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The controls on the composition of biodegraded oils in the deep subsurface-

Part 4. Destruction and production of high molecular weight non-hydrocarbon species and destruction of aromatic hydrocarbons during progressive in-reservoir biodegradation

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Abstract

This study extends the analysis of previously well studied biodegraded crude oil case history sample sets. The analytical window is extended into the high molecular weight, aromatic

hydrocarbon and non-hydrocarbon fraction of crude oils, using a 12 T ultra-high resolution mass spectrometer (FTICR-MS).

Biodegradation is pervasive across compound groups and extent of degradation appears dependent on compound abundance and hence availability. Oil constituents with molecular weights up to m/z 600 (carbon number 44) are affected by in-reservoir biodegradation. Apart from special, specific compound groups possibly related to the active reservoir biomass, all hydrocarbon and single heteroatom-containing compound classes are depleted by biodegradation. Production of various highly alkylated species indicate that transformation of crude oil components often involves derivatization and preservation rather than just complete destruction of high molecular weight compounds. Whereas one case study shows good correlation between depletion of S1 species and a strong increase in SO2 species, a nitrogen enriched oil suite shows an analogous trend in the transformation of N1 species to the corresponding NO2 species. Increase in O2 species are seen in both sample sets indicating partial oxidation is a major overall process in in-situ reservoir biodegradation. These variations are important geochemically but also impact transport, interfacial and corrosion properties of oils.

Nitrogen isotope systematics indicate that nitrogen-containing compounds might act as nitrogen nutrient sources or mainly as carbon sources for the microorganisms causing in-situ reservoir biodegradation depending of level of biodegradation. Distributions of some heterocyclic species add a very biodegradation resistant parameter set, to the petroleum geochemists arsenal.

Keywords: In-reservoir biodegradation; FTICR-MS; carbon-nitrogen nutrient source; oil destruction; oxidation; high molecular weight compounds; polar species; nitrogen isotope systematics; biodegradation resistant parameter set

1. Introduction

Recent studies have indicated that the biodegradation process in petroleum reservoirs is driven by slow anaerobic microbial activity at the interface between oil and water in petroleum reservoirs (Head et al., 2003; Bennett et al. 2013). The overall process occurs over a multi-million year timescale and involves direct reaction of hydrocarbons and water to produce carbon dioxide and methane, and leaves a residual heavy oil (Larter et al., 2003; 2006; Adams et al., 2013; Larter and Head, 2014). The process is characterized geochemically by the production of compositional gradients in a variety of compound types within oil columns, with the most rapid and evident destruction and/or production of new compounds seen at the base of the oil column in the oil-water-transition-zone (OWTZ). To date, our work has demonstrated compositional gradients in hydrocarbons, both aliphatic and aromatic, and low molecular weight non-hydrocarbons, such as alkylated aromatic nitrogen and sulfur compounds, including alkylcarbazoles and alkylidibenzothiophenes (Huang et al., 2003; Marciano et al., 2013; Adams et al., 2013; Bennett et al. 2013).

Bennett et al. (2013) performed an integrated geochemical and microbiological study of a transect through an approximately 600 m deep and 20 m thick oil column and oil-water transition zone from a biodegraded oil reservoir in Western Canada. The oils were variably degraded throughout the oil column, ranging from PM 3-5 on the Peters and Moldowan (1993)

and Peters et al. (2005) scale, and from 152 to 823 for Mn2 (Manco number 2; Larter et al., 2012). Dead oil viscosities of the mechanically extracted oils at 20 °C ranged from ca. 50 kCp (0.05McP) at the top of the oil column, to ca. 1.4 McP at the oil water transition zone (OWTZ) contact and continued to increase into the OWTZ, where the oil viscosities exceed 10.0 McP (at 20 °C). Gradients of decreasing concentrations of both hydrocarbons and low molecular weight aromatic non-hydrocarbons were measured. The degradation of hydrocarbons and non-hydrocarbons continues below the oil column into the oil-water transition zone, where the fraction of the pore volume occupied by oil decreases downwards transitionally over several meters towards the fully water saturated part of the reservoir. The behaviour of different compound classes appears as a cascading set of decreasing component concentration gradients related to biodegradation, with the most biodegradation-susceptible species, e.g. *n*-alkanes, disappearing first at the highest point within the degradation zone at the base of the oil column, and with more resistant components, such as alkylaromatic species, disappearing deeper within the OWTZ.

Bennett et al. (2013) also assessed the bacterial abundance within the reservoir, using quantitative PCR, showing that substantial bacterial populations are present in a zone immediately below the oil column in the OWTZ, with maximum cell abundance at the zone of maximum hydrocarbon depletion extent, just at the top of the OWTZ. The maximum bacterial abundances seen in the OWTZ of the reservoir are in line with the trend of bacterial abundance with depth that emerged from analysis of microbial cells in deep subsurface sediments (Parkes et al., 1994). The bacterial abundance is about 2 orders of magnitude higher within the burnout zone (biodegradation zone at the base of a biodegraded oil column), consistent with the notion

that microbial activity and abundance in the deep subsurface is elevated at geochemical interfaces (Parkes et al., 2005).

Petroleum non-hydrocarbons also are biodegraded in reservoirs. Several studies have shown chemical concentration gradients driven by basal biodegradation zones in reservoirs affecting C9-C14 alkyl, benzo and dibenzothiophenes (Marcano et al., 2013), and alkyl carbazoles and benzocarbazoles (Huang et al., 2003). The systematics of the in-reservoir degradation of these species is very similar to the biodegradation of other alkyl aromatic compounds in a similar molecular weight range.

The non-hydrocarbon fraction of crude oils (comprising mostly of nitrogen, sulfur and oxygen (NSO)-containing organic compounds) can make up a significant proportion of petroleum, with sulfur contents commonly a few percent in some heavy oils and in extreme cases in Alberta, rising to over 10% or more. Although the importance of the heteroatom compounds has been recognized in many of the processes involved in the origin and alteration of petroleum (Hunt, 1995; Tissot and Welte, 1984), their geochemistry as a broad group, is poorly understood under subsurface biodegradation conditions and it is here that we focus this paper. The potential value of NSO species in crude oils, for the definition and understanding of geochemical properties and processes such as biodegradation and any associated water washing, lies in the much greater range of physicochemical properties exhibited by these compounds, compared to hydrocarbons. While low molecular weight hetero-aromatic compounds can be analyzed by GC-MS, the bulk of the non-hydrocarbon fraction in crude oils is not accessible to GC-based analytical methods. However, recent advances, such as ultra-high resolution mass spectrometry (Fourier transform ion cyclotron resonance mass spectrometry; Marshall and Rogers, 2004),

now allow routine analysis of these often sorptive and involatile hetero-aromatic compounds. While truly quantitative data are not yet available from such instruments, the instruments are sufficiently stable and data are sufficiently reproducible, such that qualitative and semi-quantitative analysis of processes is now possible (Oldenburg et al., 2014).

In this study, several case history sample sets of crude oils, biodegraded to different extents and analyzed geochemically, are extended into the high molecular weight, non-hydrocarbon fraction of crude oils using a 12 T ultra-high resolution Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR-MS), operated in electrospray ionization (ESI), and atmospheric pressure photoionization modes (APPI), which allows us to examine many species of variable polarity including high molecular weight aromatic hydrocarbon species.

