Biodegradation of 25-norhopanes in a Liaohe Basin (NE China) oil reservoir

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A S T R A C T
A suite of reservoir cores in an oil column from the Shuguang oil field, Liaohe Basin, NE China was characterized geochemically to investigate the behaviour of 25-norhopanes (NHs) during biodegradation. The extracted bitumens show similar organofacies type and maturity levels with biodegradation as the primary control on compositional variation. All bitumens suffered severe biodegradation influence which removed all regular steranes and most of the pentacyclic terpanes leaving NHs as dominant components in total ion chromatograms of the saturated hydrocarbon fraction. However, the NHs are not the endpoint of hopane destruction: they are biodegraded as well. Biodegradation of NHs can be illustrated by significant depletion in their concentrations and systematic variation in ratios of resistant to vulnerable components throughout the oil column. The biodegradation sequence is somewhat similar to normal pentacyclic terpanes. Among the NH series, 18α,25,29,30-tetranorhopane is the most recalcitrant to biodegradation. The 18α,22,25,29,30-tetranorhopane is more resistant to biodegradation than 17α,22,25,29,30-tetranorhopane and 34NHs are more resistant than other NHs. Degradation of NHs provides a new angle to understanding the complicated inter-relationships in subsurface biodegraded reservoirs.

1. Introduction
The origin of 25-norhopanes (NHs) in biodegraded oils remains controversial even after several decades of investigation. Some early studies suggested that NHs were pre-existing biomarkers in oils and source rocks which are unmasked when severe biodegradation removes regular hopanes (Chosson et al., 1991; Blanc and Connan, 1992). However, only a few individual NHs have ever been detected in nonbiodegraded oils and source rocks. No mass balance calculation can be reached based on other recalcitrant compounds resulting in the wide acceptance of the biodegradation origin of NHs (Seifert and Moldowan, 1979; Volkman et al., 1983; Peters and Moldowan, 1993; Moldowan and McCaffrey, 1995; Grimalt et al., 2002; Peters et al., 2005; Bennett et al., 2006). The presence of a full series of NHs in crude oils is commonly recognized as an indicator of severe biodegradation and it comprises one key compound class in the assessment of biodegradation extent (Volkman et al., 1983; Cassani and Eglinton, 1991; Peters and Moldowan, 1993; Dzou et al., 1999). Nevertheless, attempting to understand the inter-relationship between hopanes and NHs is still a challenge because there appears to be no established, consistent trend. In a continuous oil column, Moldowan and McCaffrey (1995) showed a concomitant and inverse change with depth in the concentrations of certain hopanes and their NH counterparts. Such observation led them to conclude that NHs originate from direct microbial degradation of hopanes without significant accumulation of oxidized intermediates. However, more complexity in the apparent inter-relationship of hopanes and NHs was revealed by Bennet et al. (2006) who, with others, showed that the rate of hopane disappearance generally does not match that of the formation of NHs (Wang et al., 2013). Only a portion of the biodegraded hopanes gave rise to NHs, suggesting the formation of unknown biodegradation products including hopanoid acids (Watson et al., 2002; Bennet et al., 2007) or a number of different hopane biodegradation mechanisms (Brooks et al., 1988; Peters et al., 2005). Environmental conditions or local water chemistry may affect hopane biodegradation pathways. No NH series has ever been identified in sea bottom seeps or surface oil-spill remediation studies (Seifert et al., 1984; Wenger and Isaksen, 2002), nor in pure or enrichment cultures of aerobic biodegradation experiments (Goodwin et al., 1983; Chosson et al., 1991; Bost et al., 2001;
Frontera-Suáu et al., 2002), suggesting that anaerobic bacteria are responsible for their origin. Complications in the hrance and NH correlation can result from multiple charge processes in which mixing of severely biodegraded oils with a later charge of nonbiodegraded crude oils occurs during accumulation in the reservoir (Campos et al., 1996; Grimalt et al., 2002).

