Full Length Article

Severe biodegradation of polycyclic aromatic hydrocarbons in reservoired crude oils from the Miaoxi Depression, Bohai Bay Basin

Xiong Cheng[a,b,c,*], Dujie Hou[a,b,*], Rong Mao[a,b], Changgui Xu[d]

[a] School of Energy Resources, China University of Geosciences, Beijing 100083, China
[b] Key Laboratory of Marine Reservoir Evolution and Hydrocarbon Accumulation Mechanism, Ministry of Education, China University of Geosciences, Beijing 100083, China
[c] Department of Earth and Planetary Sciences and Macquarie University Marine Research Centre, Macquarie University, Sydney, NSW 2109, Australia
[d] Exploration and Development Research Institute, Tianjin Branch of China National Offshore Oil Corporation, Tianjin 300452, China

ARTICLE INFO

ABSTRACT

Aromatic hydrocarbons are major components in crude oil. Biodegradation of polycyclic aromatic hydrocarbons, such as biphenyls, naphthalenes, phenanthrenes and aromatic steroid hydrocarbons have been well studied. However, little is known about the relative susceptibilities to biodegradation of fluorene, dibenzofuran, dibenzothiophene, chrysene and pyrene, and their alkylated homologues. In this study, gas chromatography–mass spectrometry analyses of aromatic fractions of a suite of severely biodegraded crude oils from the Bohai Bay Basin have been performed to investigate the fate and relative susceptibilities of these polycyclic aromatic hydrocarbons during severe biodegradation under geological conditions.

Changes in concentrations of varying PAH classes with increasing biodegradation suggest their relative susceptibilities to microbial alteration are in the order biphenyls > naphthalenes > dibenzofurans > phenanthrenes ~ fluorenes > dibenzothiophenes > chrysenes > pyrenes > C_{26-28} triaromatic steroid hydrocarbons. The relative susceptibilities to biodegradation of individual isomers of chrysenes, dibenzothiophenes, dibenzofurans, fluorenes, pyrenes and triaromatic steroid hydrocarbons were determined based on their compositional changes. The susceptibility to biodegradation of PAHs is not directed related to the number of aromatic rings, but may be controlled by the shapes of the substrate molecules which control the ability to penetrate the cell membrane and to fit the active site of the microbial enzyme. Sulfur-containing compounds, such as dibenzothiphenes, are more resistant to biodegradation than those non-sulfur-containing components with similar molecular structure, which is likely due to their relatively greater biotoxicity. PAHs with more alkyl substituents are generally more resistant to biodegradation, but exceptions were also observed, and this observation may due to demethylation of a substituted compound. Undoubtedly, the positions of alkyl substituents control the susceptibilities to biodegradation of PAH isomers. However, the observation that PAH isomers with β-substituents are more readily biodegraded is not universal, suggesting that the susceptibility of PAH isomers is not controlled by thermodynamic stability, but may be related to their stereochemical structures.

1. Introduction

Aromatic hydrocarbons are ubiquitous constituents of sediments and petroleum [1–4]. They result from transformations of biological precursors in the sub-surface, either through microbial processes in the initial stages of diagenesis, or during subsequent burial in which such precursors experience the effects of temperature, pressure and the catalytic action of the mineral matrix [4–6].

Aromatic hydrocarbons are major components of crude oil, representing typically 20–45% of the total hydrocarbons [3]. It has long been recognized that the relative abundance and distribution patterns of PAHs can be used to assess organic matter input, thermal maturity, depositional environment and correlation of crude oils [7–19].

In addition to organic matter source, thermal maturity and depositional environment, biodegradation can also strongly affect the distributions of aromatic hydrocarbons in crude oil. Extensive studies have been carried out to investigate the effects of biodegradation on aromatic hydrocarbons in reservoired crude oils, coals, surface spills and laboratory experiments [20–35]. In general, the biodegradation rate of aromatic hydrocarbons decreases with increasing number of aromatic rings and with increasing number of alkyl substituents [22,25–27,33–36]. The positions of the alkyl substituents also control

the susceptibility to biodegradation, e.g. alkylnaphthalenes and alkyl-phenanthrenes with β-methyl substituents are more susceptible to biodegradation than those having α-methyl substituents [22,33]; di-methylnaphthalenes with adjacent methyl substituents are more re-sistant to biodegradation [22,25]; alkylnaphthalenes with methyl sub-stituents at 1 and 6 positions have been reported to be less resistant to biodegradation [26,27].

Although the effects of biodegradation on PAHs have been widely studied [21,22,24–27,29,31,33–35], most of the work has been con-centrated on the naphthalenes, phenanthrenes and triaromatic steroid hydrocarbons. In contrast, little is known about the relative suscepti-bilities to biodegradation of fluorene, dibenzofuran, dibenzothiophene, chrysene and pyrene, and their alkylated homologues, under geological conditions. One exception is the work by Ahmed et al. [29] who ob-served the order of susceptibilities of some PAHs to microbial alteration in coals to be naphthalene > fluorene > phenanthrene > dibenzothiophene > pyrene; 1-methylfluorene > 2-methylfluorene; 3-methylfluorene > 4-methylfluorene; 2- + 3-methyldibenzothio-phene (co-elution) > 4-methyldibenzothiophene. Another exception is the work by Bao and Zhu [34] who observed that the dibenzothio-phene/phenanthrene and methyldibenzothiophene/methylphenan-threne ratios increase rapidly with increasing biodegradation degree, suggesting dibenzothiophene and methyldibenzothiophene are more resistant to biodegradation than phenanthrene and methylphenan-threne, respectively.