2. Methods

All oil samples or reservoir core solvent extracts were analyzed, without fractionation, using a 12 Tesla Bruker Apex FTICR-MS located at the Tesla Petroleomics Centre in the University of Calgary. Samples were prepared by diluting whole oils to a final concentration of 0.25 mg/mL, in a solvent composed of equal parts of toluene-methanol. Just before analysis, the samples were spiked with 1 ppm of reserpine, an internal standard containing various functional groups detectable in all ionization modes and 1 % of ammonium hydroxide (dopant) for analysis in negative ion electrospray ionization (ESI-N), or with formic acid (dopant) for analysis in positive ion electrospray (ESI-P), or without dopant for analysis in atmospheric pressure photoionization (APPI-P) positive ion mode using a krypton lamp at 10.6 eV. The samples were introduced into the mass spectrometer using a syringe pump set to deliver 240 μ L/h. The instrument was tuned

and optimized using a set of standard compounds and oils and calibrated daily. Internal standards are included in each sample suite to ensure mass accuracy. Typically, for each sample, 200 scans are collected and summed to improve the experimental signal-to-noise ratio. For a typical crude oil sample the mass range of the instrument is set to between 165.88 and 1400.00 Da. The resolving power at 400 Da is $>500,000$ ($M/\Delta M_{fwhm}$). The mass accuracy is typically < 200 ppb.

The FTICR-MS raw data were processed using Composer (Sierra Analytics). Ragnarök (Aphorist Inc.), data processing software for quantitation, visualization and interpretation of multidimensional and multi-sample FTICR-MS data. Apart from the determination of molecular masses and ring number/ unsaturation levels for each species, assessed as double bond equivalents-DBE (derived as $DBE (C_cH_hN_nS_sO_o) = c-h/2+n/2+1$) of detectable individual species in whole oils or bitumen fractions (see the definitions below), we also derived lumped, summed abundances of whole heteroatom classes (a heteroatom class is a group of compounds with a uniform heteroatom population), DBE group distributions within a heteroatom class, carbon number distributions of an individual group of pseudohomolog compounds and also a variety of plots, including modified Kendrick plots and averaged molecular mass plots for related groups of compounds. The terminology we use in this paper is defined in the chart in Appendix 1, and is fully described in Oldenburg et al. (2014).

3. Samples and Case Histories - Background

While systematic concentration gradients in hydrocarbon components analyzed in oil reservoirs undergoing active crude oil biodegradation have been discussed before (Larter et al., 2003;

2006; 2008; Huang et al., 2003; Oldenburg, 2004; Oldenburg et al., 2009a; Fustic et al., 2012), there have been far fewer studies looking at the biodegradation induced, compositional gradients seen in petroleum non-hydrocarbons. Huang et al (2003) have presented the most comprehensive description to date, describing changes in alkylated, aromatic nitrogen compounds caused by biodegradation, while Marcano et al. (2013) and Adams et al. (2013) described changes in aromatic sulfur compounds vertically throughout an oil column that is undergoing active biodegradation.

3.1. Case histories

In this paper we focus mainly on samples from two case histories of quite different petroleum system settings showing a wide range of biodegradation level. The first case history setting is from the Western Canada Sedimentary Basin (WCSB), a foreland basin where the oils studied are from a reservoir charged from Palaeozoic and Mesozoic source rocks, sourcing high sulfur crude oils (Bennett et al., 2013; Adams et al., 2014). This sample suite consists of six oils extracted from a reservoir core in a single reservoir profile from the Peace River Oil Sands, Alberta, Canada, that spans a biodegradation range of PM level 3-5 and a Manco number range of 150 to 823, above the OWTZ (Bennett et al., 2013; Larter et al., 2012). The case history is described in detail in Bennett et al (2013) and a detailed background to the origin of the Peace River oil sands is described in Adams et al. (2014). Perhaps unsurprisingly, in this case, the major biodegradation-related compound transformations are observed in the sulfur-bearing aromatic species within the crude oils.

The second case study is from the Liaohe petroleum system from a rift basin in North East China, where the source rocks are Eocene, lacustrine, clastic source rocks sourcing crude oils low in sulfur, but with significant nitrogen (Huang et al., 2003; Oldenburg et al., 2004). This sample suite consists of five core extract oils spanning a biodegradation range of PM level 1-8, from two closely related, sandstone reservoirs of different ages and depths. This is a subsample of a well studied sample suite consisting of seventeen oils, eight from the shallower ES1 reservoir [biodegradation level PM 5 to 8] and nine samples from the deeper ES3 reservoir [biodegradation level PM 1 to 5 (Huang et al., 2004). The oils show a progressive and continuous increase in the level of oil biodegradation down through the oil columns and the samples were selected to cover a range of alteration from PM level 1-8, (Huang et al., 2003). In this example, the major visible biodegradation transformations occur in the aromatic nitrogen compound fraction of the crude oils.

Supplementing the case studies are additional samples of related oils from the North Sea petroleum province and other oils from Western Canada and Chinese basins. These samples are at varying levels of biodegradation and are included for reference and comparison.

4. Results and Discussion

4.1. Western Canada Sedimentary Basin case history

The first case history looks at a series of oils extracted from a reservoir core profile from the Peace River Oil Sands.

Figure 1 shows geophysical, fluid property and petroleum geochemical logs through the reservoir (modified after Bennett et al., 2013) and a summary of key fluxes, reservoir and biological system properties and cell counts for a generalized biodegradation system (modified after Larter and Head, 2014). Clear trends of decreasing concentrations of saturated and aromatic hydrocarbon species are seen as we move down through the oil column into the oil-water transition zone. The geochemical gradients, fluid property gradients and the distribution of bacterial cells in the reservoir indicate that most of the biodegradation activity in the reservoir has been driven from processes occurring in the oil-water transition zone.

All the hydrocarbon and nitrogen, oxygen, mixed sulfur and nitrogen, mixed sulfur and oxygen, sulfur and multiple sulfur heteroatom class components show a relative decrease in overall class concentrations with increasing depth in the reservoir towards the oil-water transition zone, as analyzed in APPI positive ion mode (Fig. 2). This is consistent with the same trends in bulk non-hydrocarbon compound concentrations seen in the Iatroscan SARA analysis profile for the same reservoir (Bennett et al. 2013 and Figure 1C).

Using ESI negative ion mode, the compound class relative distributions of the acidic (deprotonatable) constituents throughout the reservoir show a different behaviour (Fig. 3).

While nitrogen and sulfur bearing heteroatom class components decrease in relative abundance with increasing depth and increasing biodegradation level in the reservoir, oxygen bearing heteroatom class components, including mixed sulfur and oxygen species, increase in relative abundance with increasing biodegradation level.

4.1.1. Sulfur-containing species

APPI-P data for petroleum within the reservoir show the variation in relative abundance of species containing one sulfur atom, as a function of double bond equivalents (ring number and/or aromaticity) at different reservoir positions (Fig. 4). As is seen from GC-MS analysis of individual aliphatic and alkylated aromatic species in the reservoir (Figure 1), absolute component abundances decrease with increasing levels of biodegradation in this reservoir (a similar trend was found for individual alkylated dibenzothiophenes (Fig. 7 in Bennett et al., 2013)). Higher molecular weight alkylated aromatic sulfur-containing species also show a systematic reduction in relative concentration with increasing biodegradation level. In addition, DBE 6 species - likely alkylated benzothiophenes - are preferentially degraded compared to the species with higher ring numbers (saturated or aromatic) such as the DBE 8 and 9 species likely containing two more saturated rings or an additional aromatic ring (likely dibenzothiophenes), respectively as shown in the triangular plot in Figure 5. A reduction in absolute concentration of alkylated aromatic sulfur bearing heterocycles in biodegrading oil systems with progressive biodegradation has been shown for several reservoirs in the Canadian oil Sands (Marcano et al., 2013; Bennett et al. 2013; Adams et al., 2014). It seems reasonable to infer therefore, that the relative changes seen in the apparent abundance of alkylated aromatic sulfur heterocycles, as seen in the FTICR-MS data, do reflect absolute reductions in concentration of these species in this reservoir, with progressive petroleum biodegradation.

