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Distribution of acids and nitrogen-containing compounds in biodegraded oils of the Liaohe Basin by negative ion ESI FT-ICR MS

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ABSTRACT

Seven reservoir core (tar sand) bitumens of identical source and similar maturity from the Liaohe Basin of northeast China possess a natural sequence of increasing severity of biodegradation. This set of samples provides us an opportunity to study the change in oil composition or compound class distributions with biodegradation severity by negative ion electrospray Fourier transform-ion cyclotron resonance mass spectrometry (FT ICR-MS). The bitumen extracts from two columns (Es3 and Es1) were separated into maltene and asphaltene fractions for analysis of heteroatomic species by ESI FT-ICR MS. The maltene fractions were found to mainly contain N1, N1O1, N1O2, N1O3, O1, O2, O3 and O4 classes, while the asphaltene fractions mainly contain N1, N2O1, N1O1, N1O2, N1O3, N1O4, O2, O3, O4 and O5 classes. These species identified by FT-ICR MS in asphaltene fractions are likely to be chemisorbed/coprecipitated compounds, or the species precipitated due to high polarity during deasphaltene process. The susceptibility of compound classes and homologous series to biodegradation was studied based on the relative abundances. The results indicate that microorganisms alter the distribution of acids and nitrogen-containing compounds by selective removal and preservation of certain classes of compounds according to their susceptibility to biodegradation. For example, O2 and N1O2 classes increase significantly while N1 and N1O1 classes decrease with biodegradation. The differences in the susceptibility to microbial alteration within acyclic acids, 4–5 ring acids and 1–2 ring acids are discussed and the differences in the susceptibility of homologous series of heteroatom-containing polycyclic aromatic hydrocarbons are also discussed in this work.

1. Introduction

Most of the world's crude oils are biodegraded (Larter et al., 2006). Anaerobic biodegradation of crude oil is a common phenomenon occurring in subsurface oil reservoirs (Aitken et al., 2004). It is widely accepted that microbial degradation significantly alters the molecular composition and physical properties of crude oil, leading to a decrease in low molecular weight saturates and aromatics and an increase in polars, oil density, viscosity, sulfur content and acidity (Evans et al., 1971; Connan, 1984; Meredith et al., 2000; Peters et al., 2005). Hydrocarbons are preferentially destroyed during biodegradation. The selective consumption of saturate and aromatic hydrocarbons during biodegradation is well documented (Connan, 1984; Hunt, 1995; Wenger et al., 2002; Peters et al., 2005). In general, normal alkanes are preferentially consumed, followed by branched alkanes, monocyclic paraffinic and monoaromatic hydrocarbons, multi-ring naphthenic and polynuclear aromatic hydrocarbons and finally non-hydrocarbons (Fedorak and Westlake, 1984a,b; Huang et al., 2003). Differences in the susceptibility of hydrocarbons to microbial alteration can also be observed within homologous series and even between isomers (Peters et al., 2005).

However, the biodegradation mechanism of the polar species is much less established than hydrocarbons. The analysis of polar compounds, in general, suffers from their thermal instability, low volatility and ineffective separations and detection by chromatography and mass spectrometry. Most of the previous studies are limited to low molecular weight (<500 Da) species that are amenable to gas or liquid chromatographic analysis (Hughey et al., 2002a; Hsu et al., 2003; Kim et al., 2005). The most commonly used analytical technique for complex mixture analysis, combined gas chromatography-mass spectrometry (GC–MS) (Hsu and Drinkwater, 2001), often lacks sufficient chromatographic resolution and mass resolving power to positively identify individual compounds beyond middle distillates, especially for severely biodegraded oil

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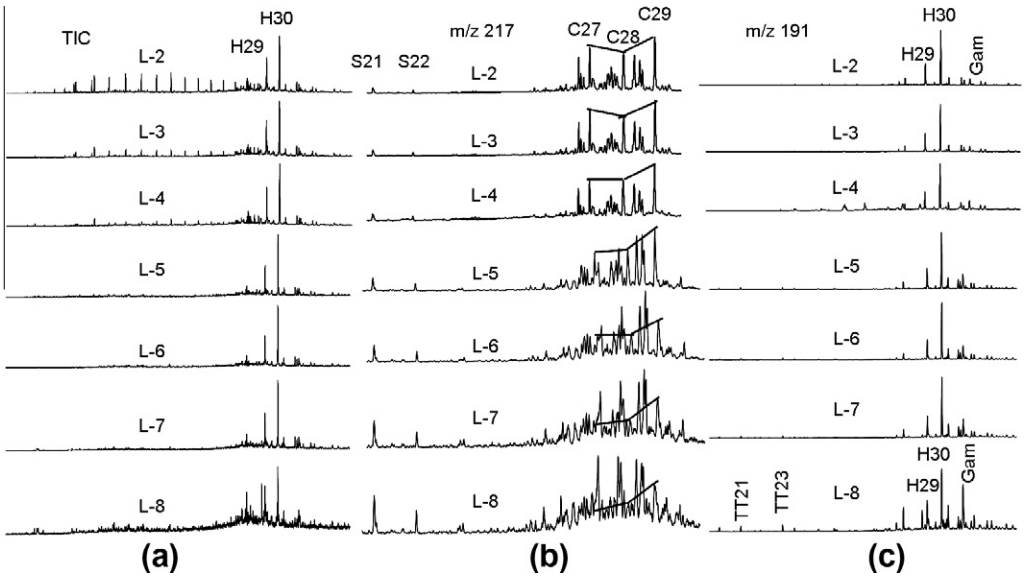

Fig. 1. GC–MS mass chromatograms of (a) TIC, (b) *m*/*z* 217, displaying steranes and (c) *m*/*z* 191, displaying terpanes in tar sand bitumens. S21 = C_{21} pregnane; S22 = C_{22} homo-pregnane; C27 = C_{27} ααα20R cholestane; C28 = C_{28} ααα20R ergostane; C29 = C_{29} ααα20R stigmastane; TT21–23 = $C_{21\text{-}23}$ tricyclic terpanes; H29–30 = $C_{29\text{-}30}$ 17α(H) 21β(H) hopanes; Gam = gammacerane.

samples containing fair amounts of polar components (Hughey et al., 2002b). Chromatograms often display an unresolved complex mixture (UCM) or hump (Behar and Albrecht, 1984; Meredith et al., 2000). In addition, most of the reference compounds are not commercially available for identification and confirmation (Galimberti et al., 2000).

The introduction of electrospray ionization (ESI) for hydrocarbon analysis in the late 1980s presented a new opportunity for characterizing polar, heteroatomic compounds in high boiling, heavy petroleum fractions (Fukuda et al., 1996; Hsu et al., 1998; Zhan and Fenn, 2000; Hughey et al., 2001, 2002a,b; Qian et al., 2001a,b, 2004; Oldenburg et al., 2009; Shi et al., 2010a,b). ESI coupled with high field (9.4 T) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) further provides ultra-high resolution in characterization of heteroatomic species in crude oils and their products (Marshall et al., 1998; Hughey et al., 2001, 2002b; Hsu and Drinkwater, 2001). Thus, ESI FT-ICR MS has become a powerful tool for research on biodegradation mechanisms of crude oils (Kim et al., 2005).

Asphaltenes are usually considered to be the components of highest molecular weight in petroleum. Resins and asphaltenes are usually distinguished from each other by their solubility in organic solvents (Strausz et al., 1999). The asphaltene fraction is defined typically as those species that are insoluble in low carbon number *n*-alkanes, such as *n*-heptane, *n*-hexane or *n*-pentane, while resins are soluble in such solvents (Tissot and Welte, 1984). Expelled asphaltenes resemble but are chemically distinct from their source kerogen, of smaller size and with less condensed aromatic nuclei (Behar and Albrecht, 1984; Pelet et al., 1986) and lack the weaker chemical bonds (di Primio et al., 2000). Most biodegradation studies have been conducted on saturated and aromatic hydrocarbon fractions and the increase of asphaltenes in heavy oil is often attributed to the selective removal of alkanes and some aromatic hydrocarbons (Tissot and Welte, 1984). However, a few studies have revealed that asphaltenes may be altered by microorganisms. Connan (1984) and Magot et al. (2000) suggested that microorganisms can feed on asphaltenes. Jenisch-Anton et al. (2000) indicated that geomacromolecules are degradable during aerobic biodegradation and new functionalities can be introduced into macromolecules by oxidation of side chains. In the study by Liao et al. (2009), the elemental (C, H, N, O, S) and isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of several tar sand bitumens which represent a natural biodegradation sequence, were measured. FT-IR was used to study the oxygenated functionalities of both resins and asphaltenes and Py-GC–MS was used to elucidate how alkyl side chains of asphaltenes were altered during biodegradation. The study by Liao et al. (2009) indicated that biodegradation may alter the composition and/or structure of asphaltenes.

In this paper, a series of tar sand extracts from the Liaohe Basin, representing a natural sequence of biodegradation (slightly to severely biodegraded), were separated into maltene and asphaltene fractions for subsequent analysis by negative ion ESI FT-ICR MS. These tar sand extracts were previously described and used by Liao et al. (2009). The influence of biodegradation on the distribution of neutral nitrogen and acidic compounds in both maltene and asphaltene fractions was explored. Furthermore, how such compositional variations may affect the elemental (C, H, O, N) and isotopic ($\delta^{13}C$, $\delta^{15}N$) compositions of resin and asphaltene fractions were also discussed.

