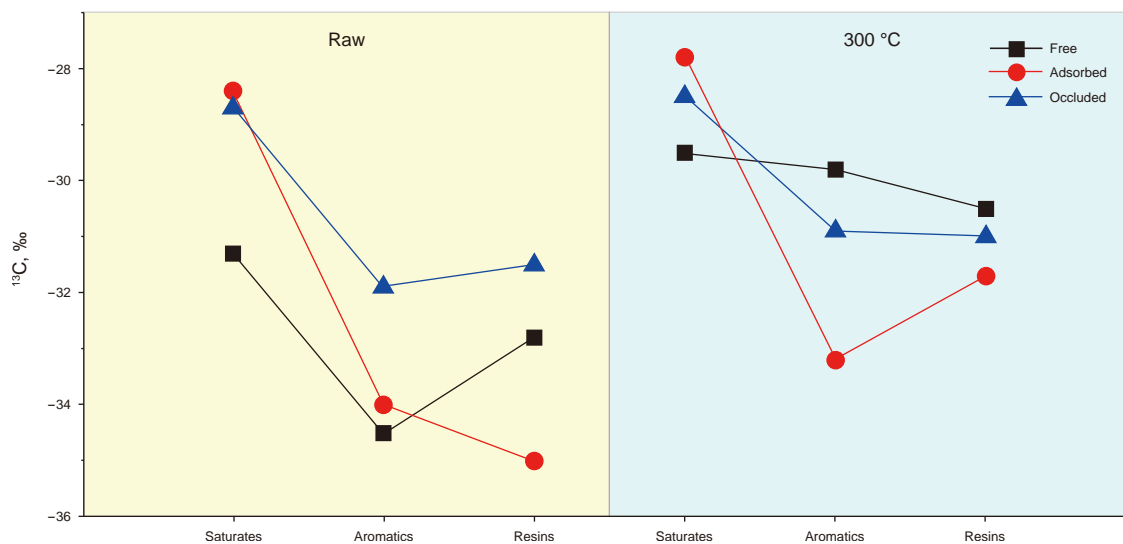


Fig. 6. Carbon isotopic compositions of free and asphaltene-adsorbed/occluded compound classes from DCM extracts of solid bitumen veins in the NW Sichuan Basin. “Raw” denotes the extracts from the original bitumen sample and “300 °C” refers to the extracts after gold tube pyrolysis of the isolated and purified asphaltenes at 300 °C for 24 h.

4.2. Maturation effect on ¹³C values of hydrocarbons in asphaltene structure

The ¹³C values of free/adsorbed saturated and aromatic hydrocarbons exhibited a decrease with increasing thermal stress, yet remained higher (enriched in ¹³C) than the ¹³C values of

parent asphaltenes (Fig. 7). A number of thermal simulation studies have demonstrated that during reactant cracking, the ¹³C value of hydrocarbon products undergoes a decrease followed by an increase (Galimov, 2006; Hill et al., 2003; Lorant et al., 1998; Tocqué et al., 2005). Actually, the evolution of ¹³C values with increasing thermal stress reflects a transition between two