Very similar trends for heteroatom compound species containing two sulfur atoms per molecule were observed (Fig. 6). Interestingly, whereas most of the DBE groups in the S1 class show clear differences in the degree of depletion through the oil column, between the top

samples (top and 2), the middle samples (3 and 4) and the two samples from the oil water transition zone (TZ), only the bottom two samples can be distinguished in terms of a different composition, for most of the DBE groups (especially >DBE9) for the S2 class. This may indicate that alkylated S2 class species are in general, more resistant to biodegradation compared to S1 species and that compound groups with low DBE numbers are more affected by biodegradation compared to their higher DBE group class members.

4.1.2. Nitrogen-containing species

The ESI-N data for single nitrogen-containing heteroatom compounds show the variation of species relative abundance, as a function of double bond equivalents and reservoir position (Fig. 7). Essentially all DBE compound groups show a decrease in relative concentration with increasing depth within the reservoir. The most dramatic reductions in relative abundance changes are seen in compounds that are plausibly alkylated carbazoles, followed by alkylated benzocarbazoles and to a lesser extent, alkylated dibenzocarbazoles. For all detectable single nitrogen atom bearing heteroatom compounds species, this result mirrors the removal with increasing biodegradation, seen by GC-MS analysis of low alkylation carbazoles and benzocarbazoles elsewhere, including in Canadian oil sands reservoirs (Huang et al., 2003; Oldenburg et al., 2004, Marcano et al., 2013). In addition, again there is clear evidence that lower DBE compound group members are more strongly affected by biodegradation compared to their more benzannulated analogs. In the triangular plot of Figure 8b this is illustrated by a relative depletion of alkylated carbazoles compared to their benzannulated homologs. Even though it is a narrow biodegradation level range, the trend of the changes in the pyrrolic DBE 9,

12, 15 compounds distributions follow the same as observed for the larger biodegradation level range (PM 1 to PM 8) of the other case study from the Liaohe Basin (Fig. 8a), which will be discussed under 4.2. The effect of biodegradation on the distribution of pyrrolic type species above biodegradation scale levels PM 3 (Peters and Moldowan, 1993) and Mn2 760 (Larter et al., 2012), has to be considered when applying the relative distribution of alkyl carbazoles, benzo- and dibenzocarbazoles to maturity assessments based on recently proposed maturity dependent proxies of Oldenburg et al. (2014).

4.1.3. Species containing one oxygen atom

Compounds with a single oxygen atom per heteroatom compound, likely species containing a hydroxyl group, are also affected by biodegradation (Fig. 9). As seen before with single heteroatom containing compound groups containing one nitrogen or one sulfur atom, similar trends are observed for compounds containing a single oxygen atom. All species show a decrease in relative abundance with increasing depth within the reservoir, which corresponds to increasing biodegradation extent with lower DBE group member compounds, especially components containing 4 and 5 DBEs being more strongly affected by biodegradation compared to class members with higher DBE numbers. A similar trend was observed by Kim et al. (2005), Oldenburg et al. (2009b), Martins et al. (2017) and Pan et al. (2017). In contrast, Pan et al. (2013) investigating Athabasca tar sand bitumen in the range of PM 6 to PM 7 reported a predominance of O1 species with DBEs of 5 and 6 in the maltene fraction. Another study from Angolini et al. (2015) showed an increase of O1 class from 0.25% to 1% within a biodegradation sequence from PM 0 to 6 with predominance at DBE 5 and 6 in the most biodegraded samples.

The pseudohomolog carbon number distribution of the most affected DBE groups with DBE 4 and 5 are shown in Figure 10. Of the DBE 4 species, a bimodal carbon number distribution is observed with high abundances at C₂₇₋₂₈ and around C₃₃. The former compound group (C₂₇₋₂₉) is possibly, at least partially, contributions from sterols. GC-MS analysis of BSTFA derivatized polar (DCM/methanol) fractions confirmed the presence of sterols throughout the reservoir column. Among the sterols the C₂₇ pseudohomolog is the most depleted followed by C₂₈ and C₂₉. However, most DBE 4 and 5 species seem to be affected by increasing levels of biodegradation down through the oil column, the intensity of removal, correlating with the relative concentrations of pseudohomologs. This means the most abundant species are the most rapidly degraded, independent of the molecular structure and carbon number within the compound group. This may indicate that degradation of high molecular weight organic species is pervasive and dependent more on the availability/relative concentration of precursors than by molecular structure. What is eaten is what is most abundant and of course that varies through the biodegradation process! In summary, single heteroatom N, S, O species all tend to decrease in relative abundance in oil with increasing levels of biodegradation in this case history.

4.1.4. Hydrocarbon species

This reduction in predominantly aromatic, alkylated, N, S, O heteroatom containing compound species, with increasing biodegradation level, reflects the same reduction seen in aromatic hydrocarbon content by GC-MS analysis of core extract petroleum (Figure 1A and C). This biodegradation related hydrocarbon destruction trend is also evident in APPI-P radical ion

analysis (RAD), of core extracts throughout the reservoir as seen in Figure 11. As shown for the DBE 7 and 10 hydrocarbon species, carbon number distributions of the pseudohomologs (likely alkyl naphthalenes and alkyl phenanthrenes, respectively; Figure 12A and C) suggest that biodegradation of hydrocarbons, with molecular weights higher than m/z 600 (carbon# >44), is occurring in this reservoir. In addition, in some cases a preferred degradation of the higher carbon number pseudohomologs compared to the less alkylated ones is observed, as shown for the HC RAD DBE 7 members (likely alkylated naphthalenes with summed alkylation of C_{20} , C_{25} and C_{30}) in the triangular plot in Figure 12B. The relative proportion of the alkylated naphthalenes with summed alkylation of C_{20} (total 30 carbons) increases by 5.5 % relative to the higher alkylated pseudohomologs with increasing biodegradation between the top to the bottom sample of the reservoir. Overall, as biodegradation proceeds, the average molecular mass (AMM), for each DBE compound group tends to decrease with increasing biodegradation extent. Thus as shown in Figure 12D, for hydrocarbons from the DBE 10 hydrocarbon group (likely alkyl phenanthrenes), an AMM reduction of 35 amu is seen between the top and the most biodegraded bottom oil sample analyzed in the reservoir (Fig. 12D).

While it is not possible to exclude the formation of single heteroatom compound species in this reservoir during biodegradation, it would appear, that for hydrocarbons and for single heteroatom oxygen, nitrogen and sulfur species, the primary alteration process is one of compound removal during biodegradation. The situation is however quite different, it would appear, if we look at compounds with more than one oxygen atom present in a single molecule, or compounds with mixed oxygen and sulfur heteroatom complements.