2. Samples and experimental methods

2.1. Samples and pretreatment

Seven reservoir core (tar sand) bitumens from the Western Depression of the Liaohe Basin, in northeast China, were chosen for study. Three samples (L-2, L-3, L-4) are from the deeper Es3 column (1700–1850 m) and four samples (L-5, L-6, L-7, L-8) from the shallower Es1 column (1500–1650 m). These bitumens were generated from the same source rocks of similar maturity (Lu et al., 1990; Koopmans et al., 2002; Huang et al., 2003, 2004). Both columns exhibit a successive intensified biodegradation level toward the oil–water contact. These bitumens have suffered varying degrees of biodegradation with Es3 oils in the range of 2–4 and Es1 oils in the range of 5–8 on the Peters and Moldowan scale (abbreviated as PM level in following text; Peters et al., 2005). For convenience, tar sand bitumens are coded according to their biodegradation levels on the PM scale, e.g., tar sand bitumen of PM level 3 is coded as L-3. *n*-Alkanes were consumed progressively

in the deeper Es3 column (L2, L-3 and L-4) with increasing depth (Fig. 1) while regular steranes were altered progressively in the more severely biodegraded oils of the shallower Es1 column (L-5, L-6, L-7 and L-8). Although on a local scale diffusion and mixing may result in homogeneous composition over geological time (England et al., 1987), at large scales mixing is usually inefficient and the observed compositional gradients in the biodegraded oils of two columns can be attributed mainly to biodegradation (Huang et al., 2004).

A finely crushed oil sand sample (50 g) of reservoir core was added to 50 ml 93:7 dichloromethane (DCM):methanol in a 200 ml glass vial. The vial was sealed and sonicated for 30 min. The precipitate was separated by centrifugation and the extracted bitumen was removed to a clean vial where most of the solvent was evaporated under a stream of nitrogen. The details of deasphaltening procedure are described in Liao et al. (2009). Briefly, asphaltene was precipitated by adding 40 fold cold *n*-hexane into the vial containing the oil sand extract, sonicated for 15 min and separated by centrifuge. The maltene fraction was removed and the isolated asphaltene was washed repeatedly with hexane until the hexane washing solvent remained colorless. About 1 ml DCM was added to redissolve the asphaltene, the solution was sonicated and the asphaltene was re-precipitated and washed as described above. The whole process was repeated three times to purify the asphaltene. Both maltene and asphaltene fractions were analyzed by ESI FT-ICR MS. The maltene was fractionated into saturate, aromatic and resin fractions by silica gel/alumina column chromatography eluting with *n*-hexane, toluene, and 1:1 chloroform:methanol, respectively. The saturated hydrocarbon fraction was analyzed by GC–MS.

2.2. ESI FT-ICR MS analysis

Each of the maltene and asphaltene fractions was dissolved in toluene to produce a 10 mg/ml solution for ESI FT-ICR MS analysis. A total of 20 μl of the solution was diluted with 1 ml of 1:1 toluene:methanol (v:v). All solvents were twice distilled analytical reagent grade. The MS analysis was performed using a Bruker Apex Ultra FT-ICR MS equipped with a 9.4 T superconducting magnet. The sample solution was injected at 150 μl/h into the electrospray source using a syringe pump. The operating procedure for the negative ESI FT-ICR MS analysis has been described by Shi et al. (2010a,b). Briefly, the operating conditions for negative ion formation consisted of a −4.0 kV emitter voltage, −4.5 kV capillary column induction voltage and −320 V capillary column end voltage. Ions were accumulated in a hexapole (hexapole 1) for 0.1 s, then passed through a quadrupole, and collisionally cooled and accumulated in collision cell (hexapole 2) filled with argon bath gas for 1 s. The delay was set to 1.2 ms to transfer the ions from hexapole 2 to an ICR cell by electrostatic focusing. The mass range was set at *m/z* 200–1000 and the data size was set at 2 M words. The time domain data sets of 256 acquisitions were co-added.

2.3. Mass calibration and data analysis

The FT-ICR mass spectra were calibrated using alkyl carbazole series having relatively high abundance of negative ion ESI mass spectral peaks. Shi et al. (2010a) provided the details of calibration procedure. The peaks in the mass range of 200–800 Da with relative abundance greater than five times the standard deviation of the baseline noise were exported to a spreadsheet. Data analysis was performed using custom software, which has been described elsewhere (Shi et al., 2010a,b). Briefly, the data analysis was performed by selecting two mass segments near the most abundant peak of the spectrum, followed by identification of individual peaks. The peaks of at least one of the heteroatom classes, such as the carbazole series, were selected as reference for mass calibration. The accurate masses measured were converted onto Kendrick mass scale (Hsu et al., 1992) for determining elemental composition (or double bond equivalent (DBE) values and carbon number) by matching the measured mass defects with Kendrick mass defects (KMD) of known compositions in data base within a window of ±0.001 Da. The number of rings plus double bonds in molecules is designated as DBE value (Hughey et al., 2001, 2004).

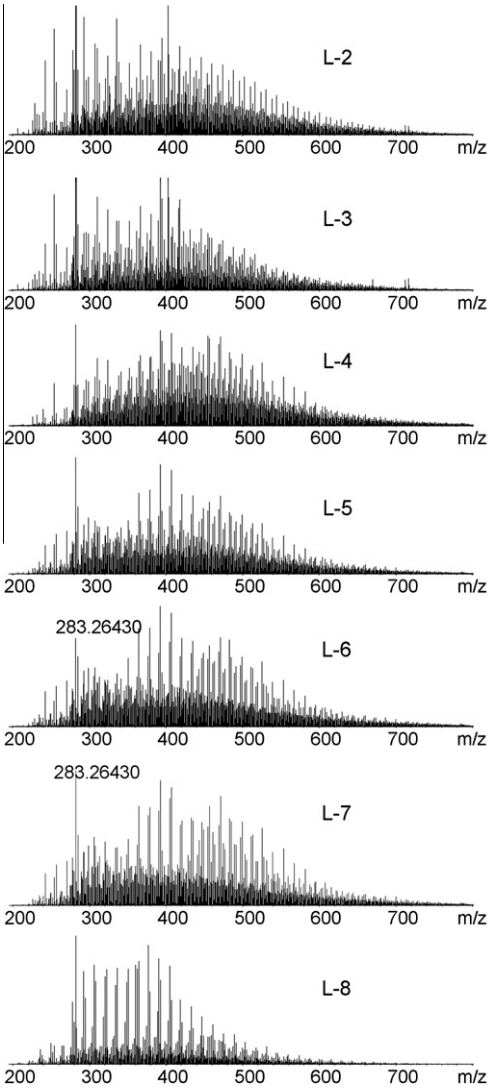

Fig. 2. Negative-ion electrospray FT-ICR mass spectra of the maltene fractions separated from biodegraded tar sand bitumens (*m/z* 200–800).

2.4. GC–MS analysis

Analysis of the saturate biomarkers was performed employing an Agilent 6890 N GC coupled with an Agilent 5973 N mass spectrometer. A DB-5 (Agilent) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) was used for the GC–MS analysis. The injector temperature was kept at 290 °C. The column was held at 40 °C for 5 min, ramped to 300 °C at 4 °C/min, and then held at 300 °C for 20 min. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. The ion source was kept at 260 °C and operated in the electron impact ionization (EI) mode with electron beam energy of 70 eV. The relative

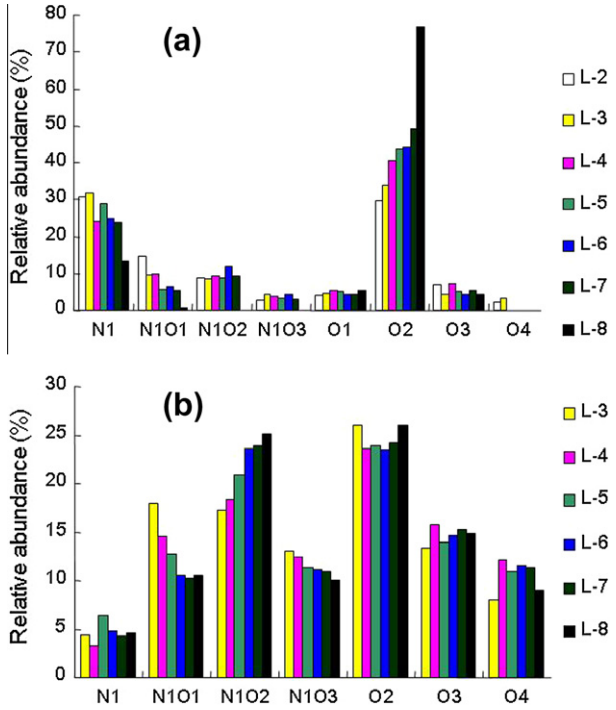

Fig. 3. Distribution of Heteroatom classes determined by negative-ion ESI FT-ICR mass spectra: (a) maltene fractions; (b) asphaltene fractions (no L-2 asphaltene fraction available).

abundance of biomarkers was calculated from the peak area in the chromatograms.