The NHs are generally regarded as end products of biodegradation. Whether they are also biodegradable within an oil reservoir has not been convincingly demonstrated. Aerobic biodegradation experiments performed in the laboratory for two oils containing NHs show that the biodegradation rate of NHs is similar to that of hopanes after a 5 week incubation using a microbial enrichment culture (Bost et al., 2001). However, biodegradation of NHs underoxic conditions through aerobic mechanisms may be not applicable to the fate of NHs under reservoir conditions, as oil biodegradation within reservoirs most likely occurs under anoxic conditions through an anaerobic microbial metabolism (Head et al., 2003; Larter et al., 2003; Huang et al., 2004). A severely biodegraded oil column was thoroughly investigated using routine geochemistry in the present study. The objective of the study is to monitor the behaviour of NHs within biodegraded column and to verify biodegradation influence in a natural case. Our results may provide a new angle to interpret the inconsistencies between hopenes and NHs under severe biodegradation situation.

2. Geological background

The Liaohe Basin, a part of the Bohai Bay super basin series in NE China, is the largest heavy oil production area in China. Structural evolution, depositional facies, stratigraphic correlation and petroleum geology have been thoroughly investigated by numerous studies (Ge and Chen, 1993; Yuan, 2004; Xie, 2005). It is a Cenozoic rift basin, and the Eocene Shahejie (Es) Formation is the most important sequence. The Shahejie Formation can be divided into four members. The fourth (oldest) member of the formation (Es4) was deposited in a semi-enclosed saline environment which has minor petroleum source rock potential at the northern part of the Western Depression. The lower part of the third member of the formation (Es3) was deposited in a deep freshwater environment, which has excellent source potential throughout the entire basin. The upper part of the Es3 member consists of interbedded shales and sandstones. The Es1+2 member is composed mainly of poorly sorted coarse sediments, and these comprise the most important petroleum reservoir in the basin (Fig. 1). The overlying Dongying Formation serves as regional seal for oil accumulated in the Shahejie Formation.

Most heavy oil in the Liaohe Basin is produced from the Western Depression (Fig. 1). The Shuguang oil field, situated in the middle part of the western slope of the Western Depression, is one of the basin’s most prolific oilfields. Samples used in the present study are from the Du84 Block within the Shuguang oil field. Finely crushed core (ca. 20 g) was Soxhlet extracted with dichloromethane (DCM) for 24 h. The solvent was evaporated to ca. 1 ml using a Büchi Rotavapor and transferred to a 10 ml vial. The remaining solvent was removed under a gentle stream of nitrogen and the bitumen weight obtained. An aliquot of the extracted bitumen (ca. 50 mg) was desalpated using cold hexane and the precipitated asphaltene was determined. The maltenes were fractionated using column chromatography (silica gel:alumina, 3:1) into saturated hydrocarbons, aromatic hydrocarbons and resins eluted with n-hexane, benzene and DCM:methanol (1:1), respectively. The summed weight of eluted fractions plus asphaltene divided by the initial loaded weight is designated as the recovery efficiency. The recovery normalized percentage of each fraction is the bulk composition (saturated and aromatic hydrocarbons, resins and asphaltene or SARA) of oil or extracted bitumen.

Detailed gas chromatography-mass spectrometry (GC-MS) was performed on an Agilent 6890 gas chromatograph coupled to an Agilent 5975i MS mass spectrometer. A HP-5MS column (60 m × 0.25 mm, 0.25 µm film thickness) was used. For the saturated hydrocarbon fraction, the GC oven temperature was programmed from 50°C (1 min) to 120°C at 20°C/min, then to 310°C at 3°C/min (held 25 min). For the aromatic hydrocarbon fraction, the oven temperature was programmed from 80°C (1 min) to 310°C (hold 15 min) at 3°C/min. Helium was used as the carrier gas with a constant flow rate of 1 ml/min. The gas chromatograph was equipped with an injector at 300°C and a transfer line at 230°C. Compounds were ionized electronically (EI) with ionization energy of 70 eV. The mass spectrometer was operated in both full scan and selected ion monitoring (SIM) mode for saturated and aromatic hydrocarbon fractions. Internal standards, d4-C40, 20R sterane and d8 dibenzothiophene, were added to the oil for saturated and aromatic hydrocarbon fraction quantification purposes. Peak area was used for concentration and molecular parameter calculations. Response factors for the components of interest relative to the internal standards were assumed to be 1.0 and no calibration was applied.