4.1.5. Naphthenic acid species

ESI-N FTICR-MS data for core extract petroleum, analysed as a function of reservoir depth for heteroatom compounds containing two oxygen atoms per molecule, show how the relative abundances of different species vary by DBE group (Fig. 13). The most abundant species present in the petroleum have DBE group numbers of 2 and 3 and are likely, alkylated single and double ring naphthenic acids and show a dramatic increase in relative abundance of these species, with increasing reservoir depth and increasing level of biodegradation. The inset diagram shows the relative abundance ratio of total O2 heteroatom compounds, to total N1 heteroatom compounds which, as might be expected from the discussion above, shows a substantial increase throughout the petroleum column with increasing reservoir depth. The DBE 1 carbon number distributions of the pseudohomologs (likely fatty acids and/or alkenediols; Figure 14A) show strong even-odd predominance (EOP) between C₂₇ and C₃₇ throughout the reservoir. Terrestrial plants are usually considered as the main source, but some microalgae and/or bacteria are also suspected to be a minor source of these acids (Nichols et al., 1986; Volkman et al., 1989). As the former are unlikely to be the source in this setting, microorganisms are the suspects. The strongest effect of progressive biodegradation within this sequence (PM level 3-5), is observed between pseudohomologs C₂₂ and C₃₂ of the O2 DBE 1 compound group with no even-over-odd predominance occurring for the smaller species. Speculatively, these pseudohomologs might be metabolites from ongoing degradation processes. Whereas the two most abundant DBE groups 2 and 3 of the O2 class (likely mainly one and two ring naphthenic acids), show a strong increase in pseudohomologs between C₂₂ and C₄₀ (Figs. 14B and C) with increasing degree of biodegradation, O2 class DBE 7 members

only increase slightly in abundance in this carbon number range (Fig. 14D). However, a strong increase in relative abundance was observed for the pseudohomolog C₂₀. This relative increase of a single pseudohomolog with the molecular formula C₂₀H₂₈O₂ with increasing degree of biodegradation could be speculated to be related to the oil degrading microorganisms and belong to a terpenoid family component possibly similar to retinoic acid. However, this is highly speculative and further detailed investigations regarding possible molecular structures have to be carried out in future work. The production of carboxylic acids with increasing biodegradation in crude oil is well documented in the literature (e.g. Atkins et al., 2004; Kim et al., 2005; Jones et al., 2008; Hughey et al., 2008; Barrow et al., 2009; Oldenburg et al., 2009b; Liao et al., 2012; Pan et al., 2013, Vaz et al., 2013; Angolini et al., 2015, Martins et al., 2017; Pan et al., 2017), but the specific species produced vary dramatically between settings.

In this Canadian case history, with biodegradation levels in the range PM 3-5, one and two ring naphthenic acids are the dominant O₂ species produced, although substantial acidity occurs in other species as well. However, in other crude oil systems more complex variations in naphthenic acid distributions are observed. Thus, for example, ESI-N FTICR-MS data for O₂ heteroatom compound species for a suite of related oils from another, non-Canadian, marine shale sourced petroleum system show substantial evolution in the abundance of naphthenic acids and also, naphthene ring size, as a function of increasing biodegradation level from PM 0 to 9 (Fig. 15).

The total acid number values (TAN), for the different oils are also indicated in Figure 15.

Whereas oil A doesn't have any typical biodegradation related signatures, oil B is enriched especially in single and double ring naphthenic acids, similar to Peace River core extracts

discussed above. At higher degrees of biodegradation (oil C), the two ring naphthenic acids strongly dominate the relative abundance plots of oxygen species, whereas at severe levels of biodegradation (oil D; PM level 9) three ring naphthenic acids and even four ring naphthenic acids are highly enriched. DBE 4 species could also be related to diamondoid acids, as previously reported (Rowland et al., 2011). These variations are important geochemically, but are likely also to impact the corrosivity of the naphthenic acid mixture, as metal surface sorption properties of the different components will vary dramatically even as total acidity rises with increasing biodegradation. Increasing levels of petroleum biodegradation will also increase the concentrations of a variety of non-hydrocarbon species that ultimately will affect many of the transport and interfacial properties of the petroleum fluids. The response of individual petroleum types to biodegradation will vary and a rigorous examination of the detailed molecular variation of petroleum non-hydrocarbons as a function of corrosion impacts and interfacial properties with both oil-water-metal and oil-water systems is required.

4.1.6. Mixed sulfur-oxygen-containing species

ESI-N FTICR-MS data for core extract petroleum analysed as a function of depth, for heteroatom compounds containing two oxygen atoms and one sulfur atom per molecule show that all species appear to increase in relative abundance with increasing depth in the reservoir (increasing level of biodegradation) (Fig. 16). The most abundant species present in the petroleum have DBE numbers of four, five, seven, and eight and are unidentified. However, based on previous comparison studies with GC-MS data (e.g. Oldenburg et al., 2014) these compound groups are likely related to alkylated thiophenic type species containing a carboxylic

acid group (e.g. DBE 4: thiophenes carboxylic acid; DBE 5: tetrahydrobenzothiophenes carboxylic acid; DBE 7: benzothiophenes carboxylic acid; DBE 8: tetrahydrodibenzothiophenes carboxylic acid). These species are most likely oxidation products of alkylated thiophenes, tetrahydrobenzothiophenes, benzothiophenes and tetrahydrodibenzothiophenes. For DBE 4 and 5 group species a strong increase in abundance is observed in the range between C_{23} and C_{35} , whereas for DBE 7 and 8 compounds, the range expands to C_{40} (Fig. 17). There is a good correlation between the observed depletion in S1 RAD heteroatom compound class depletion (Fig. 4) and the increase in SO_2 species that suggests that the SO_2 species are indeed likely oxidation products of the S1 constituents. For instance, Figure 4 illustrates the strong depletion of alkylated benzothiophenes (DBE 6) and tetrahydrodibenzothiophenes (DBE 7) with depth, which corresponds to an observed increase in the more oxygenated products at DBE 7 (alkylated benzothiophene carboxylic acids) and DBE 8 (alkylated tetrahydrodibenzothiophene carboxylic acids). This is not observed for the non-oxidized precursors of the SO_2 DBE 4 and 5 group species. The reason for this might be related to ion suppression effects occurring for the small thiophene (DBE 3) and tetrahydrobenzothiophene (DBE 4) molecules relative their benzannulated homologs. The ionization potential of these species in APPI-P ionization mode, to produce radical ions is lower, compared to the larger more aromatic species, as the ionization potential decreases with increasing numbers of fused benzene rings (Kuroda, 1964; Harada et al., 1999). The production of carboxylic acids with increasing biodegradation in crude oil is well documented in the literature (e.g. Atkins et al., 2004; Kim et al., 2005; Hughey et al., 2008; Barrow et al., 2009; Oldenburg et al., 2009b; Liao et al., 2012; Pan et al., 2013, Vaz et al., 2013; Angolini et al., 2015, Martins et al., 2017; Pan et al., 2017), but this is the first time that

mixed heteroatom, non-hydrocarbon species have been seen to increase in relative abundance with increasing level of petroleum biodegradation. This is very important, as mixed heteroatom species would appear to be very common in oil sands bitumen and related heavy oils, and with increasing concern over oil spillage during transportation and water body interaction with these materials, it will be important to obtain quantitative data and toxicological information on these diverse mixtures of previously unidentified species, as they have largely unknown toxicological and water solubility properties (Overton et al., 2016).

In summary, for the Canadian case study, it would appear that while single heteroatom non-hydrocarbon compounds and mixed heteroatom compounds with a single oxygen or nitrogen atom, are destroyed or partially oxidized during biodegradation, some groups of compounds, principally containing two oxygen atoms, are generated during the biodegradation process and these include mixed sulfur and oxygen species, as well as the expected carboxyl bearing alkylated naphthenic acids. It seems that biodegradation is attacking essentially all species present in the crude oil with the most abundant species being preferentially attacked at any biodegradation level. In addition, some preserved possible biomarker products in biodegraded crude oils may be indications of the biodegradation biome.