3. Results and discussion

Both Kim et al. (2005) and Oldenburg et al. (2009) had presented some FT-ICR MS results with different sample sets on how biodegradation can affect the composition of polar compounds. Oldenburg et al. (2009) even concluded that organic nitrogen compounds may be nutrient sources of microorganisms during biodegradation based on the results of the elemental composition, isotopic composition and FT-ICR MS analysis. In our work, tar sand extracts were first separated into maltene and asphaltene fractions before negative ion ESI FT-ICR MS analysis. Fig. 2 shows the broadband negative ion ESI FT-ICR mass spectra of the maltene fractions separated from biodegraded tar sand bitumens of the Liaohe Basin. Both the number of components and the average molecular weight of polar compounds decrease with biodegradation. The most severely biodegraded oil, L-8, contains the least number of peaks. L-2 contains more than 10,000 peaks (>6σ of baseline noise) at 200–800 Da, of which about 5000 were assigned molecular formulas by accurate masses. Since biodegradation could selectively reduce compounds with different carbon skeletons, it is not surprising that some heteroatomic species were selectively removed. However, the reduction of the number of peaks with increasing biodegradation could also be related to the limit of dynamic range in FT-ICR MS. Naphthenic acids have good selectivity in ESI ionization source, other species would be suppressed and some of them might be undetectable due to limited dynamic range of FT-ICR MS. The content of asphaltene in L-2 is low and the separated asphaltene was not available. Hence, only 6 asphaltene samples were analyzed by ESI FT-ICR MS in this research. The distributions of heteroatomic species in maltene and asphaltene fractions are discussed below.

3.1. Compound class distribution

The relative abundance of compound classes in the maltene and asphaltene fractions is presented in Fig. 3. The compound classes identified are characterized by the type and the number of hetero-atoms. N1, N1O1, N1O2, N1O3, O1, O2, O3 and O4 classes were found to be present in maltene fractions. Relative abundance of each class was calculated by normalizing the peak area to the total area. The relative abundance of N1 class in maltene fractions decreases with biodegradation in both columns Es3 and Es1 (Fig. 3a), especially in the range of L-5 to L-8, in which the oils are at heavy to severe biodegradation stages. There is also a decrease in the relative abundance of N1O1 class that was almost completely removed at the severe biodegradation stage of L-8. As discussed above, this decrease could be due to selective removal of N1O1 class during biodegradation and/or ion suppression of other classes in ESI. There is only a very slight increase in the relative abundance of the O1 class in both columns of Es3 (L-2 to L-4) and Es1 (L-5 to L-8) with biodegradation. Fig. 3a clearly shows a significant increase in the O2 class with biodegradation. O2 is the most abundant heteroatom class in our studies. This is different from the results reported by Shi et al. (2010b) on a non-biodegraded oil from Liaohe Basin in which the N1 class was most abundant. This difference can be attributed to the selective removal of N1 class and/or the generation of O2 class during biodegradation.

The compound classes identified from the mass spectra of the asphaltene fractions were N1, N2O1, N1O1, N1O2, N1O3, N1O4, O2, O3, O5 and O5. For convenience, only the relative abundance of the same classes as in maltene fractions is calculated and presented in Fig. 3b. N1O2 and N1O3 classes are richer in asphaltene fractions than in maltene fractions (Fig. 3). Moreover, N1O4 and O5 classes were presented in asphaltenes, but absent in maltenes. Basically, these multi-heteroatom compounds are preferentially precipitated in the deasphaltene process due to their higher aromaticity and polarity that decrease their solubility in *n*-hexane (Chang and Fogler, 1994; Shi et al., 2010b).

Because of the extremely complex composition of crude oil, it is impossible to isolate asphaltene compounds with specific chemical structures (Chang and Fogler, 1994). Typically, asphaltenes were defined as the crude oil fractions that are insoluble in aliphatic solvents but soluble in aromatic solvents; thus, asphaltenes separated from crude oil by low molecular weight alkanes contain the most polar and the highest molecular weight species of crude oil (Chang and Fogler, 1994). Peng et al. (1999) also indicated that low molecular weight asphaltenes usually comprise chemisorbed and coprecipitated resins along with low molecular weight asphaltene fragments. Since it may be difficult to completely ionize asphaltene fractions by ESI due to the wide molecular weight range, the species analyzed by FT-ICR MS may only account for lower molecular weight compounds in asphaltene fractions.

The N1O1 and N1O3 classes decrease with biodegradation in both maltene and asphaltene fractions, while N1O2 class increase with biodegradation. The increases could be contributed to not only the oxidation of hydrocarbons, but also the preferential removal of other heteroatomic classes. The selective consumption of heteroatomic classes during biodegradation may provide an explanation to the variations in both elemental and isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of resins and asphaltenes observed by Liao et al. (2009) and Oldenburg et al. (2009).

3.2. The distribution of O-containing compounds

3.2.1. O1 species

In the slightly biodegraded bitumen (L-2), the O1 class is dominated by DBE = 1 with a carbon number range of 20–32. These species appear to be alcohols. They decrease rapidly to zero in

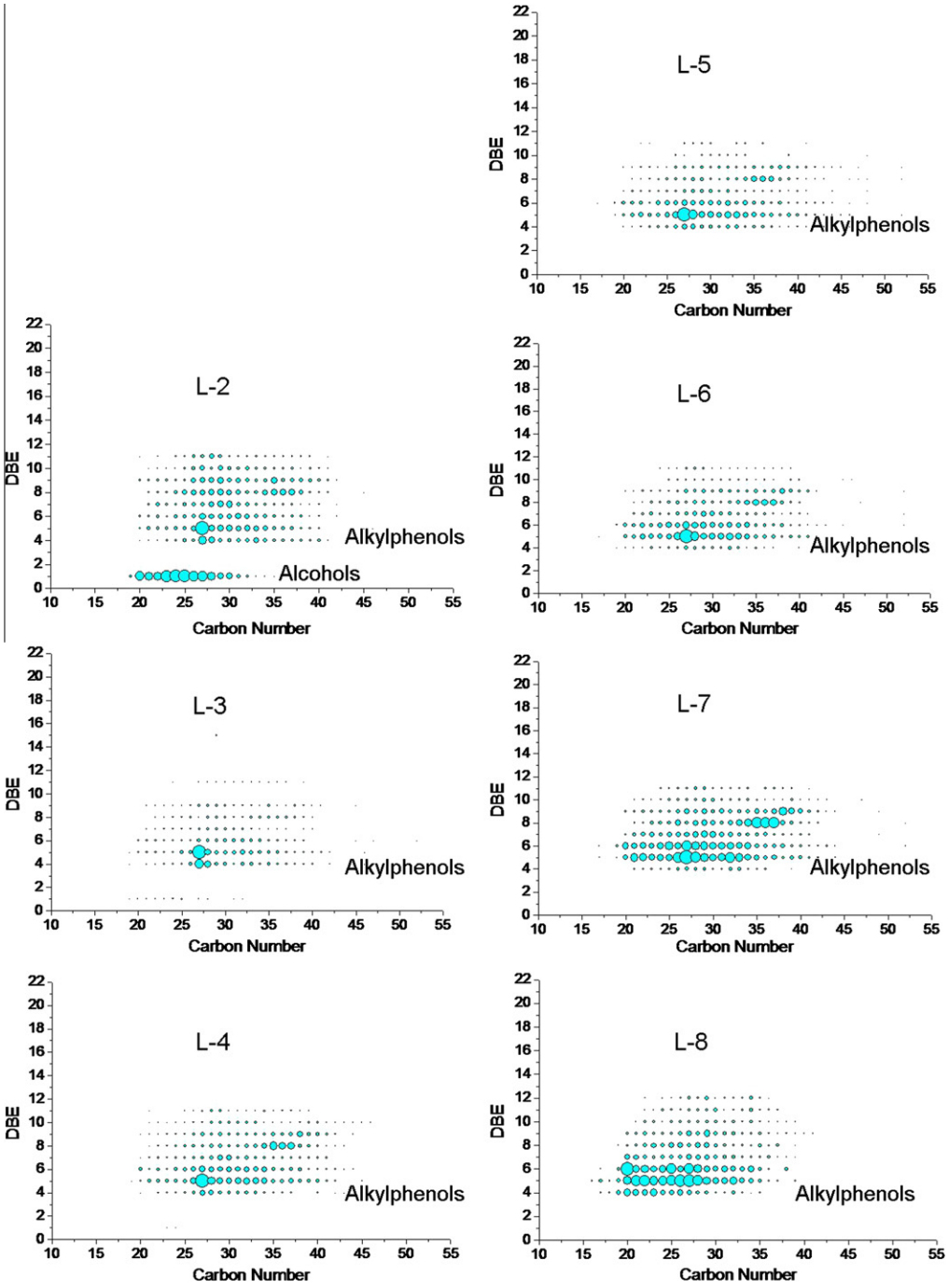

Fig. 4. Iso-abundance plots of DBE versus carbon number of O1 class in maltene fractions. The size of circles represents the relative abundance of O1 species in the spectra.