The stable carbon isotopes were analyzed using a Thermo Scientific Flash HT elemental analyzer coupled with MAT 253 isotope mass spectrometer via a continuous flow technique. The oxidation tube of the Flash HT elemental analyzer was filled with oxidized copper, chromium oxide and silver cobalt oxide. The reaction temperature was 980°C and helium (99.999%) was used as the carrier gas with a flow rate of 100 ml/min. Oxygen (99.995%) with a flow rate of 250 ml/min was used as auxiliary gas for combustion. Compounds were ionized electronically (EI) with ionization energy of 70 eV. IAEA-600 caffeine and USGS24 graphite were used as standards for isotopic calculation to precisions of ± 0.1‰.

4. Results

4.1. Bitumen content and bulk composition

The bitumen contents vary from 4.9–17.9 wt% in the studied oil column (Table 1), decreasing with increasing depth. The average extractable yield is 13.7 wt% in Es1+2 reservoir while it is only 6.7 wt% in the Es3 portion. The lowest bitumen content occurs in
the deepest sample (819 m), which may correspond to the occurrence of the oil-water contact (OWC).

The SARA data indicate that the core extracts have enriched resin and asphaltene contents (44.8–58%) whereas the saturated hydrocarbons ranged between 24.7% and 33.7% and the aromatic hydrocarbon fraction ranged from 16.1–24.5%. These values are commonly observed in heavy oil fields resulting from biodegradation. Variation of the bulk fractions can be noted at different sample depths. Within the Es1+2 reservoir, the downward decreasing abundance of saturated hydrocarbons is complementary to the increasing asphaltene content, reflecting hydrocarbon removal by biodegradation and enrichment of the remaining extract in asphaltenes. Bulk fractions in the Es3 reservoir do not show dramatic variations in this case study due to the extremely high level of biodegradation (Table 1).

4.2. Oil family classification

The first step to investigate biodegradation influence on oil compositions requires determining the origin of the oil and thermal maturity variation. If the studied oils belong to the same family (originate from a single source rock system) with similar maturity, compositional variation can be confidently attributed to the influence of biodegradation. However, source input and thermal maturity determination of organic matter in oil can be problematic when oil suffers severe biodegradation influence, which has removed most of the susceptible components, leaving a pronounced unresolved complex mixture (UCM) in both saturate and aromatic hydrocarbon chromatograms.

Among hydrocarbons, aromatic steroids are highly resistant to biodegradation, being degraded only under extreme conditions (Lin et al., 1989). The representative triaromatic steroid hydrocarbons (TAS) detected on m/z 231 mass chromatograms of the aromatic fraction are shown in Fig. 2 (left). The C20 and C21 components are only present in D84-5999 oil, but are absent from all core extracts and another oil sample (D84-57-23), consistent with the interpretation of severe biodegradation (Volkman et al., 1983). Since TAS are derived from biological precursors like sterols or sterones during diagenesis and catagenesis (Mackenzie et al., 1982), the distribution of long chain TAS components are source input related and can be applied to oil family identification. Due to the co-elution of C26 20R with C27 20S, the other four isomers were used to differentiate homolog variations. All samples contain higher amounts of C28 (20R, 20S) TAS than C27 20R and C26 20S counterparts and the values of C28 (20S + 20R)-TAS/(C26 20S + C27 20R)-TAS are in narrow range between 2.0 and 2.2 (Fig. 3).

The stable isotopic composition of crude oil is mainly dependent on the δ13C value of the kerogen which, in turn, depends on organic input and the depositional environment (Schoell, 1984; Sofer, 1984; Chung et al., 1992). All core extracts and D84-5999 oil were examined for bulk δ13C and uniform stable carbon isotopic values are revealed (Table 1). The whole extracted bitumens have δ13C values from −30.3‰ to −29.7‰ with an average value of −30.1‰, which is the same as that in D84-5999 oil. Huang and
Larter (2014) reported some whole oil isotopic values from the Lengdong oil field, Liaohoe Basin. They noticed that oil from the Es3 source rock has stable carbon isotopic value around –30.0‰, while oil from the Es4 source rock is about 5‰ heavier. The data present in this case study are consistent with the Es3 origin.