4.2. North East China case history

The second suite of samples discussed here is a set of 5 related oils (out of 17 samples studied) from the Liaohe Basin in Northeast China. The oils show a progressive and continuous increase in the level of oil biodegradation down through the oil columns and the samples were selected to cover a range of alteration from PM level 1-8, (Huang et al., 2003). Previously, broad studies

of pyrrole-type species in petroleum were limited to low molecular weight alkylated carbazoles, benzo- and dibenzocarbazoles, with less than three alkyl carbon substituents, due to the analytical restrictions of GC-MS (Larter et al., 1996). Huang et al. (2003), studied alkyl carbazoles and benzocarbazoles in petroleum reservoir core extracts isolated from several oil columns within the Lengdong oilfield, from the Liaohe Basin, China, to investigate their occurrence and the effect of biodegradation on their concentrations and distributions. Bulk petroleum composition and molecular data indicate the occurrence of systematic biodegradation gradients within the oil columns, the extent of biodegradation ranging from light (PM level 1) to moderate (PM level 5) in the deeper ES3 reservoir columns, and from moderate (PM level 5) to heavy (PM level 8), seen in the shallower and more heavily biodegraded, ES1 reservoir columns. These authors suggested that in reservoir petroleum biodegradation played a significant role in controlling the distribution of alkyl carbazole and benzocarbazole compounds in reservoir oils.

Pyrrolic nitrogen species are ubiquitous components of crude oils and source rock extracts (Helm et al., 1960, Frolov et al., 1989), with alkylated carbazoles and benzocarbazoles being the dominant species seen using classical petroleum geochemical GC-MS approaches (Larter et al., 1996; Huang et al., 2003). The ultimate biological origins of those compounds remain unclear. Their precursors have been proposed to be alkaloids, proteins and pigments in algae and terrestrial plants (Snyder, 1965, Hesse, 1974), although thermal maturation processes in source rocks undoubtedly generate a wide variety of aromatic heteroatomic species from a variety of non-specific precursors. More recently, Oldenburg et al. (2007) investigated nitrogen isotope compositions of the two dominant nitrogen containing compound species in petroleum

neutral/acidic pyrrolic species versus basic pyridinic components of oils from the Liaohe Basin, which revealed that the basic fractions were depleted significantly in ^{15}N compared to the neutral fractions - by more than 5‰. The authors concluded that the source of the pyridine-type N differs significantly from that of pyrrole-type N-compounds in petroleum and/or that the mechanisms of formation of these two types of compounds are quite different. Alkyl carbazoles are generally regarded as resistant to biodegradation at low to moderate levels of biodegradation, but at biodegradation levels greater than PM level 4 they may be microbially altered in a way similar to that observed for aliphatic and aromatic hydrocarbons (Huang et al., 2003). The resistance of nitrogen containing compounds to biodegradation compared to hydrocarbons also was reflected in the changes of the total nitrogen content of the oils from the Liaohe Basin. The total nitrogen content was reported to at first increase, at low to moderate levels of biodegradation, and then to remain constant at higher biodegradation levels (Oldenburg et al., 2006).

4.2.1. Nitrogen-containing species

Oil constituents containing a single nitrogen atom, analyzed in ESI negative ion mode show strong depletion in most DBE groups (Fig. 18). The small diagram in the top right of Figure 18, compares the relative concentration profile of the $\text{C}_0\text{-C}_3$ alkylated carbazoles measured with GC-MS analysis (red symbols), for a larger suite of samples from the ES1 and ES3 reservoirs (Huang et al., 2003; Oldenburg et al., 2006), with the FTICR-MS intensity profile of the summed N1 DBE 9 components (blue symbols) also shown.

The main plot in Figure 18 shows the relative depletion with increasing biodegradation level, of alkylated pyrrolic compounds with increasing degree of biodegradation. DBE 9 pseudohomologs (likely alkylated carbazoles) are preferentially depleted with increasing biodegradation, followed by DBE 12 and DBE 15 species, most probably benzo- and dibenzocarbazoles, respectively. The triangular plot in Figure 8a illustrates this biodegradation preference well. More biodegradation resistant compound groups were also detected with DBE groupings of 10 and 13, which we speculate could be alkylated tetrahydrobenzocarbazoles and tetrahydrodibenzocarbazoles, respectively.

The small diagram in the top right hand corner of the figure compares the relative concentration profile of the C₀-C₃ carbazoles, measured with GC-MS for the ES1 and ES3 reservoirs with the analogous profile from the ESI-N FTICR-MS data, of the summed N1 DBE 9 group components, data also reflecting, most probably, alkylated carbazoles. Note, these data are from two different sets of samples, from slightly different depths from the same wells. The samples for FTICR-MS analysis were taken near previously analyzed GC-MS samples (Huang et al., 2003; Oldenburg et al., 2006). Especially in the less biodegraded ES3 formation oils, a good correlation between GC-MS and FTICR-MS results trending down through the reservoir is observed which again supports the semi-quantitative capabilities of the FTICR-MS technique and also to some extent reassures us in terms of making tentative assignments of structural type, based on FT-MS data alone.

The effects of biodegradation on the distribution of acidic nitrogen containing compound classes, including oxidized species, by subtracting the intensities of pseudohomologs of the least biodegraded sample (PM level 1) from those of the most severely degraded samples (PM

level 8), of the classes N1, NO, NO2, and NO3 are illustrated in Figure 19. Whereas the pyrrolic N1 and NO classes show a strong reduction in intensities of the higher alkylated pseudohomologs of all DBEs with increasing biodegradation, the least alkylated pseudohomologs show a relative increase in their intensities. This might reflect a preference for biodegradation of components with longer alkyl chains relative to the less alkylated pseudohomologs as previously suggested by Kim et al. (2005).

The pseudohomologs of the NO2 and NO3 compound classes in this reservoir are becoming relatively enriched during biodegradation. This might indicate that these compound classes are not being altered during biodegradation, but might be concentrated, either by removal of other species, or speculatively, by formation of new species through partial oxidation of alkylated pyrrolic and pyridinic type compounds that have become carboxylated and/or hydroxylated. While we do not have confirming evidence of this, this seems the more likely explanation.

In comparison to pyrrolic constituents (alkylated carbazoles), the alkylated pyridinic pseudohomologs show a quite different trend. Figure 20(A) illustrates the changes in the N1 DBE 9 pseudohomolog carbon number distribution pattern, with increasing level of biodegradation. A strong relative increase is seen, especially of the C₃₅ pseudohomolog, with increasing biodegradation up to PM level 6, followed by a relative decrease in intensity of these species at PM level 8. The predominance of the C₃₅ pseudohomolog might tentatively indicate an isoprenoid skeleton derived structure. At DBE 10 (not shown here), a similar specific pseudohomolog distribution pattern, with a pseudohomolog predominance at C₄₀ is observed, which also supports an isoprenoidal structural inference. In a previous study of oils from this area, Oldenburg et al. (2004), suggested the occurrence of pyridinohopanoid compounds

(carbon number range C_{33} - C_{36}), using GC-MS at the limits of the technique (in terms of analytical accessibility of high molecular weight compounds). A comparison of the analogous, relative concentration profile of those pyridinohopanoids measured with GC-MS methods for the ES1 and ES3 reservoirs, together with the intensity profile of the summed N1 DBE 9 group, inferred from FTICR-MS analysis (likely pyridinic components), are also shown in Figure 20(B). Note, these data are from two different sets of samples from the same two stratigraphically separate reservoirs as reported here. Even though these are different sample sets, they are from the same petroleum systems and reservoirs and indeed, similarities in the general reservoir profiles are evident when comparing GC-MS measured pyridinohopanoid compound concentrations, (C_{33} to C_{36}) with N1 DBE 9 intensities (C_{17} to C_{71}).