L-2 to L-4 (Fig. 4). No species with DBE values <4 were detected in L-5 to L-8, suggesting that these O1 compounds (DBE $\geqslant$ 4) are phenolic (on aromatic rings) rather than hydroxyl (on saturate rings). In maltene fractions of L-3 to L-7, the most abundant species (DBE = 5 and carbon number of 27) is interpreted to be unsaturated isoprenoid phenol, consistent with isoprenoid phenol having a DBE of 4 and carbon number of 27 recently identified by Zhang et al. (2011). Alkylphenols, with a DBE of 4, can be produced during the anaerobic degradation of aromatic hydrocarbons (Vogel and Grbic-Galic, 1986; Grbic-Galic and Vogel, 1987), which are considered as intermediates in a number of biodegradation pathways of petroleum components. They may be completely consumed during further biodegradation under both aerobic and anaerobic conditions (Harwood et al., 1998).

3.2.2. O2 class

There have been many reports on the detailed analysis of carboxylic acids, particularly for naphthenic acids, in crude oils. The compounds identified include linear fatty, isoprenoid, monocyclic, polycyclic and aromatic acids (Seifert and Teeter, 1970a,b; Meredith et al., 2000). Atlas (1984) proposed that carboxylic acids may be intermediates of biodegradation. However, Behar and Albrecht

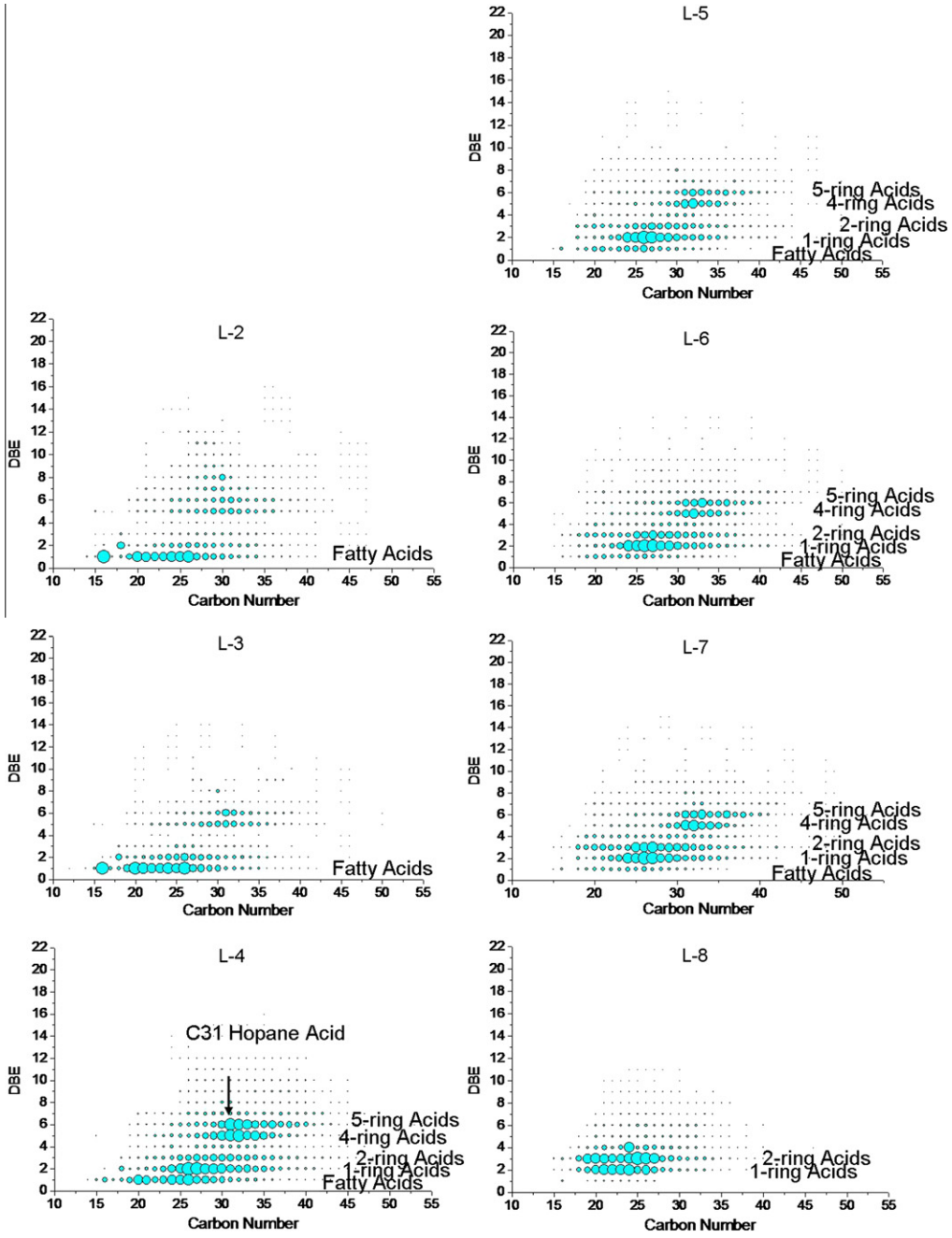

Fig. 5. Iso-abundance plots of DBE versus carbon number of O2 class in maltene fractions. The size of circles represents the relative abundance of O2 species in the spectra.

(1984) reported that acid concentrations in some oils decreased with increasing degrees of biodegradation. Watson et al. (2002) suggested that carboxylic acids in crude oils could have three different sources: the primary acids from the source rock, the oil biodegradation products and the acids biosynthesised by microorganisms. The carboxylic acids in crude oil may be continuously removed by secondary alterations such as water washing, further biodegradation and thermal alteration (Mackenzie et al., 1983).

Figs. 5 and 6 shows that fatty acids and isoprenoid acids (DBE = 1) are dominant O2 species in both maltene and asphaltene fractions in lightly biodegraded oils (L-2 and L-3). The progressive decrease of fatty acids with biodegradation suggests the highest susceptibility of fatty acids to biodegradation among O2 compounds. Aerobic biodegradation pathway of *n*-alkanes involves the oxidation of the terminal methyl group with the formation of alcohols, and dehydrogenation of the alcohols via aldehydes to carboxylic acids (Watson et al., 2002). Anaerobic alkane activation mechanism differs completely from that of aerobic alkane activation (Widdel and Rabus, 2001; Wilkes et al., 2002). Anaerobic degradation of *n*-alkanes, branched alkanes and pristane was detected in enriched bacterial communities that reduce nitrates (Bregnard et al., 1997; Rabus et al., 1999; Ehrenreich et al., 2000) and sulfates (Aeckersberg et al., 1991; Rueter et al., 1994; So and Young, 1999). Anaerobic biodegradation of long chain *n*-alkanes to form methane and CO_2 by methanogenesis (Zengler et al., 1999; Anderson and Lovley, 2000) was demonstrated. The anaerobic activation of

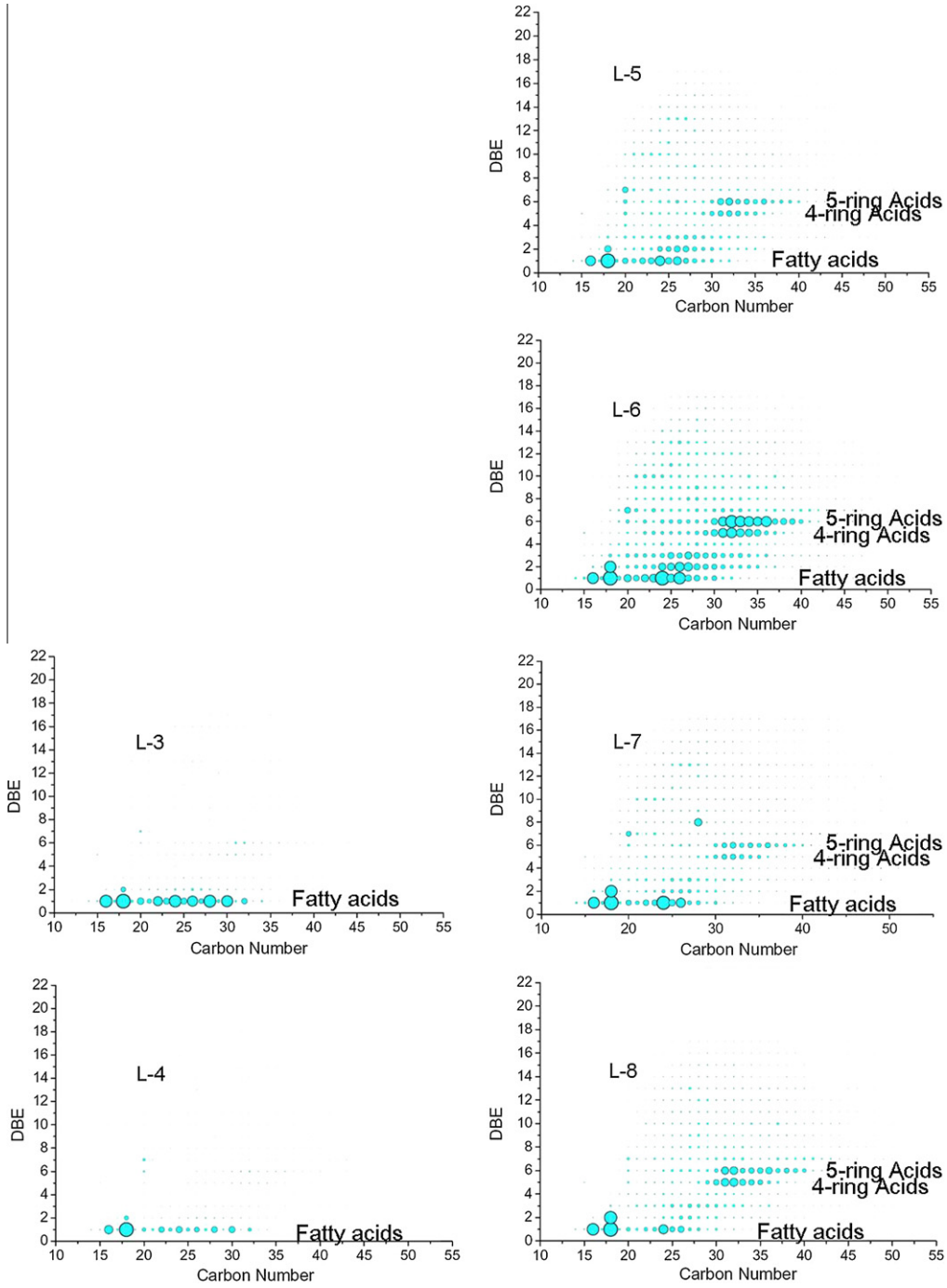

Fig. 6. Relative abundance as a function of DBE value of O2 species in asphaltene fractions (no L-2 asphaltene fraction available). The size of circles represents the relative abundance of O2 species in the spectra.