The saturated hydrocarbon fraction in our sample suite is isotopically depleted in 13C relative to the other fractions with an average value of –34.3‰. The average isotopic difference between aromatic and saturated hydrocarbon fractions is as large as 4.9‰, typically characteristic in lacustrine oils (Collister and Wavrek, 1996). The stable isotopic compositions of resin and asphaltene are only slightly heavier than that of aromatic hydrocarbon fraction with an average δ13C value of –29.0‰ and –28.5‰, respectively. The δ13C values of aromatic hydrocarbons, resin and asphaltene for D84-5999 oil are –29.2‰, –28.6‰ and –28.3‰, respectively, which are very close to those in the core extracts. The uniform stable carbon isotopic compositions further verify the same source input and both oils and core extracts can be classified into a single oil family.

Due to the severe biodegradation influence on the samples, mostly of the commonly used molecular maturity parameters are not valid in this case study except the steroid hydrocarbon aromatization parameter [TAS/(TAS + MAS)] (MAS is total C27 monoaromatic steroid hydrocarbons), which is based upon the decrease in MAS relative to TAS with increasing maturity. The [TAS/(TAS + MAS)] ratios are in the range of 0.43–0.55 with an average value of 0.48 and 0.53 in the Es1+2 and Es3 reservoirs, respectively (Fig. 3). Subtle maturity variation may be due to continuous charging from increasingly mature source rock, but the overall uniform profile suggests the original source characteristics of the oils have been maintained without a significant late oil charge.

Based on aromatic steroid hydrocarbon distributions and bulk isotopic analysis, the studied core extracts have similar organofacies type and maturity levels and can be regarded as constituting a single oil family. Biodegradation is inferred to be the main control on compositional variation. However, minor late charge and mixing in the Es3 causing some complication in compositional variation cannot be ruled out.

4.3. Biodegradation level assignment

The saturated hydrocarbon fractions of all core extracts contain no n-alkanes or branched alkanes such as pristane and phytane, corresponding to a minimum of level 5 on the Peters and Moldowan (1993) biodegradation scale (abbreviated as PM 5). The sterane distribution is another critical indicator for biodegradation level assignment in severely biodegraded samples. Highly biodegraded oils exhibit the selective elimination of steranes with respect to diasteranes, while pregnanes are more resistant to biodegradation than both steranes and diasteranes (Seifert and Moldowan, 1979; Bennett et al., 2006). The representative sterane distribution is illustrated in Fig. 2 (right). D84-5999 oil suffers the least biodegradation influence in the studied sample suite. C27–C29 steranes are dominated on m/z 217 mass chromatograms with relatively minor amounts of C27–C29 diasteranes and C21–C22 pregnanes. In contrast, C27–C29 steranes are almost absent from D84-57-23 oil and all core extracts, and diasteranes and pregnanes are relatively concentrated on m/z 217 mass chromatograms. The formation of diasteranes and pregnanes is controlled by depositional environments and lithologies in addition to thermal maturity. However, their low concentration is possibly due to overall low thermal maturity rather than the lack of clay mineral catalysis. Some peaks appearing at regular sterane retention times are pentacyclic triarpenes, which contribute minor m/z 217 fragments (Bennett et al., 2013). The biodegradation level in the core extracts is between PM 8 (steranes depleted) and PM 9 (diasteranes altered). Since long chain aromatic steroid hydrocarbons are intact in all the studied samples, the biodegradation level is less than PM 10.