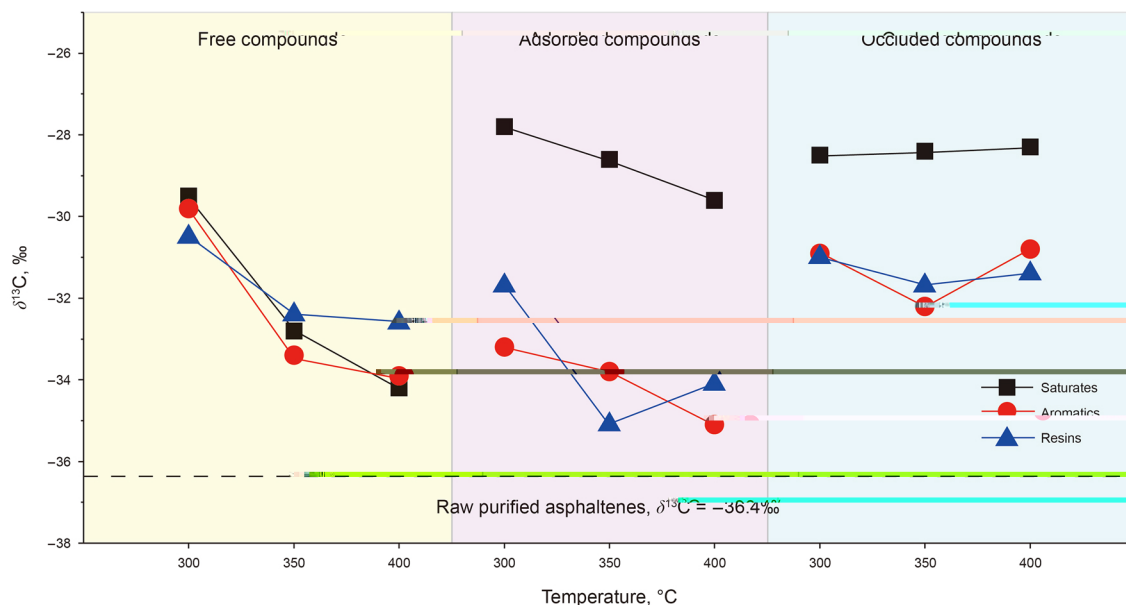


Fig. 7. Evolution of the carbon isotopic composition of compound classes during thermal maturation of purified asphaltenes in gold tubes for 24 h.

dominant mechanisms: the low-temperature release of pre-existing “residual carbon” and high-temperature cracking of the asphaltene matrix. At 300 °C, the observed enrichment in ^{13}C of the released hydrocarbons (the “residual carbon release effect”) is interpreted primarily as a thermal desorption process (Galimov, 2006). This stage involves the mobilization and release of ^{13}C -enriched saturated hydrocarbons that were previously trapped within the asphaltene structure as a result of biodegradation, without significant thermal cracking of covalent bonds. As temperature increases to 350 °C and 400 °C, true thermal cracking becomes the dominant process. Here, the systematic decrease in ^{13}C values for free and adsorbed hydrocarbons is driven by kinetic isotope effects (KIE) (Tocqué et al., 2005), wherein ^{12}C - ^{12}C bonds within the asphaltene macromolecules are preferentially cleaved over ^{13}C -containing bonds due to their lower activation energy, leading to the generation of ^{12}C -enriched products. The potential inflection points in ^{13}C at very high maturity, as suggested by the trend in adsorbed resins (Fig. 7), would mark the depletion of readily cleavable ^{12}C - ^{12}C bonds, causing the residual pool to become progressively enriched in ^{13}C .

The yields of free maltenes increase with temperature, while the yields of asphaltene change inversely. Asphaltene-occluded saturated hydrocarbons exhibit significantly higher yields (0.7%–1.3%) compared to adsorbed saturated hydrocarbons (0.3%) across all experimental temperatures (Table 2). This systematic trend supports the independence of occluded hydrocarbons. The consistently higher yields of occluded saturated hydrocarbons (even at 400 °C) reflect their protection within asphaltene nanoaggregates, which limits thermal cracking. In contrast, adsorbed saturated hydrocarbons (0.3%) are more susceptible to degradation due to their exposed location on asphaltene surfaces. This aligns with prior studies showing that occluded biomarkers resist thermal alteration better than adsorbed/free counterparts (Cheng et al., 2017). In addition, the low yields of adsorbed saturated hydrocarbons confirm minimal contamination from residual free hydrocarbons during extraction. This underscores the reliability of dispersive solid-phase extraction (DSPE) protocol, which selectively isolates occluded components on the premise of excluding adsorbed components.