n-alkanes and branched alkanes is similar to the anaerobic activation of toluene by the addition of *n*-alkane via a radical mechanism to fumarate (Wilkes et al., 2002). Carboxylic acids can be metabolized by β-oxidation pathway leading to the formation of CO_2 as end product (Singer and Finnerty, 1984; Watkinson and Morgan, 1990; Wilkes et al., 2002). Fig. 6 shows that there are considerable amounts of fatty acids in asphaltene fractions at all biodegradation levels. The presence of these organic acids suggested that intermolecular hydrogen bonding in asphaltenes is common. Those hydrogen bonding acids may be precipitated together with asphaltenes during deasphalting process.

The O2 species with DBE values of 2–7 are naphthenic acids of 1–6 naphthenic rings, which have been confirmed by GC–MS and MS using various ionization techniques (Shi et al., 2010b). The species with a DBE of 5 are likely 4-ring steroidal acids (Seifert et al., 1972; Rodrigues et al., 2000; Peters et al., 2005) and/or secohopanoic acids (Nascimento et al., 1999; Shi et al., 2010b). The species with a DBE of 6 are likely 5-ring hopanoic acids (Jaffé and Gallardo, 1993; Watson et al., 2002). Watson et al. (2002) indicated that hopanoic acids can be formed by the oxidation of corresponding hopanes during aerobic biodegradation of crude oils with side chain oxidation. However, hopanoic, secohopanoic and steroid

acids are even present in lightly biodegraded oils (L-2), shown in Fig. 5. Study by Behar and Albrecht (1984) also showed that the non-biodegraded oils contain pentacyclic hopanoids and tricyclic terpenoids in acid fractions. Thus it indicates that these 4–5 ring naphthenic acids identified in our biodegraded oils should at least partly be primary acids from source rock. These 4–5 ring naphthenic acids become progressively dominant O2 species with biodegradation due to the progressive consumption of fatty acids. But 4–5 ring naphthenic acids are no longer dominant species in maltene fraction of L-8. The relative abundances of 4–5 ring naphthenic acids are very low in maltene fraction of L-8 relative to 1–2 ring naphthenic acids (Fig. 5).

Fig. 1 shows how the distribution of steranes (a) and hopanes (b) in crude oils are progressively altered by microbial organisms when the oils were severely biodegraded. The homo-pregnane (S_{22}), and C_{23} tricyclic terpane (TT23) are considered to be more recalcitrant to biodegradation than most regular steranes and terpanes (Peters et al., 2005). So the ratios of C_{27}–C_{29} regular steranes to C_{22} homo-pregnane and the ratios of terpanes to C_{23} tricyclic terpane are calculated to show the variations in the distribution of C_{27}–C_{29} regular steranes and terpanes. Table 1 lists the ratios of C_{27}–C_{29} regular steranes to S_{22} and the ratios of terpanes to TT23 calculated from the peak areas on the *m*/*z* 217 and *m*/*z* 191 mass chromatograms, respectively. The ratios of C_{27} cholestane to C_{22} homo-pregnane (C_{27}/S_{22}), C_{28} ergostane to C_{22} homo-pregnane (C_{28}/S_{22}) and C_{29} stigmastane to C_{22} homo-pregnane (C_{29}/S_{22}) decrease significantly from L-5 to L-8. The relative abundance of 4-ring acids increase with biodegradation in the range of PM level 2–4, due to the consumption of fatty acids. Then 4-ring acids decrease significantly relative to 1–2 ring acids. However, it is hard to compare the bioresistance between steranes and 4-ring naphthenic acids in this work. The ratios of C_{27} 18α trisnorhopane to tricyclic terpane (Ts/TT23), C_{27} 17α trisnorhopane to tricyclic terpane (Tm/TT23), gammacerane to C_{23} tricyclic terpane (Gam/TT23) and moretane to C_{23} tricyclic terpane (Mor/TT23) are not significantly altered in the range of PM level 2–8. However, the ratios of hopanes to C_{23} tricyclic terpane (H29–30/TT23) and homohopanes to C_{23} tricyclic terpane (H31–34S/TT23, H31–34R/TT23) are almost constant in the range of PM level 2–7, but decreased significantly in L-8, while the relative abundance of hopanoid acids are quite low relative to 1–2 ring acids at PM level 8 (Fig. 5). Behar and Albrecht (1984) indicated that pentacyclic acids and hydrocarbons undergo similar biodegradation pathways with a preferential attack of the higher homologs bearing a linear side chain, and the alkanes appear to be more resistant than corresponding acids. Our work seems consistent with the observation by Behar and Albrecht (1984).

The relative abundances of 3-ring acids to 5-ring acids are very low, consistent with very low relative amount of tricyclic terpanes to hopanes in the crude oils of the Liaohe Basin shown in Fig. 1. In the biodegraded oils studied by Kim et al. (2005), 2–3 ring naphthenic acids become dominated O2 species progressively, accompanying the decreasing of 4–5 ring naphthenic acids. It indicates 2–3 ring naphthenic acids may have higher bioresistance than 4–5 ring naphthenic acids.

The relative abundances of 1–2 ring naphthenic acids in maltene fraction increases with biodegradation and finally becomes dominant O2 species in maltene fraction of the severely biodegraded oil L-8 (Fig. 5). Study by Kim et al. (2005) also showed that there are abundant 1–2 ring naphthenic acids in severely biodegraded oils. With carbon numbers between 18 and 34, the monocyclic (1 ring) acids are expected to contain long alkyl chains with cyclopentane and cyclohexane substructures. The long chain alkyl cyclopentanes and cyclohexanes are known to have similar biodegradation susceptibility as branched alkanes (Peters et al., 2005). In addition, cyclopentanes and cyclohexanes are usually absent or in trace quantities by PM levels 4–5. Bicyclic (2 ring) acids are expected to have the structure of bicyclic terpanes. Although bicyclic terpanes are less susceptible to biodegradation than branched alkanes, they usually are completely absent before the onset of biodegradation of steranes and hopanes (Peters et al., 2005). Thus 1–2 ring naphthenic acids seem to have less susceptibility to biodegradation than corresponding alkanes. The carbon numbers of 1–2 ring naphthenic acids decrease significantly in the range of PM level 7–8. It indicates that alkyl side chains of 1–2 ring naphthenic acids are shortened. Side chain oxidation of hopanes was observed in laboratory aerobic biodegradation experiment of Watson et al. (2002).

Table 1
Biomarker ratios based on peak area calculation.

Parameters	Tar sand extract						
	L-2	L-3	L-4	L-5	L-6	L-7	L-8
Ts*/TT23	2.09	1.80	1.27	1.29	1.36	1.21	1.34
Tm/TT23	4.10	4.61	4.11	4.08	4.28	3.93	3.79
Gam/TT23	9.50	10.67	12.21	12.00	13.50	12.55	10.96
Mor/TT23	6.53	7.62	6.79	6.55	7.96	6.85	4.46
H29/TT23	14.83	17.62	13.24	12.46	14.18	11.30	4.87
H30/TT23	42.33	48.22	37.98	34.78	41.03	30.68	11.08
H31S/TT23	7.67	8.53	5.59	5.95	7.50	5.53	2.23
H31R/TT23	5.42	6.21	3.95	4.45	5.37	3.98	1.74
H32S/TT23	4.49	5.02	3.99	3.75	4.38	3.58	1.68
H32R/TT23	3.53	3.83	3.07	2.90	3.59	3.48	2.52
H33S/TT23	2.88	3.14	2.58	2.74	2.88	2.98	1.79
H33R/TT23	2.01	2.48	1.88	2.02	2.30	2.14	1.83
H34S/TT23	1.56	2.15	1.60	1.49	1.69	1.72	1.34
C27/S22	16.68	18.71	14.55	5.85	2.57	0.69	0.38
C28/S22	26.37	24.40	21.24	7.80	3.56	1.60	1.30
C29/S22	33.23	33.52	26.15	11.19	6.89	4.77	3.41

* TT23 = C_{23} tricyclic terpanes; Ts = C_{27} 18α trisnorhopane; Tm = C_{27} 17α trisnorhopane; Gam = gammacerane; Mor = moretane; H29–30 = $C_{29\text{-}30}$ 17α(H) 21β(H) hopanes; H31–34S = $C_{31\text{-}34}$ 22S 17α(H) 21β(H) homohopanes; H31–34R = $C_{31\text{-}34}$ 22R 17α(H) 21β(H) homohopanes; C27 = C_{27} ααα20R cholestane; C28 = C_{28} ααα20R ergostane; C29 = C_{29} ααα20R stigmastane; S22 = C_{22} homo-pregnane.