4.4. Distributions of hopanes and 25-norhopanes

Representative mass chromatograms of m/z 191 (Fig. 4, left) and m/z 177 (Fig. 4, right) show distribution patterns of regular pentacyclic terpanes and their demethylated homologs in the studied sample suite. Pentacyclic terpanes in D84-5999 oil include the C27 and C29–C35 homologues, rearranged hopanes such as 18x 30-norneohopane (29Ts) and C30 diahopane (30D) and gammacerane (G), with C30 17α hopane (30H) as the dominant component. The extended homologues are resolved at the C-22 position into the S and R epimers. Gammacerane is moderately concentrated in some samples but it was biodegraded as well. No predominance of C35 17α homohopanes (35H) over the C34 homolog (34H) has been observed. This reflects typical lacustrine sourced organic matter deposited under fresh-saline water conditions. Slightly enriched gammacerane is most likely caused by biodegradation rather than stratified saline water. In contrast to intact steranes (Fig. 2, right), the NHs are prominent in the m/z 177 chromatogram, implying that the hopanes are demethylated prior to complete removal of the steranes in the crude oils of the Shuguang oil field. The NHs occur preferentially among low molecular weight compounds (C31). The demethylated counterparts of 18x–22,29,30-trisnorhopane (Ts) is 18x–22,25,29,30-tetranorhopane (NTs) and the
Fig. 2. Representative m/z 231 mass chromatograms from aromatic hydrocarbon fraction showing the distributions of C20–C28 triaromatic steroid hydrocarbons (left) and m/z 217 mass chromatograms from saturated hydrocarbon fraction showing the distributions C21–C29 steranes (right) in the studied oil column. Numbers are carbon number of compounds. 20, 21, 26–28S and 26–28R: C20–C28 triaromatic steroid hydrocarbons; 21P–22P: C21–C22 pregnanes.
homohopanes and their demethylated counterparts. The NHs, with C32 homohopanes (31H and 32H) are 25-norhopanes (30-34NHs). The chromatogram is dominated by unidentified components in the pentacyclic terpane range. and gammacerane are relatively enriched. C29 chromatograms. The bottom sample of oil column (well D84-65-67, m = 734 m in well D84-65-67 are slightly less degraded, implying a local basal water effect. The upper part of the Es1+2 reservoir and decrease significantly in the Es3 reservoir (Fig. 5). Overall concentration of 29H is quite low throughout the whole oil column with all samples having 29H < 500 µg/g extract. The concentrations of 28NH are higher than 2200 µg/g extract in the Es1+2 reservoir with the highest 28NH/29H ratio up to 70 at 676 m. No parent-product relationship can be established in the present case study. In the Es3 reservoir, concentrations of 28NH decrease dramatically from 2690 µg/g extract at the top to 279 µg/g extract at the bottom (Fig. 5a). Such a concentration gradient in the more severely biodegraded Es3 portion is most likely caused by biodegradation of 28NH.

Comparison of the concentrations of the compound pair 30H and 29NH is illustrated in Fig. 5b. The highest concentration of 30H is 1223 µg/g extract at 662 m where the corresponding 29NH is 1626 µg/g extract. While concentrations of 30H are less than 300 µg/g extract in other Es1+2 samples, the concentrations of 29NH vary from 1998–3932 µg/g extract, again indicating a complicated internal relationship. Concentrations of 30H are mainly below 1000 µg/g extract in the Es3 reservoir with the lowest amount of 178 µg/g extract for the bottom sample, whereas the concentrations of 29NH show a sharply decreasing trend from 3725 µg/g extract at depth 734 m to 177 µg/g extract at the bottom.

Similar trends in concentration profiles can be observed from C15–C35 homohopanes and their demethylated counterparts. The concentrations of C31 and C32 homohopanes (31H and 32H) are low and scattered throughout oil column, while the concentrations of 30NH and 31NH are high in the Es1+2 reservoir, then decrease dramatically from top of the Es3 reservoir toward bottom (Fig. 5c and d).

5. Discussion

5.1. NHs are biodegradation products

Whether NHs are formed from hopanes through biodegradation or are simply concentrated during biodegradation due to the removal of other, more susceptible components is the first issue to be resolved for a deep understanding of the geochemical behaviour of NHs. Although NHs have been reported in source rocks and nonbiodegraded oils (Blanc and Connan, 1992), their distribution typically consists of only one or a few compounds rather than the full series. Most terpane components including 29Ts and gammacerane are only slightly higher than analytical background (noise). The m/z 177 chromatogram clearly shows the removal of C28–34 NHs, with the relative concentration of the more biodegradation resistant 25-nor-29Ts (28NTs). Biodegradation of NTs and NTm is less obvious than other NHs.