The carbon isotopic compositions of the occluded hydrocarbons derived from pyrolysis products were found to be similar to that of the original occluded components, with saturated hydrocarbons displaying a stable ^{13}C value of around -28.5 (± 0.2). Mild oxidation degradation has systematically confirmed that hydrocarbons occluded by macromolecules (kerogen or asphaltene) are shielded from secondary alterations (e.g., biodegradation) due to the protective effect of macromolecule structure (Cheng et al., 2017; Liao et al., 2006a; Yang et al., 2009). Thermal simulation experiments in semi-closed systems revealed that biomarker parameters (e.g., sterane/hopane parameters) of kerogen-occluded hydrocarbons remained stable across maturity stages ($R_0 = 0.49\%$ – 1.67%), unlike free hydrocarbons with obvious thermal evolution (Cheng et al., 2016). In addition, our prior gold-tube experiments showed that saturated and aromatic biomarkers in asphaltene-occluded hydrocarbons exhibited minimal compositional changes at 300–400 °C, supporting their thermal stability (Fang et al., 2022a, 2024). While ^{13}C values of hydrocarbons in different occurrence states (free, asphaltene-adsorbed/occluded) were not explicitly reported in earlier studies, the variation law of distribution characteristics of biomarkers in different occurrence states is consistent with that of ^{13}C values (Fig. 5), suggesting isotopic stability of occluded hydrocarbons.

The strong correlation between occluded biomarker stability and ^{13}C invariance is mechanistically explained by the hierarchical protection effects of asphaltene. T_s/T_m and $C_{31} 22S/(22S + 22R)$ ratios showed non-ideal maturity trends (Table 3), likely due to sedimentary-organic facies effects (T_s/T_m) and pre-existing equilibrium ($C_{31} 22S/(22S + 22R)$ ratio), consistent with their known limitations in mature systems (Peters et al., 2004; Wang et al., 2000). The multistage aggregation structure of asphaltenes (Evdokimov and Fesan, 2016; Mullins, 2011) restricts thermal diffusion of occluded compounds (e.g., $\Delta\text{Tri}/\text{Pentacyclic terpane} = 0.15$; $\Delta C_{21}\text{-}C_{22}\text{P}/C_{27}\text{-}C_{29}\text{S} = 0.03$; Table 3), while free hydrocarbons undergo significant thermal cracking (e.g., $\Delta\text{Tri}/\text{Pentacyclic terpane} = 0.88$; $\Delta C_{21}\text{-}C_{22}\text{P}/C_{27}\text{-}C_{29}\text{S} = 1.01$; Table 3). This aligns with our observation that n -(1)-alkenes persist in occluded fractions even at 400 °C (Fig. 5), whereas free n -alkanes show progressive carbon-number shifts (Fig. 5). The stability of

biomarkers thus mirrors ^{13}C invariance because occluded compounds are shielded within asphaltene nanoaggregates (~2 nm), where limited exposure to thermal/chemical reactions preserves primary molecular and isotopic signatures. Critically, adsorbed biomarkers (molecular composition and biomarker parameters) exhibit transitional properties between free and occluded fractions (Table 3; Fig. 5), reflecting their partial shielding by asphaltene peripheries. The intermediate behavior of adsorbed hydrocarbons arises from their partial protection by asphaltene peripheries: Adsorbed components reside on asphaltene surfaces, making them more susceptible to thermal/chemical alterations than occluded (core-shielded) but less than free (unbound) hydrocarbons; Adsorbed components may exchange with free fractions during thermal stress, explaining their transitional isotopic/molecular signatures. This supports a hierarchical protection model: free > adsorbed > occluded in susceptibility to alteration. For source correlation, only occluded fractions meet dual criteria: thermal stability and carbon isotopic consistency. These findings validate asphaltene-occluded biomarkers as robust proxies for oil-source studies, particularly in reservoirs with secondary alterations.