The changes in the distribution pattern of the NO2 class DBE groups with increasing level of biodegradation, measured in ESI-P ion mode, is shown in the main graph of Figure 21. Not only does the overall distribution pattern change from PM 1 to the more severely biodegraded samples, but the relative fraction of the DBE 10 heterocyclic compound groups strongly increases with increasing levels of biodegradation up to PM 8. As this class is measured in ESI-P ion mode, only ionized pyridinic compounds are expected to be detected. The small embedded diagram at top right of the figure, compares the DBE group distribution of the pyridinic N1 class of the most severely biodegraded oil sample (PM 8) with the same sample pyridinic NO2 class DBE group distribution. Similarities between the two DBE group distribution patterns are evident with a shift of plus 1 in the most abundant DBE group, as we move from the N1 class to the NO2 class distribution. The formation of carboxyl group containing N1 class pseudohomologs would increase the DBE by one, resulting in NO2 pseudohomologs with one

higher DBE number. This might well be an indication of carboxyl forming process as the dominant chemical process.

The processes involved within the pyridinic compound classes during biodegradation are best illustrated by the relative changes in the distribution of pyridinic N1 DBE 9, NO DBE 9 and NO2 DBE 10 species, with increasing degree of biodegradation (Fig. 22). This is shown as the relationship between the pyridinic N1 DBE 9 group heterocyclic compounds to the NO2 heterocyclic compounds, DBE 10 group. The diagram shows an apparent relative increase in the N1 DBE 9 group with increasing levels of biodegradation, up to PM level 4, followed later by an increase in the relative concentration of the NO2 DBE 10 group at higher levels of biodegradation. The increase of N1 DBE 9 at moderate levels of biodegradation probably reflects, on the one side, concentration of resistant pyridinic N1 class components up to PM level 4, and on the other side, formation of at least the likely isoprenoid skeleton derived pyridinohopanoids C₃₅ pseudohomolog, as shown in Figure 20A. At more severe levels of biodegradation, more N1 species are transformed by oxidation into NO2 species.

4.2.2. Naphthenic acid species

Within the naphthenic acids containing O2 class most of the DBE groups show a relative depletion in intensity, with increasing level of biodegradation, with DBE 5 species being most affected (Fig. 23). This relative depletion is compensated by a relative increase in the one, two and three ring naphthenic acids (DBE 2, 3 and 4, respectively) with increasing level of biodegradation. For the latter ones, first the one ring naphthenic acids (DBE 2) are predominant until PM level 5 but succeeded by the two ring naphthenic acids at higher levels of

biodegradation. The embedded, subtraction modified Kendrick plot, illustrates the impact of biodegradation on each O₂ species by comparison of the most severely biodegraded oil (PM level 8), with the least biodegraded oil sample (PM level 1). This subtraction plot reveals the relative increase in the DBE 2, 3, and 4 groups and a depletion of DBE 1, 5 and 6 groups during the biodegradation process. The DBE 1 group reflects mainly fatty acids, whereas the DBE 5 pseudohomologs are possibly alkylated benzoic acids, tetracyclic acids and/or pentacyclic diols. The DBE 6 group pseudohomologs probably reflect the contributions of hopanoid acids which were detected independently in a previous study with GC-MS methods (Oldenburg et al., 2004). A comparison of the of C₃₀-C₃₃ $\alpha\beta$ (S)hopanoic acids previously determined by GC-MS (Oldenburg et al., 2004) with intensities of the pseudohomologs with the same carbon number of the FTICR-MS determined equivalent O₂ class DBE 6 group at different levels of biodegradation (PM5 and PM6) are supporting the suggested molecular structure of these species (Fig. 24). Some similarities are observed between the distribution patterns from both systems. The similarities in the distribution patterns may indicate that most of the peak abundances detected in the C₃₀-C₃₃ range of the O₂ class DBE 6 are actually related to hopanoic acids (however, minor contributions of other deprotonatable isomers, such as alkylated tetrahydronaphthoic acids, other pentacyclic acids and hexacyclic diols can't be ruled out). On the other hand, this is another indication that FTICR-MS approaches - even though not yet quantitative – do have some semi-quantitative potential.

4.3. Comparison of Results

In summary, there are many general similarities between the Chinese petroleum case study and the Canadian case study, and it would appear that while some single heteroatom non-hydrocarbon compounds and mixed heteroatom compounds with a single oxygen or pyrrole type nitrogen atom are destroyed or partially oxidized during biodegradation, several groups of alkylated compounds, principally containing two oxygen atoms, are generated during the biodegradation process. These include mixed nitrogen and oxygen species as well as the expected carboxyl bearing alkylated naphthenic acids. It seems that biodegradation is attacking essentially all species present in the crude oil with the most abundant species being preferentially attacked at any biodegradation level. In addition, tentatively inferred preserved biomarker products in biodegraded crude oils, may be indications of the biodegradation biome. In comparison to the sulfur-rich kerogen Type IIS- derived Canadian oils, the Chinese lacustrine oils are low in sulfur but enriched in nitrogen. Therefore, the predominant formation of SO₂ species during biodegradation in the Canadian oils and NO₂ species in the Chinese oils is related to the source of the most abundant heterocyclic species available in those particular petroleum reservoirs settings. It appears biodegradation is quite a pervasive process in terms of heterocyclic compounds with the most abundant species present commonly being the primary biodegradation targets. This also may indicate that incomplete oxidation (formation of carboxyl and hydroxyl species, among others) are major overall processes in in-situ reservoir biodegradation, with at least partial biodegradation and/or oxidation occurring over a broad range of molecular masses (>C₄₀ heterocyclic compounds) and with a wide range of precursor hetero compound species. If verified, this has major implications for electron acceptor inventories and biodegradation mass balances of crude oils. ESI-P FTICR-MS data from several

different petroleum systems containing biodegraded oils at different levels of biodegradation indicate three apparently very biodegradation resistant nitrogen and sulfur containing heterocyclic compound groups (Fig. 25). These species are tentatively identified as alkylated pyridine derivatives N1_DBE 8; sulfur containing alkylated pyridine derivatives NS_DBE10 and NS2_DBE12; for lacustrine oils from N.E. China (PM level range 1 to 8), two sample suites marine Type II kerogen sourced sulfur-rich oils (S content: 4-5%) from W. Canada (PM level 5 to 8), one sample suite marine Type IIS kerogen sourced high sulfur oils (S content: 8%) from W. Canada (PM level 3 to 5) and a suite of marine Type II kerogen sourced low sulfur oils from N. Europe that are all non-biodegraded. While varying biodegradation levels do not seem to impact the relative distributions of these groups of compounds for individual oil families, source facies clearly does impact distributions, with high sulfur kerogens generating oils with significant but variable amounts of biodegradation resistant NS and NS2 species and the lacustrine oils having lower sulfur species contributions.