In previous case studies where NHs were observed as biodegradation products, a relative decrease in the abundance of the hopanes corresponding with increased NHs was commonly observed (Moldowan and McCaffrey, 1995; Tocco and Alberdi, 2002; Bennett et al., 2006; Wang et al., 2013), although the quantity of generated NHs may not match the mass loss of corresponding hopanes. However, no such behaviour occurs in the present work due to extreme biodegradation levels. The concentrations of NHs are far higher than their parent compounds in the Es1+2 reservoir and decrease significantly in the Es3 reservoir (Fig. 5). Overall concentration of 29H is quite low throughout the whole oil column with all samples having 29H < 500 µg/g extract. The concentrations of 28NH are higher than 2200 µg/g extract in the Es1+2 reservoir with the highest 28NH/29H ratio up to 70 at 676 m. No parent-product relationship can be established in the present case study. In the Es3 reservoir, concentrations of 28NH decrease dramatically from 2690 µg/g extract at the top to 279 µg/g extract at the bottom (Fig. 5a). Such a concentration gradient in the more severely biodegraded Es3 portion is most likely caused by biodegradation of 28NH.
Fig. 4. Representative $m/z$ 191 (left) and $m/z$ 177 (right) mass chromatograms showing the distributions of 17$\alpha$-hopanes and 17$\alpha$, 25-norhopanes in oils and core extracts of the studied samples. Ts: 18$\alpha$-22,29,30-trisnorneohopane; Tm: 17$\alpha$-22,29,30-trisnorhopane; 29H: C$_{29}$ 17$\alpha$ hopane; 29Ts: 18$\alpha$ 30-norneohopane; 30D: C$_{30}$ diahopane; 29 M: C$_{29}$ 17$\beta$ moretane; 30H, C$_{30}$ 17$\alpha$ hopane; 30 M: C$_{30}$ 17$\beta$ moretane; 31-35H: C$_{31}$–C$_{35}$ 17$\alpha$ 22S and 22R homohopanes; G: gammacerane; NTs: 18$\alpha$-25,30-bisnorneohopane; NTm: 17$\alpha$-22,25,30-tetranorhopane; 28–34NH: C$_{28}$–C$_{34}$ 25-norhopanes; 28–29NM: C$_{28}$–C$_{29}$ 25-normoretanes; 28NTs: 18$\alpha$-25,30-bisnorneohopane; ND: demethylated C$_{30}$ diahopane; NG: demethylated gammacerane.
A simple mass balance calculation also disputes the relative concentration interpretation as illustrated by concentration profiles of some biodegradation resistant components such as pregnanes, diasteranes, tricyclic terpanes and TAS (Fig. 6). Concentration of C27–C29 diasteranes is 106 µg/g oil in D84-5999 and varies from 86–201 µg/g extract in the core extracts. Concentration of C21–C22 pregnanes is 50 µg/g oil in D84-5999 and varies from 31–97 µg/g extract in the core extracts. Slightly more variable but a similar concentration range can be observed from tricyclic terpanes and TAS. The concentrations of tricyclic terpanes are in the range from 1203–1784 µg/g extract in the Es1+2 reservoir with an average value of 1450 µg/g extract. Those in the Es3 reservoir vary from 1248–1837 µg/g extract with an average value of 1605 µg/g extract. The concentrations of TAS are from 614–907 µg/g extract with an average value of 777 µg/g extract and from 728–976 µg/g extract with an average value of 810 µg/g extract in the Es1+2 and Es3 reservoirs, respectively. If NHs are pre-existing biomarkers having their concentrations enhanced by selective biodegradation of more readily degradable compounds, other biodegradation resistant components should also be concentrated or depleted in a similar manner. Our data are consistent with the interpretation that NHs are biodegradation products of hopanes and they cannot be simply concentrated in the studied magnitude of variation.