4.3. Indication for the source of solid bitumen from the Kuangshanliang area

The $\delta^{13}\text{C}$ values of asphaltene-occluded saturated hydrocarbons (approximately -28.5 ‰) are evidently higher (enriched in ^{13}C) than the ^{13}C values ($-38.4 \sim -34.0$ ‰) of Lower Cambrian bitumens or oil seeps from the same area (Fang et al., 2022c; Huang and Wang, 2008; Wang et al., 2014; Wu et al., 2012). The free fractions in bitumen obtained by conventional fractionation methods are characteristically ^{13}C -poor, which is often attributed to the preferential consumption by cyanobacteria of ^{12}C in CO_2 through photosynthesis, producing organic matter with lower (enriched in ^{12}C) carbon isotope values (Huang and Wang, 2008). However, secondary alterations, such as biodegradation, can entirely alter the carbon isotope composition of sedimentary organic matter. Consequently, the biogenic sources and sedimentary environments of organic matter cannot be directly inferred from the isotopic compositions of the bitumen sample that have undergone moderate-to-severe alteration.

2022HTj e9TeQe@Te6QE,/,Tfe35T33(,E,E(54(E/,TzT5z/3/Iz,kET5z3k3E(,Trz1(HLj

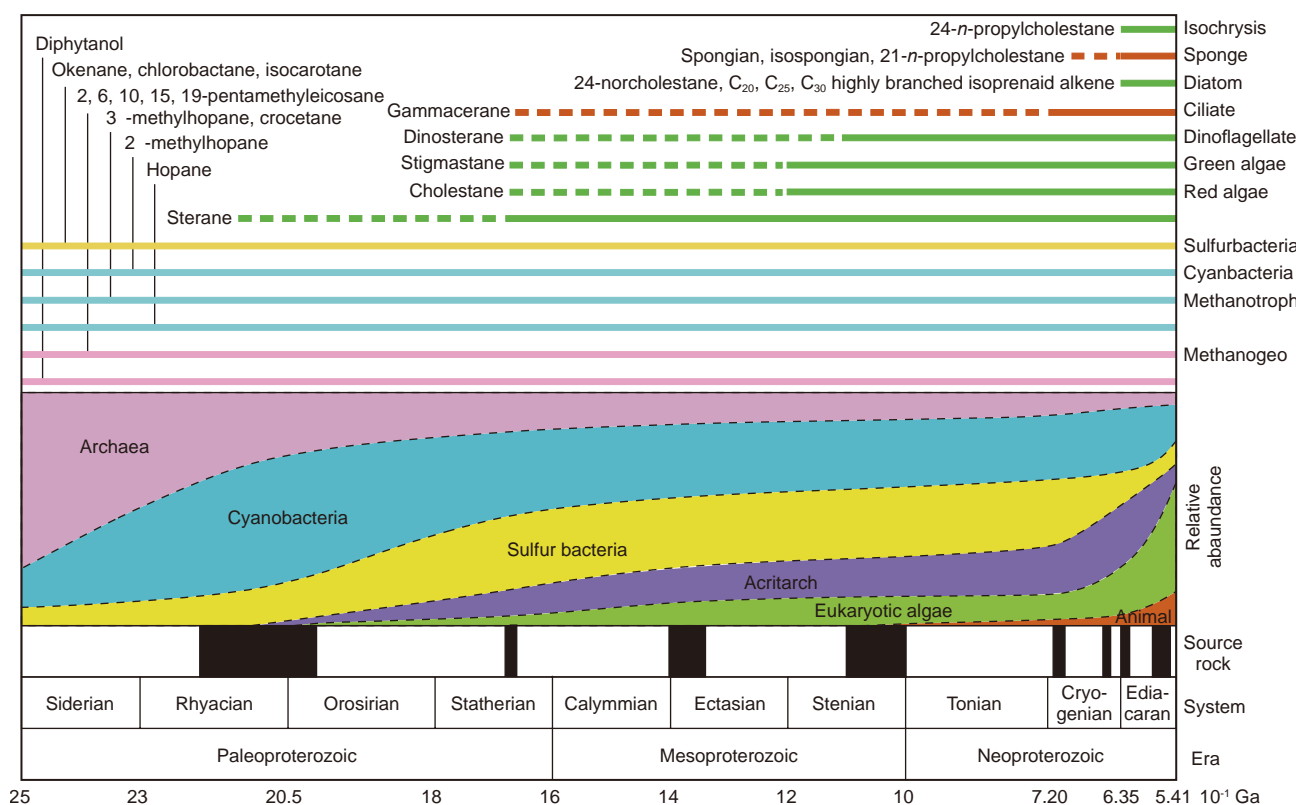


Fig. 9. Proterozoic biological evolution trends and their biomarker evidence (Zhao et al., 2019).