Despite the broad range of biodegradation of the various oil families, all oils are clearly distinguishable by their source facies, independent of the level of biodegradation. While the data set is relatively small, it would appear that source maturity has only a minor impact on the parameters compared to interfamilial variations. These species add a very biodegradation resistant parameter to the petroleum geochemists arsenal.

As an aside, in earlier studies we also examined the nitrogen isotope systematics of biodegrading oils from the same Chinese petroleum system and we briefly reflect on possible inferences there that relate to the operation of the petroleum biodegradation biome. Three polar fractions (low polar pyrrole-type, pyridine-type base compounds and a non-defined

fraction of high polarity oil constituents) were separated by liquid chromatography and analyzed for $\delta^{15}\text{N}$ as described by Oldenburg et al. (2007). Nitrogen isotope systematics of nitrogen-containing fractions show an increase in $\delta^{15}\text{N}$ between 1.7 and 3.5 ‰ of all three fractions from an early stage of biodegradation (between PM levels 2 and 4) and remains constant at levels above PM level 5 (Figure 26). This isotopic fractionation at an early stage of biodegradation appears to indicate an enrichment in the ^{15}N isotope and that perhaps the organic nitrogen is being used as a nutrient for the degradation biome. At later stages of biodegradation when easily biodegradable hydrocarbons such as *n*-alkanes are completely destroyed, highly alkylated nitrogen-containing compounds might be used by the microorganisms mainly as a carbon source with no nitrogen isotope preference.

5. Conclusions

The potential value of NSO species in crude oils, for the definition and understanding of geochemical properties and processes such as biodegradation and any associated water washing, lies in the much greater range of physicochemical properties exhibited by these compounds in comparison with hydrocarbons. Several biodegraded oil suites from various petroleum system settings were investigated for the effects of biodegradation on the distribution of high molecular weight hydrocarbons and non-hydrocarbons using ultra-high resolution mass spectrometry. The two main case history sample sets described here are one set of lacustrine oils from the Liaohe Basin, NE China (PM level 1-8) and one set of sulfur rich oils from Western Canada (PM level 3-5) which were previously studied extensively by Huang et al. (2004) and Bennett et al. (2013), respectively, using common geochemical methods like GC-

MS. Both sample sets show depletion of most compound classes as biodegradation proceeds, and it appears that biodegradation is pervasive in terms of the heterocyclic compounds, with the most abundant species present being the primary biodegradation targets at any level of biodegradation. Microorganisms seem to attack oil constituents with molecular weights at least as high as m/z 600 (carbon number 44) and functionally prefer low DBE species over high DBE species within a heteroatom group compound class. More highly alkylated hydrocarbon pseudohomologs are preferentially degraded, which is reflected in a decrease in the average molecular mass (AMM) of hydrocarbon species with increasing degree of biodegradation. Some particular compound groups, like even-over-odd predominant fatty acids, sterols, pyridinohopanes, and others, are produced and partially consumed, and are likely related, in part at least, to biomarkers of the biodegradation biome. A third group of compound classes, mainly mixed heteroatom species with more than one oxygen atom are produced during biodegradation, indicating that transformation rather than full destruction of high molecular weight compounds occurs. Chinese lacustrine sourced oils, low in sulfur but enriched in nitrogen show a good correlation between the depletion of N1 species and the production of NO2 species. An analogous trend was observed for the sulfur-rich Canadian marine sourced oils as seen in the likely transformation of S1 species to SO2 species. If verified, that a significant fraction of oil is transformed rather than mineralized to CO_2 and water, this will have major implications for electron acceptor inventories (strong reduction of the amount of electron acceptors needed for biodegradation) and biodegradation mass balances (strong reduction of amount of oil actually destroyed) of crude oils. Production of naphthenic acids (O2 class) and an increase with naphthene ring size (from 1 to 3 rings) as a function of increasing biodegradation

level was obvious. Our observations reveal that oxidation processes (formation of carboxyl and hydroxyl species) are the main overall processes occurring in in-situ reservoir biodegradation. Moreover, these surfactant-like species may well impact interfacial, corrosion, transport and environmental distribution properties of an oil, and the impact will vary with the type of petroleum. This is very important and a rigorous examination of the various impacts on oil properties, beyond the well-studied viscosity property domain, is required. In addition, as mixed heteroatom species would appear to be very common in oil sands bitumen and related heavy oils, and with increasing concern over oil spillage during transportation and water body interaction with these materials, it will be important to obtain quantitative data and toxicological information on these diverse mixtures of previously unidentified species with largely unknown toxicological properties.

An extended set of sample suites from several petroleum systems with various levels of biodegradation was used for the study of biodegradation resistant parameters. Groups of nitrogen and mixed nitrogen-sulfur species appear to clearly distinguish oil families by their source facies, independent of the level of biodegradation. Source maturity has a minor impact on the parameters compared to interfamilial variations. These species add a very biodegradation resistant parameter to the petroleum geochemists arsenal.

Finally, and more speculatively, nitrogen isotope systematics within the Chinese oil sample suite shows selective enrichment of ^{15}N isotope at low levels of biodegradation (PM level 2 to 4) which may indicate that nitrogen is used by the microorganisms as a nutrient source. This preference disappears at more severe levels of biodegradation. Because readily biodegradable hydrocarbons such as *n*-alkanes are completely destroyed at this stage, it is possible, but not

proven, that highly alkylated nitrogen-containing species are being used as a source of carbon and nitrogen.

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Figure captions

Figure 1. Geophysical, fluid property and petroleum geochemical logs through a reservoir from the Peace River oil Sands, in Western Canada (modified after Bennett et al., 2013), including a generalized summary of key fluxes, reservoir and biological system properties and cell counts (Larter and Head, 2014). Part A, shows the lithology, stratigraphy and section depth related to a reference point in the cap rock. The resistivity log shows the development of the oil leg, grading down towards the oil water transition zone (OWTZ), with polar compounds content increasing down towards this interval and oil viscosity increasing over an order of magnitude down towards this interval. Modified after Bennett et al. (2013), this figure also shows the results of a quantitative GC-MS analysis of saturated and aromatic hydrocarbons in the oil throughout the reservoir and oil water transition zone Part B, including a summary of the properties of the reservoir section and the oil water transition zone, and a summary of the bacterial cell counts and putative oil charge rates and biodegradation rates (after Larter and Head, 2014). Part C, SARA analysis by latroscan, shows substantial decreases in aromatic hydrocarbon content and increases in Polars II non-hydrocarbons in reservoir core extracts, with increasing depth in the reservoir (after Bennett et al., 2013).

Figure 2. Heteroatom and hydrocarbon classes relative abundance distribution changes in the marine sourced oil reservoir system (WCSB) described in Figure 1, as a function of reservoir position for species detectable using APPI-P FTICR-MS analysis. [The dot indicates radical heteroatom or hydrocarbon classes; the other classes are found as protonated species. Samples are colour-coded and referenced to reservoir depth in the cartoon in the top left portion of the figure.]

Figure 3. Heteroatom class distribution changes with depth and increasing biodegradation level in a reservoir containing marine shale sourced petroleum (WCSB). Data are obtained using FTICR-MS in ESI-N mode. [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 4. Distribution of S1-heteroatom class (radical ions: RAD) at different depths of the WCSB sample suite, sorted into groups of double bond equivalent (DBE) compounds, plotted versus abundance of each DBE group, measured in APPI positive ion mode. S1 class DBE 3 group pseudohomologs are most likely dominated by alkylated thiophenes (T), based on abundant evidence of these species being dominant components in the lower carbon number regions accessible by GC-MS studies, whereas DBE 6 group species are probably predominantly benzothiophenes (BT) and DBE 9 group species are likely largely dibenzothiophenes (DBT). [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 5. Triangular plot illustrating the effect of biodegradation changes of the WCSB sample suite on the relative distribution of DBE 6 (likely alkylated benzothiophenes), DBE 8 and 9 (likely alkylated octahydrotribenzo- and alkylated dibenzothiophenes, respectively). [Molecular structures illustrate possible core structures that correspond to a specific DBE number and compound group].