5.2. Biodegradation of 25-norhopanes

The effects of biodegradation are most commonly recorded by the variations in the concentrations of the compounds of interest. Systematic depletion of NHs in the Es3 reservoir is a good indicator of biodegradation influence (Fig. 5). However, the concentration itself for NHs can be illusive due to very complicated processes and/or mechanisms involved in their formation (Seifert and Moldowan, 1979; Goodwin et al., 1983; Volkman et al., 1983; Connan, 1984; Peters and Moldowan, 1993; Moldowan and McCaffrey, 1995; Peters et al., 1996; Bennett et al., 2006, 2007; Wang et al., 2013). A visual inspection of the m/z 177 chromatograms in our studied sample indicates that biodegradation of NHs is the best interpretation (Fig. 4). Here more evidence is derived from ratios between reactive and refractory components.
The hopane distribution pattern is generally controlled by source rock lithology and depositional environment. For example, high 29H/30H ratio is indicative of carbonate source rock and high 35H/34H is commonly linked to large scale bacterial activity in highly saline environments (Peters and Moldowan, 1991; 1993; Peters et al., 1996; Bost et al., 2001; Wang et al., 2013). Many of these studies offer two opposite views on the homohopane biodegradation order, i.e., conservation of the 35H or preferential biodegradation of 35H (Requejo and Halpern, 1989; Chosson et al., 1991; Peters et al., 1996). Detailed biodegradation behaviour of each terpane component is out the scope of the present study.

Normalized relative abundance of NHs with their carbon number shows a large variation (Fig. 7a). The 28NH is the dominant component in the upper part of the Es1+2 reservoir and the relative abundance of NHs decreases with increasing carbon numbers. In the lower part of the Es1+2 and Es3 reservoir, the 29NH becomes the most enriched NH component. Different biodegradation behaviour and/or conversion rate of 29H and 30H to their demethylated counterparts may cause such variation, however, when 25-norhopanes were accounted, biodegradation of NHs seems most likely occur although no clear trend in the 28NH/29NH ratio has been observed. The summed relative abundances of 28NH and 29NH decrease from around 76.0% in the Es1+2 reservoir to 62.4% at the bottom of the Es3 reservoir and ratios of 28-29NHs/30-34NHs decrease from 3.5 to 1.7 accordingly. Meanwhile, the relative abundance of 34NH increases from about 1.0% at the top to 4.5% at the bottom and the ratios of 34NH/33NH increase from 0.4 at the top to 1.2 at bottom (Fig. 7b). The relative enrichment of 30–34NHs indicates that higher molecular weight NHs are more biodegradation resistant than lower molecular weight counterparts and the increasing 34NH/33NH ratio supports the notion that the 34NH is more resistant to biodegradation than other NHs. There are two different mechanisms to explain homohopane biodegradation sequence, i.e., bacterial attack of the side chain favors the higher molecular weight homologues (35H > 34H > 33H > 32H > 31H), while bacterial attack of the cyclic core results in preferential degradation of the lower molecular weight homohopanes (Peters et al., 1996). In the present case study, biodegradation of NHs appears to attack the cyclic core preferentially, resulting in the enrichment of 34NH.

As the formation of NHs consumes hopanes, the ratios of 28NH/29H and 29NH/30H are the most commonly used biodegradation indicators. Ratios of 28NH/29H and 29NH/30H are extremely high...
in the Es1+2 reservoir, while they are relatively low in the Es3 reservoir (data not shown). These ratios are not ideal biodegradation indicators due to the biodegradation of NH themselves. Possible late charge can cause a more complicated situation and much lower 28NH/28NTs and 29NH/30H ratios as slightly more regular hopanes are detected in the Es3 reservoir. This multiple charge process was proposed previously to explain the coexistence of n-alkanes and NHs in oils (Volkman et al., 1983; Campos et al., 1996; Grimalt et al., 2002). Although no absolute biodegradation resistant components are available in the reservoir, biodegradation of 29Ts is far slower than other pentacyclic terpanes and biodegradation of 28NTs seems not obvious in the present sample suite. The ratio of 28NTs/29Ts can provide a more reliable biodegradation intensity measurement. This ratio is generally < 1 in the most of the oil column, while it reaches 5.63 in the bottom sample (Fig. 8a).