Species with DBE >7 are likely multi-ring naphthenic acids and aromatic acids. Aerobic metabolism of aromatic compounds is characterized by the extensive use of molecular oxygen which is essential for the hydroxylation and cleavage of aromatic ring structures (Harwood et al., 1998). The biodegradation of aromatic compounds generally involves a ring-opening reaction and the formation of dihydroxy intermediates which are then cleaved to form aliphatic carboxylic acids for monoaromatic compounds and aromatic acids for polycyclic aromatic hydrocarbons (Gibson and Subramanian, 1984; Cerniglia, 1992). In the anaerobic metabolism of low molecular weight aromatic compounds, on the other hand, aromaticity is broken by reduction followed by hydrolytic ring opening as observed in most cases (Harwood et al., 1998). In previous studies, the bioresistance of alkyl benzenes and polynuclear aromatic hydrocarbons (PAHs) increases with numbers of aromatic rings and alkyl substituents (Volkman et al., 1984; Williams et al., 1986; Peters et al., 2005), but decrease with the length of side chain (Huang et al., 2004).

3.3. The distribution of N-containing compounds

In the maltene fractions, DBE values of the N1 class are in the range of 6–17 (Fig. 7). The most abundant N1 species with DBE = 9, 12 and 15 are likely carbazoles (DBE = 9), benzocarbazoles (DBE = 12) and dibenzocarbazoles (DBE = 15) (Hughey et al., 2002b). However, in the asphaltene fractions, the N1 class contains higher DBE values in the range of DBE = 8–21 (Fig. 8), indicating higher aromaticity in the molecules. The N1 class is dominated by homologous series of dibenzocarbazoles (DBE = 15) and

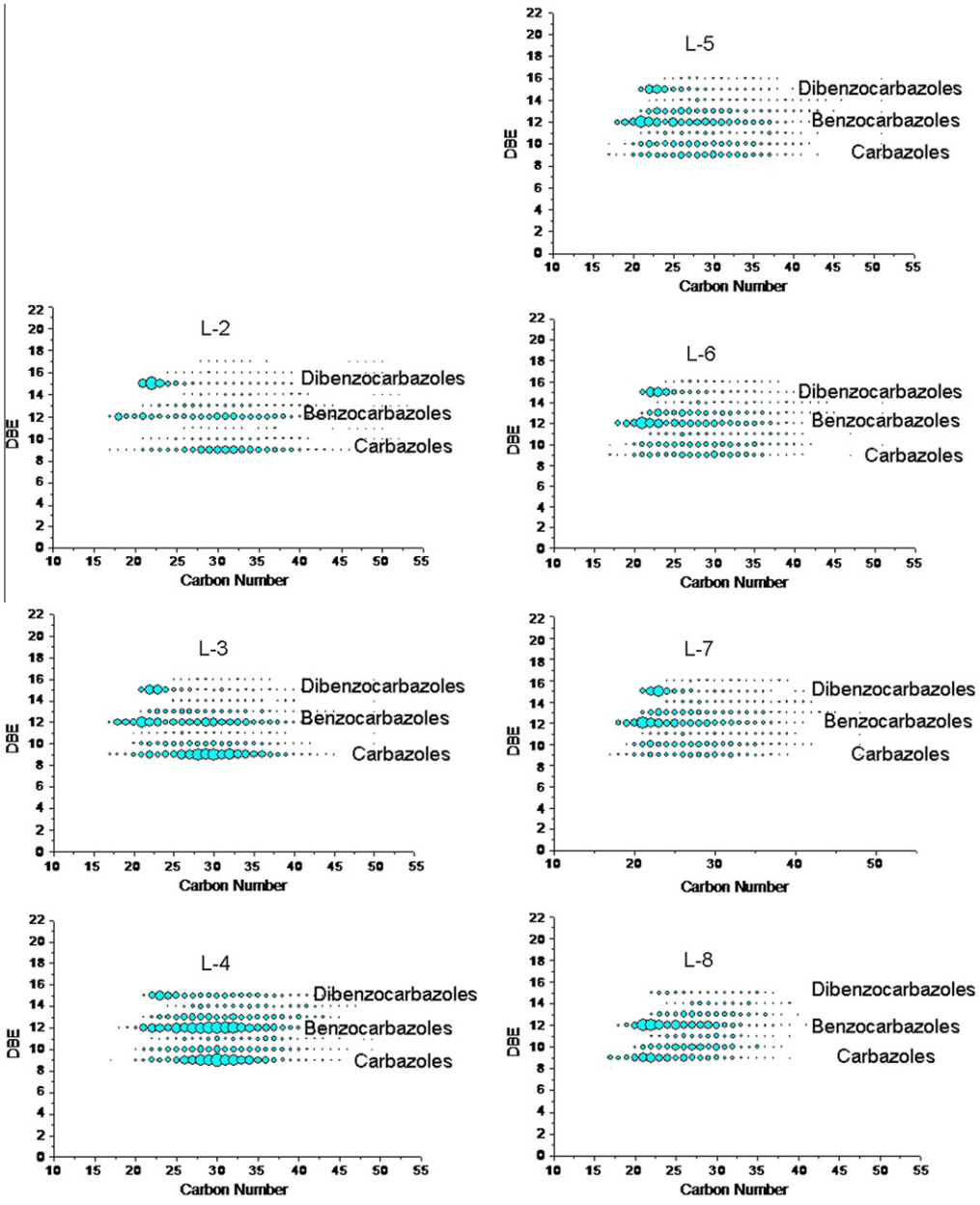

Fig. 7. Iso-abundance plots of DBE versus carbon number of N1 class in maltene fractions. The size of circles represents the relative abundance of N1 species in the spectra.

benzonaphthocarbazoles (DBE = 18) that are readily precipitated by *n*-hexane in deasphaltene process due to higher polarity and higher aromaticity (Shi et al., 2010b).

Fig. 7 shows the decrease of all N1 compounds with biodegradation in maltene fractions. The relative abundance of carbazoles decrease more significantly than benzocarbazoles and dibenzocarbazoles with biodegradation in L-4 to L-7. Huang et al. (2003) studied the susceptibility of carbazoles and benzocarbazoles of crude oils from the Liaohe Basin by GC–MS and reached similar conclusions. Huang et al. (2003) further noted that the susceptibility of alkylated carbazoles to biodegradation decreases with increasing number of the alkyl substituents (Huang et al., 2003). Fig. 7 shows that there is progressive decrease in carbon number with biodegradation. The carbon numbers of the carbazoles in the maltene fraction are mainly in the ranges of 16–53 in L-2, while the carbon numbers are 16–42 in L-8. It suggests that homologous series of carbazoles with longer alkyl side chains are preferentially altered by microorganisms. It is also consistent with the FT-ICR MS results of Kim et al. (2005).

Figs. 9 shows that the relative abundances of N1O1 species in both maltene and asphaltene fractions decrease with biodegradation. N1O1 compounds are considered to be intermediates of N1 species biodegradation (Kim et al., 2005). Shi et al. (2010a) suggested that the oxygen functional group in the N1O1 compounds is a hydroxyl rather than a cyclic ether group because such compounds are present in polar resin fractions rather than in non-polar aromatic hydrocarbon fractions. In maltene fraction of L-2, N1O1 compounds with DBE values of 10, 13 and 15 are predominant. The predominance of N1O1 compounds with DBE of 10 and 13 progressively diminish with biodegradation in the range of PM levels