abundance of C₂₇ steranes in occluded steranes is obviously higher (Fig. 4). The dominance of C₂₉ steranes in free hydrocarbons from the bitumen has been postulated to reflect the contribution of cyanobacteria or green algae during the Early Paleozoic and preceding periods (Ge et al., 2018; Grantham, 1986; Volkman, 1986; Wang et al., 2014). However, a recent study has demonstrated that the dominance of C₂₉ steranes in free hydrocarbons from the bitumen is the result of biodegradation (Fang et al., 2021). Therefore, the distribution of steranes in occluded hydrocarbons may be a more reliable indicator of the source of sedimentary organic matter.

The C₂₇ sterane dominance can be traced back to the evolution of life in the Precambrian period. The evolution of eukaryotes from prokaryotes occurred during the Proterozoic period (Fig. 8). However, it was not until an interval between global glaciations known as Snowball Earth, which occurred during the Neoproterozoic Oxygenation Event (NOE), that eukaryotic algae radiated to become the major oxygen producers (Brocks et al., 2017). C₂₇ steranes are the dominant compounds in rock extracts from the Kwagunt Formation (~0.74 Ga) of the Chuar Group and the Red Pine Shale (~0.74 Ga) of the Uinta Mountain Group in North America, indicating that eukaryotic algae had become the primary source of sedimentary organic matter at that time (Summons et al., 1988; Vogel et al., 2005). The appearance of fossils of complex macroscopic algae, benthic algae, and metazoans during the Doushantuo interval in the Yangtze region, southern China, marks a significant milestone in the evolution of life on Earth (Chen et al., 2014; Yuan et al., 2011). Furthermore, the Neoproterozoic black shales in which C₂₇ sterane is dominant have also been identified in the Yangtze region, providing evidence that eukaryotic algae contributed to the formation of Precambrian sedimentary organic matter (Wang et al., 2008).

Although due to the high maturity and the limitations of the samples, the biomarkers in the source rocks of the Doushantuo Formation have not been recognized as consistent distribution characteristics. The distribution of steranes in occluded hydrocarbons suggests that the primary biogenic source of bitumen is likely to be eukaryotic algae, which is consistent with the biological source of high-quality source rocks in Doushantuo Formation (Zhu et al., 2021). From the perspective of biological evolution, the expansion of eukaryotic algae in the Neoproterozoic (Wang et al., 2019) suggests a greater capacity for the production of organic matter compared to prokaryotic bacteria (Fig. 9). Consequently, eukaryotic algae provide a material basis for the widespread distribution of Neoproterozoic source rocks and are an important source of Precambrian sedimentary organic matter including paleo-oil reservoirs of the Dengying Formation and derived surface bitumens. These evidences support the consistency of ¹³C values of asphaltene-occluded hydrocarbons with those of source rocks of Doushantuo Formation.

5. Conclusions

- (1) The ¹³C values of the saturated hydrocarbons of the free maltenes extracted from solid (vein) bitumen of NW Sichuan are significantly higher (enriched in ¹³C) than those of the aromatic and polar components. All asphaltene-associated “occluded” compound groups have higher ¹³C values (are enriched in ¹³C) than the free ones. This holds especially for the saturated hydrocarbons.
- (2) Thermal maturation experiments demonstrate that the ¹³C values of the free and asphaltene-adsorbed components decrease with temperature. At low thermal stress, the release of isotopically heavy “residual carbon” compounds

from the asphaltene matrix is the dominant process. With increasing thermal stress, asphaltenes decompose, resulting in the preferential scission of ^{12}C - ^{12}C bonds and release of isotopically lighter compounds. This process ultimately explains the decrease in the ^{13}C values of free and adsorbed hydrocarbons with thermal evolution.