Biodegradation of Ts and Tm has been reported in the literature, with Ts being more resistant to biodegradation than Tm (Seifert et al., 1984; Peters and Moldowan, 1993). Because both Ts and Tm were biodegraded, a wide range of Ts/Tm ratios from 0.48–1.02 is present in the studied oil column. Different susceptibility between Ts and Tm seems not to be discernible at such extremely severe biodegradation levels. However, the NTs/NTm ratios increase from 0.28 to 0.51 in the Es1+2 reservoir and from 0.20 to 1.02 in the Es3 reservoir. The increment in NTs/NTm ratios downward is consistent with biodegradation influence which intensifies toward the base of oil column (Larter et al., 2003, 2006; Huang et al., 2004; Bennett et al., 2013), implying preferential biodegradation of NTm versus NTs (Fig. 8a).

As 28NTs is extremely recalcitrant, ratios of other NHs to 28NTs can be applied to assess the extent of biodegradation. At relatively low biodegradation levels, 28NTs is in low concentration and ratios of other NHs to 28NTs should be high. This ratio is expected to decrease with increasing biodegradation intensity. The component has similar ability to resist biodegradation as 28NTs, the ratio shows no variation. Since Tm is also very resistant to biodegradation (Peters and Moldowan, 1993), ratios of NTm/28NTs are quite constant in the range of 0.47–0.68 in the Es1+2 reservoir. They decrease from 1.48 to 0.22 throughout the Es3 reservoir; consistent with downward intensity of biodegradation influence and the slightly more vulnerable nature of NTm as compared to 28NTs. Ratios of 28NH/28NTs vary from 5.85 to 3.74 in the Es1+2 reservoir, while they decrease sharply from 11.2 to 0.28 in the Es3 reservoir. Similar variation can be observed from 29NH/28NTs ratios, which are in the range from 3.76 to 7.88 in the Es1+2 reservoir and decrease from 15.51 to 0.31 in the Es3 reservoir. Less dramatic but following the same trend are the demethylated homohopanes. Ratios of 30NH/28NTs are from 1.76 to 0.92 in the Es1+2 reservoir and they change from 4.42 to 0.16 throughout the Es3 reservoir (Fig. 8b). All these ratios suggest that biodegradation might be responsible for NH removal and intrinsic level of biodegradation reaches a maximum at the base of the Es3 reservoir. Biodegradation is expected to affect different NH compounds to different degrees but the overall biodegradation sequence of NHs is somewhat similar to biodegradation of regular pentacyclic terpanes.

6. Conclusions

Oils and core extracts from the Shuguang oil field, Liaohe Basin NE China show similar organic input and thermal maturity. Biodegradation is the main controlling factor for compositional variation but minor late charge cannot be ruled out. Mass chromatograms show that core extracts suffered at least PM8 level of biodegradation which intensifies downward in both Es1+2 and Es3 reservoirs.

Our semi-quantitative data show that NHs result from the demethylation of regular hopanes during biodegradation in reservoir, but they are not the end product of the biodegradation process. Biodegradation of NHs seems inevitable to interpret their dramatically depleted concentrations within the Es3 reservoir.

Biodegradation patterns of NHs are similar to regular pentacyclic terpanes. Systematic increment of the relative proportion of 34NH over other demethylated hpane homologs support the conservation of 34NH during biodegradation and preferential degradation of NTm over NTs can be confirmed by an increase in the NTs/NTm ratio. 28NTs is the most recalcitrant component and ratios of other NHs to 28NTs decrease dramatically with increasing biodegradation intensity.

Biodegradation of NHs provides a new parameter to understand the complications in the balance of the quantitative relationship among hopanes and NHs. Some previously noted inconsistencies

Fig. 8. Molecular component ratios indicating different biodegradation susceptibility.
may be partially solved once the biodegradation of NHs is taken into account.

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