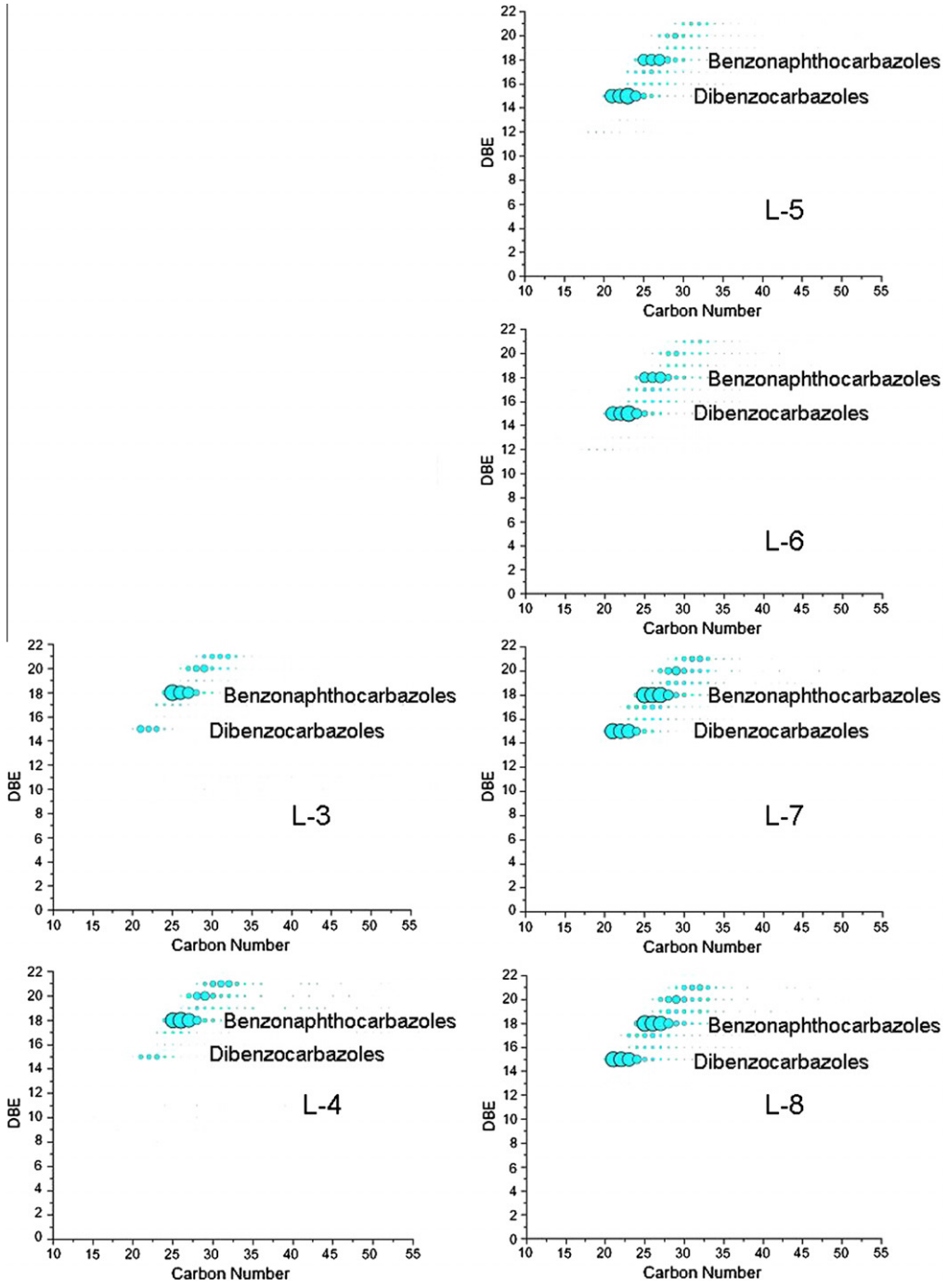

Fig. 8. Iso-abundance plots of DBE versus carbon number of N1 class in asphaltene fractions (no L-2 asphaltene fraction available). The size of circles represents the relative abundance of N1 species in the spectra.

2–4, resulting in the dominance of N1O1 compounds with DBE of 15 in L-5 to L-7 (Fig. 9a). This suggests the increasing number of aromatic rings fused to a carbazole structure increases the resistance to biodegradation. However, it is difficult to identify the structure of all N1O1 compounds with high certainty. The N1O1 species with DBE = 15 are identified as homologous series of dibenzocarbazoles with a hydroxyl groups attached to the core structure because the only structure possible for DBE = 15 with carbon number of 20 is hydroxyl dibenzocarbazole. Previous studies (Benedik et al., 1998; Bressler and Fedorak, 2000; Riddle et al., 2003) suggested that the aromatic or cyclic ring containing a nitrogen atom is readily broken by bacteria during the aerobic biodegradation of carbazoles, and then the nitrogen atom is removed and replaced with hydroxyl or carboxyl groups. Kim et al. (2005) suggested that the biodegradation pathway of N-containing aromatic compounds in anaerobic biodegradation included a ring-opening reaction that decreases DBE similar to aromatic hydrocarbons. In asphaltene fractions, N1O1 compounds with DBE values of 13, 15 and 18 are predominant over other compounds. Similar to N1O1 species in maltene fraction, the relative abundance of N1O1 species

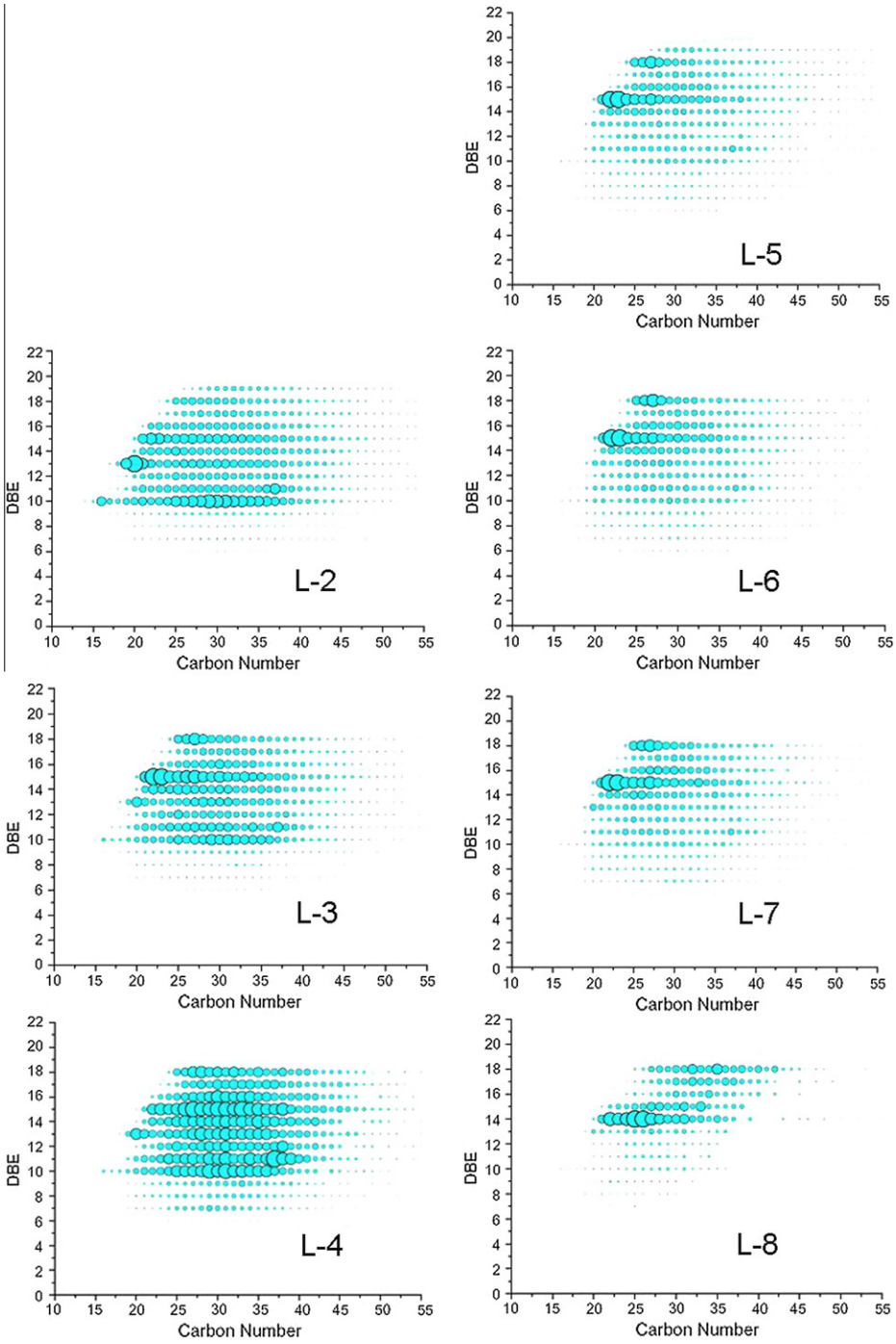

Fig. 9. Iso-abundance plots of DBE versus carbon number of N1O1 class in maltene fractions. The size of circles represents the relative abundance of N1O1 species in the spectra.

in asphaltene fractions also decreases with biodegradation (Figs. 3 and 9), suggesting the consumption of N1O1 species during biodegradation.

In both maltene and asphaltene fractions, N1O2 species increase with biodegradation (Fig. 3). N1O2 class is richer in asphaltene fractions than in maltene fractions. The N1O2 species appears to be biodegradation products of N-containing compounds. N1O2 compounds can be formed from ring opening of hydroxyl dibenzocarbazoles with additional of a carboxyl group. In maltene fractions of oils with PM > 4, the DBE spread of the N1O2 class becomes narrower with biodegradation in both maltene and asphaltene fractions, leading to the increase in relative magnitude of N1O2 compounds with lower DBE values (DBE = 10 to 15). In asphaltene fractions, the DBE ranges of the dominant N1O2 species also changed from 10–20 to 10–15 gradually. It seems that microorganisms preferentially removed N-containing aromatic acids with higher DBE values during biodegradation. However, increasing number of aromatic rings fused to a carbazole structure usually increases the resistance to biodegradation. Thus further studies are still needed to elucidate how N1O2 species are altered in biodegradation (see Figs. 10 and 11).