- (3) The ^{13}C value of asphaltene-occluded components remains unaltered throughout the thermal maturation experiment, thereby reflecting the isotopic composition of the original organic matter. This evidence confirms that the asphaltene structure effectively protects and isolates the occluded components. The ^{13}C values of asphaltene-occluded components remain constant regardless of maturity (R_o 1.5%) and are in agreement with the $^{13}\text{C}_{\text{org}}$ (the bulk organic carbon isotope) value of the source rock of the Doushantuo Formation. Future studies should include bitumen samples from different locations to further verify the robustness of asphaltene-occluded hydrocarbons as a universal tool for oil-source correlation in altered systems.

CRediT authorship contribution statement

Peng Fang: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis. **Jia Wu:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Xue-Min Xu:** Writing – review & editing, Supervision, Methodology. **Bin Shen:** Writing – review & editing, Supervision. **Wei-Lin Sun:** Writing – review & editing. **Xiao Jin:** Validation, Data curation. **Feng Chen:** Writing – review & editing, Data curation. **Ning-Ning Zhong:** Writing – review & editing, Resources, Conceptualization. **Ming-Hui Zhou:** Writing – review & editing.

Declaration of interests

The authors declare that they have

- two Venezuelan oils. *Org. Geochem.* 37 (3), 291–303. <https://doi.org/10.1016/j.orggeochem.2005.10.010>.
- Liao, Z., Graciaa, A., Geng, A., et al., 2006c. A new low-interference characterization method for hydrocarbons occluded inside asphaltene structures. *Appl. Geochem.* 21 (5), 833–838. <https://doi.org/10.1016/j.apgeochem.2006.02.005>.
- Liu, S., Deng, B., Sun, W., et al., 2018. Sichuan Basin: A superimposed sedimentary basin mainly controlled by its peripheric tectonics. *Chin. J. Geol.* 53 (1), 308–326. <https://doi.org/10.12017/dzlx.2018.018> (in Chinese).
- Lorant, F., Behar, F., 2002. Late generation of methane from mature kerogens. *Energy Fuel.* 16 (2), 412–427. <https://doi.org/10.1021/ef010126x>.
- Lorant, F., Prinzhofer, A., Behar, F., et al., 1998. Carbon isotopic and molecular constraints on the formation and the expulsion of thermogenic hydrocarbon gases. *Chem. Geol.* 147 (3), 249–264. [https://doi.org/10.1016/S0009-2541\(98\)00017-5](https://doi.org/10.1016/S0009-2541(98)00017-5).
- Ma, A., Zhang, S., Zhang, D., 2008. Ruthenium-ion-catalyzed oxidation of asphaltenes of heavy oils in Lunnan and Tahe oilfields in Tarim Basin, NW China. *Org. Geochem.* 39 (11), 1502–1511. <https://doi.org/10.1016/j.orggeochem.2008.07.016>.
- Meredith, W., Snape, C.E., Carr, A.D., et al., 2008. The occurrence of unusual hopenes in hydropyrolysates generated from severely biodegraded oil seep asphaltenes. *Org. Geochem.* 39 (8), 1243–1248. <https://doi.org/10.1016/j.orggeochem.2008.01.022>.
- Michels, R., Landais, P., 1994. Artificial coalification: Comparison of confined pyrolysis and hydrous pyrolysis. *Fuel* 73 (11), 1691–1696. [https://doi.org/10.1016/0016-2361\(94\)90154-6](https://doi.org/10.1016/0016-2361(94)90154-6).
- Monthieux, M., Landais, P., Monin, J.-C., 1985. Comparison between natural and artificial maturation series of humic coals from the Mahakam delta, Indonesia. *Org. Geochem.* 8 (4), 275–292.