Here we report the changes in concentrations and relative abun-dance of the less studied polycyclic aromatic hydrocarbons (di-benzothiophenes, dibenzofurans, chrysenes, pyrenes and triaromatic steroid hydrocarbons) in biodegraded crude oils from the Miaoxi Depression, Bohai Bay Basin. The aims of this study were (1) to identify the relative susceptibilities to biodegradation of varying PAH classes and individual isomers, and (2) to elucidate the factors that control their susceptibilities to biodegradation.

2. Samples and methods

2.1. Sample descriptions

Thirteen biodegraded crude oil samples obtained from the Miaoxi Depression, Bohai Bay Basin are reservoired in the Guantao and Minghuazhen formations of Neogene age, and buried hill reservoir of Proterozoic age [37]. They are derived from the Eocene Shahejie For-mation (Es) source rock [37]. Previous study of the effects of biode-gradation on saturate hydrocarbons showed that the crude oils have been severely biodegraded, which is evident by the transformation of hopanes to 25-norhopanes, and by the alteration of the highly resistant tricyclic terpanes in the most degraded samples [37].

In this study, we focused on six crude oils (Table 1), as they re-present a reference oil (PL15-1B) and five severely biodegraded oils with varying extents of biodegradation, based on the results of Cheng et al. [37]. Fig. 1 shows the total ion chromatograms (TICs) of saturate fractions and distributions of tri-, tetra- and pentacyclic terpanes in these samples. The PL15-1B reference oil is slightly biodegraded and

contains some severely biodegraded residues, as shown by the absence of n-alkanes, the relatively intact acyclic isoprenoids, bicyclic sesqui-terpenoids and regular steranes, and the presence of a minor amount of C_{29} 25-norhopane (Fig. 1). However, the relatively intact nature and high abundance of the naphthalenes and phenanthrenes in the TIC of the aromatic fraction shows that the mixing of initial biodegraded re-sidues and the later slight biodegradation do not affect the validity of the PL15-1B as a reference oil to assess the alteration of PAHs in the other oils. The 17α(H)-hopanes and regular steranes in the PL15-1A and PL91A oils were considerably altered, indicating that they have been subjected to level 7 biodegradation based on the Peters and Moldowan biodegradation scale [38]. The ratios of C_{29} 25-norhopane to C_{30} 17α(H)-hopane for the PL15-1A and PL91A oils are 0.69 and 0.90 (Table 1), respectively, showing that the degree of biodegradation of the PL91A oil is slightly greater than for the PL15-1A oil. The PL15-8A, PL15-8B and PL15-8C oils are the most severely biodegraded oils, and are characterized by almost complete removal of 17α(H)-hopanes and regular steranes, and by partial depletion of tricyclic terpanes (Fig. 1 and Table 1). The distributions of tricyclic terpanes (Fig. 1) indicate that the PL15-8C oil is more biodegraded than the PL15-8B, which in turn is more biodegraded than the PL15-8A oil [37].

2.2. Methods

Aliquots of the crude oils (31.3–36.8 mg; Table 1) were deasphal-tened with excess n-hexane, and then the maltenes were separated into saturate and aromatic fractions and resins by column chromatography over activated silica gel and alumina with different solvents [37].

Prior to gas chromatography–mass spectrometry (GC–MS), an in-ternal standard, anthracene-d_{10} (0.1042 μg/μL, 20 μL), was added into each aromatic fraction for quantification. GC–MS analyses of the aro-matic fractions were performed using a HP 6890 gas chromatography interfaced to a HP 5973 MSD (ionization energy 70 eV). A HP-5 ms fused silica capillary column (30 m × 0.25 mm i.d., film thicknesses 0.25 μm) was used and the oven temperature was programmed to start at 40 °C (hold for 2 min), and increase to 320 °C at a rate of 4 °C/min with a final hold of 20 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Parent and alkylated polycyclic aromatic hydro-carbon chromatograms were recorded as follows: naphthalenes (m/z 128, 142, 156, 170, 184, 196); biphenyls (m/z 154, 168, 182); phe-nanthrenes (m/z 178, 192, 206, 220); chrysenes (m/z 228, 242, 256); dibenzothiophenes (m/z 184, 198, 212, 226); dibenzofurans (m/z 168, 182, 196); fluorenes (m/z 166, 180, 194); pyrene and fluoranthene (m/z 202); methylpyrene, benzo(a)fluorene and benzo(b)fluorene (m/z 216); triaromatic steroid hydrocarbons (m/z 231). Quantification was based on peak areas of the PAHs versus the internal standard integrated in the mass chromatograms using Chemstation. The relative response factor for individual PAHs with the internal standard was assumed to be 1 [39,40].

3. Results

TICs of the aromatic fractions in the crude oils are shown in Fig. 1

Table 1
Basic information of selected crude oils and parameters, showing the increasing biodegradation levels of the samples.

Sample ID	Depth (m)	Formation	Oil weight (mg)	$D_{29}/C_{30}H$	$C_{21}TT/C_{24}TT$	$C_{23}TT/C_{24}TT$	PM level
PL15-1B	1475–1478	N_2m^L	36.8		1.36	1.60	
PL15-1A	1164–1170	N_2m^U	34.2	0.69	1.32	1.60	7
PL91A	1104–1111	N_1g	36.7	0.90	1.25	1.59	7
PL15-8A	1031–1048	N_2m^U	31.3	5.88	0.82	1.45	8
PL15-8B	1348–1362	N_2m^L	33.4	5.91	0.43	1.22	8
PL15-8C	1747–1758	N_2m^L	35.8	6.16	0.2	0.61	8

Oil weight: the weights of the oils used for fractionation; D_{29}: C_{29} 25-norhopane; $C_{30}H$: C_{30} 17α(H)-hopane; TT: tricyclic terpane; PM level: biodegradation level based on Peters and Moldowan (1993) biodegradation scale.