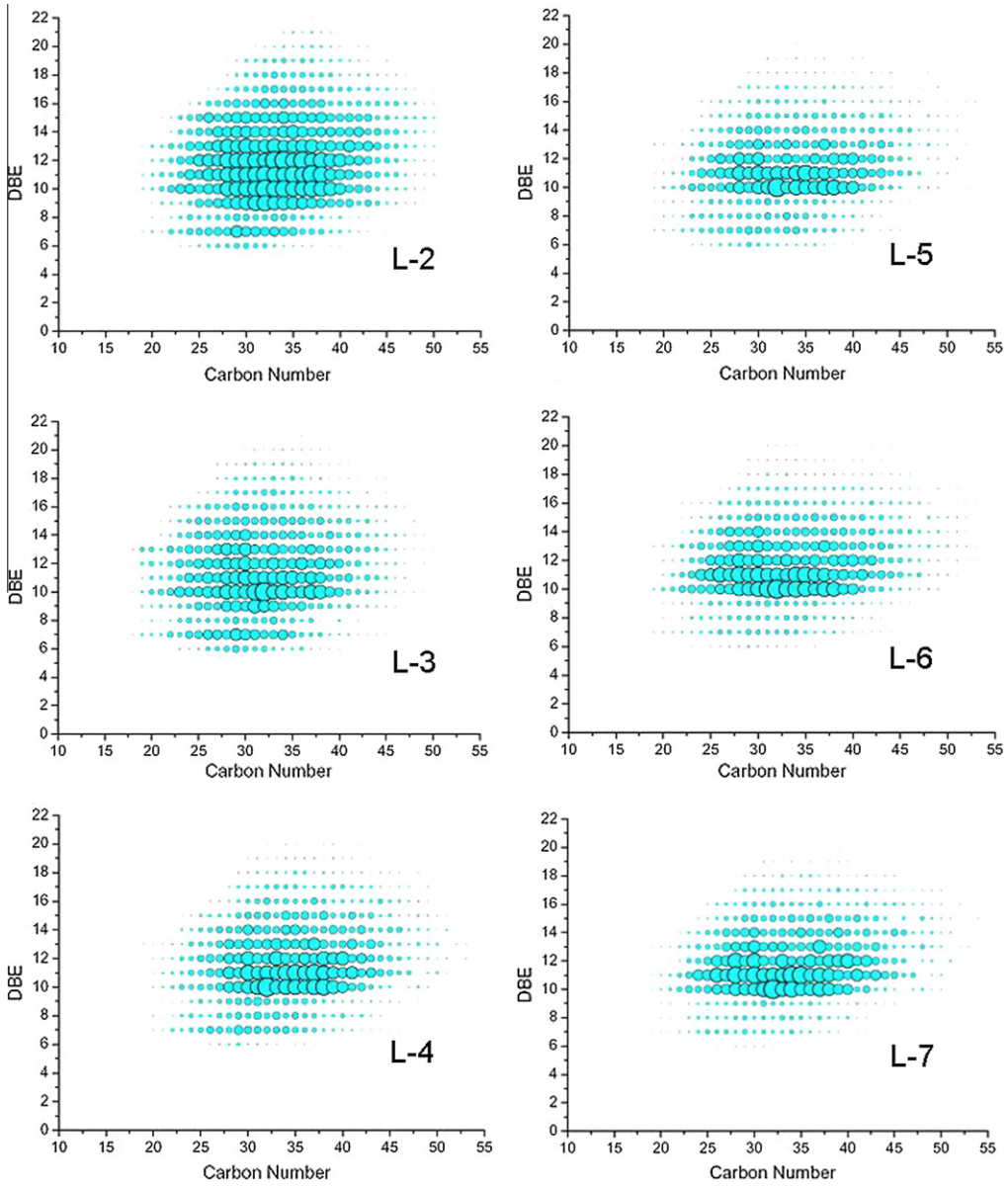

Fig. 10. Iso-abundance plots of DBE versus carbon number of N1O2 class in maltene fractions. The size of circles represents the relative abundance of N1O2 species in the spectra.

4. Conclusions

The FT-ICR MS results based on seven tar sand bitumens of various biodegradation levels suggest that microorganisms alter the distributions of acids and N-containing compounds by selective removal and preservation of certain compound classes according to their susceptibility to biodegradation. Differences in the susceptibility to microbial alteration can be observed not only within different classes, but also within homologous series. The O2 and N1O2 classes increase significantly with biodegradation while N1 and N1O1 classes decrease with biodegradation. In the O1 and O2 classes, acyclic compounds have higher susceptibility than cyclic compounds. The relative abundance of naphthenic acids increases with biodegradation, accompanying the consumption of acyclic acids. 1–2 ring acids seem to have lower susceptibility than 4–5 ring naphthenic acids. The relative abundance of 1–2 ring acids increase with biodegradation and become the dominant acids at L-8, but 1–2 ring acids are also altered with decrease in alkyl side chain length. The susceptibility of N1 species to biodegradation follows the order of carbazoles > benzocarbazoles > dibenzocarbazoles, while the N1O1 species follow similar order of hydroxyl carbazoles > hydroxyl benzocarbazoles > hydroxyl dibenzocarbazole. For N1 species and N1O1 species, more fused ring numbers and more alkyl side chains can usually lead to lower susceptibility in biodegradation, but longer side chain can lead to higher susceptibility. Quite a few heteroatom containing species are identified by ESI FT-ICR MS in asphaltene fractions. These species are likely to be chemisorbed/coprecipitated compounds in asphaltene fractions, or the species precipitated due to high polarity during deasphaltene process. The compositions of these species are also altered to some extent with increasing biodegradation.

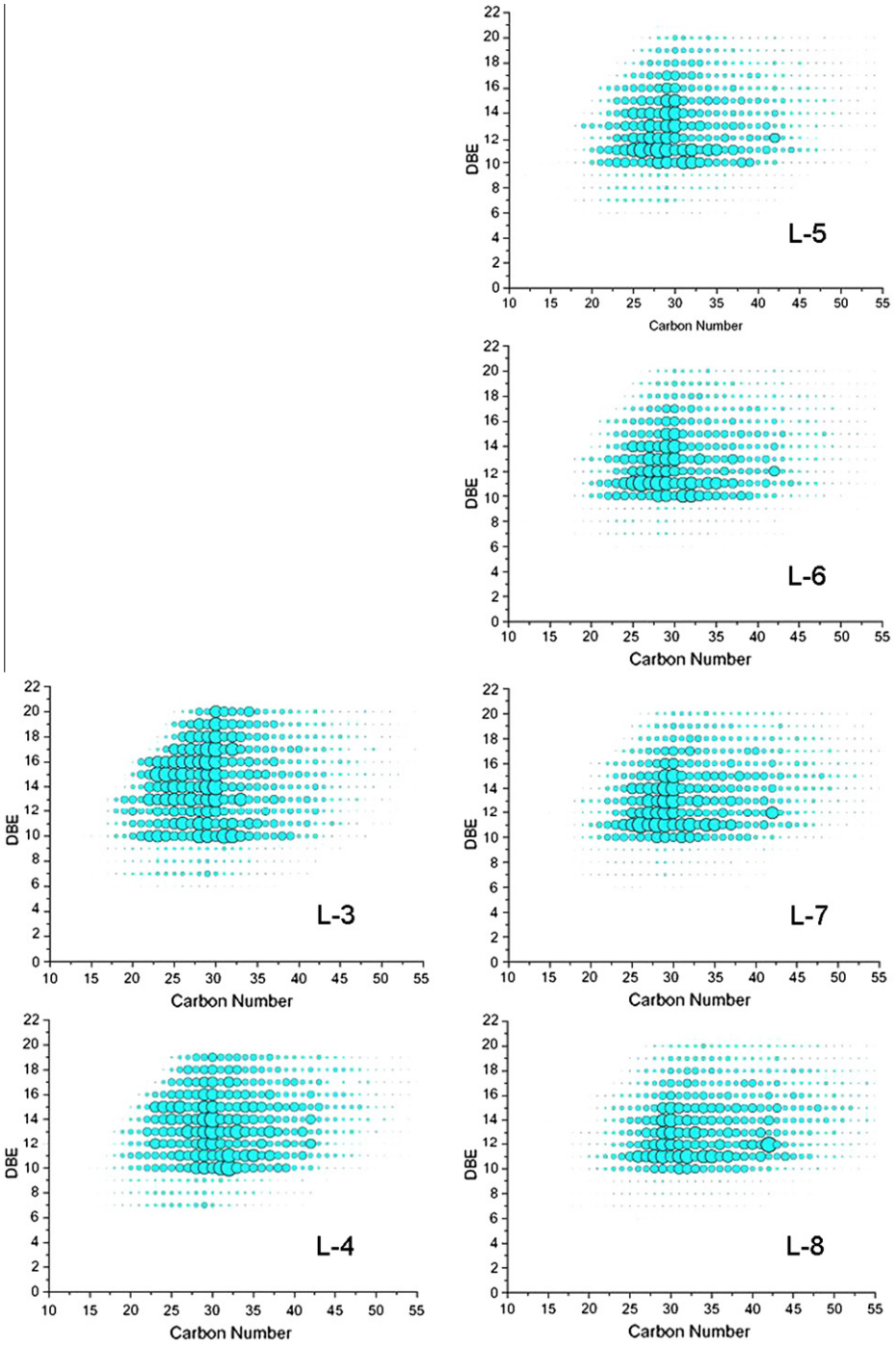

Fig. 11. Iso-abundance plots of DBE versus carbon number of N1O2 class in asphaltene fractions (no L-2 asphaltene fraction available). The size of circles represents the relative abundance of N1O2 species in the spectra.

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