Figure 6. Distribution of S2-heteroatom class RAD at different depths of the WCSB sample suite, sorted into groups of double bond equivalents (DBE), plotted versus abundance of each species DBE group, measured in APPI positive ion mode. [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 7. Distribution of N1-class (probably pyrrolic, nitrogen species) of the WCSB sample suite measured in ESI negative ion mode. The plot shows the distributions of these molecular types, sorted into groups of double bond equivalent pseudohomologs (DBE). N1 class DBE 9 group pseudohomologs are most likely dominantly alkylated carbazoles (C), based on abundant evidence of these species being dominant components in the lower carbon number regions accessible by GC-MS studies. The DBE 12 group species are likely predominantly benzocarbazoles(BC) and DBE 15 group species are likely largely dibenzocarbazoles (DBC). [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 8. A) Triangular plot illustrating the effect of biodegradation changes of the WCSB and Chinese sample suites on the relative distribution of DBE 9 (likely alkylated carbazoles), DBE 12 and DBE 15 (likely alkylated benzo- and alkylated dibenzocarbazoles, respectively). B) Zoomed-in portion of the triangular plot A to visualize the trend observed for the Canadian samples. [Molecular structures illustrate possible core structures that correspond to a specific DBE number and compound group.]

Figure 9. Distribution of O₁-class (species containing one deprotonatable hydroxyl functional group) of the WCSB sample suite measured in ESI negative ion mode. The plot shows the distributions of these molecular types, sorted into groups of double bond equivalent pseudohomologs (DBE). [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 10. Distribution of DBE 4 and 5 compound groups of the acidic O₁ heteroatom class (likely alcohols, i.e. containing a hydroxyl functional group) of the WCSB sample suite, sorted by pseudohomolog carbon number C# versus the percentage of total ions (percentage of sum of all assigned peaks measured in ESI negative ion mode). [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 11. Distribution of hydrocarbon radical ions (HC RAD) class at different depths of the WCSB sample suite, sorted into groups of double bond equivalents (DBE), plotted versus

abundance of each DBE group, measured in APPI positive ion mode. [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 12. A, C) Distribution of DBE 7 and 10 compound groups of the hydrocarbon radical ions (HC RAD) class (likely alkyl naphthalenes and alkyl phenanthrenes, respectively) of the WCSB sample suite, sorted by pseudohomolog carbon number C#, versus intensity. B) Triangular plot illustrating the effect of biodegradation changes on the relative distribution of HC RAD DBE 7 pseudohomologs with different carbon number (likely alkylated naphthalenes with C20, 25, and 30 alkyl groups). D) Averaged molecular mass (AMM) for each DBE group for the HC RAD class measured in APPI positive ion mode.

Figure 13. Distribution of O2-class (mainly acidic species, i.e. containing one carboxyl functional group or two hydroxyl functional groups) of the WCSB sample suite, measured in ESI negative ion mode. The plot shows the distributions of these molecular types, sorted into groups of double bond equivalent pseudohomologs (DBE). The most abundant species present in the petroleum have DBE group numbers of 2 and 3 and are likely to be alkylated single and double ring naphthenic acids. The inset diagram shows the relative abundance ratio of total O2 heteroatom compounds to total N1 heteroatom compounds. [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 14. Distribution of DBE 1, 2, 3 and 7 compound groups, of the acidic O2 heteroatom class (likely fatty acids (DBE 1, partially DBE 2, i.e. C₁₈ monounsaturated fatty acids) and naphthenic

acids of the WCSB sample suite, sorted by pseudohomolog carbon number C#, versus the percentage of total ions (percentage of sum of all assigned peaks measured in ESI negative ion mode). [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 15. Distribution of O2-class (mainly acidic species, i.e. containing one carboxyl functional group or to a lesser extent two hydroxyl functional groups) measured in ESI negative ion mode of four non-Canadian marine shale sourced oils. The plot shows the distributions of these molecular types, sorted into groups of double bond equivalent pseudohomologs (DBE). These data for O2 heteroatom compound species are from a suite of oils from another marine shale sourced petroleum system. The plot shows substantial evolution in the abundance of naphthenic acids, and also naphthene ring size as a function of increasing biodegradation level. The total acid number values (milligrams KOH/gram oil) and biodegradation level based on the Peters and Moldowan scale (1993) are also indicated on the diagram for the different oils.

Figure 16. Distribution of SO2-class (interpreted as mainly acidic species, likely containing one sulfur and carboxyl functional group or one sulfur and two hydroxyl functional groups) of the WCSB sample suite, measured in ESI negative ion mode. The plot shows the distributions of these molecular types, sorted into groups of double bond equivalent pseudohomologs (DBE). [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 17. Distribution of DBE 4, 5, 7, and 8 compound groups of the acidic SO₂ heteroatom class (likely thiophenic type species containing a carboxylic acid group such as for DBE 4: thiophenes carboxylic acid; DBE 5: tetrahydrobenzothiophenes carboxylic acid; DBE 7: benzothiophenes carboxylic acid; DBE 8: tetrahydrodibenzothiophenes carboxylic acid) of the WCSB sample suite, sorted by pseudohomolog carbon number C# versus the percentage of total ions (percentage of sum of all assigned peaks measured in ESI negative ion mode). [Samples are colour-coded and referenced to reservoir depth in the cartoon in the top right portion of the figure.]

Figure 18. ESI-N FTICR-MS relative abundance data for heterocyclic compounds with a single nitrogen atom, for five selected samples of different biodegradation level selected from two reservoirs from the Liaohe Basin with related common oil charge, in a single oil field charged by a common source rock. The different heterocyclic compounds are separated according to DBE group number. [Sample labels: PM# means level on Peters&Moldowan biodegradation scale (1993)] The small diagram in the top right of the figure, compares the relative concentration profile of the C0-C3 alkylated carbazoles (red symbols) measured with GC-MS for a larger suite of samples from the ES1 and ES3 reservoirs (Huang et al., 2003; Oldenburg et al., 2006), with the FTICR-MS intensity profile of the summed N1 DBE 9 (blue symbols). The general concordance of GC-MS data for alkylated carbazoles and the N1 DBE 9 group of components seen in the FTICR-MS data, suggests that these species, tentatively identified in the FT-MS data, are likely the alkylated carbazoles. While GC-MS typically only looks at alkylated carbazole

species with a total of 12-15 carbon atoms, FTICR-MS data suggest that this group of compounds extends up to a carbon number of at least 52.

Figure 19. Modified Kendrick mass plots using ESI-N FTICR-MS data from the Chinese case history. These plots compare the relative intensities of a compound class in the least biodegraded end member sample (PM 1) and the most severely biodegraded end member sample (PM 8). These are plotted as the ion intensity for the component in the most degraded sample, minus the ion intensity for the same component in the least degraded sample, plotted as a function of pseudohomolog C# and DBE group for heterocyclic compounds with a single nitrogen atom (A); for heterocyclic compounds with a single N and a single O atom (B); for heterocyclic compounds with NO₂ (C); and for heterocyclic compounds with NO₃ (D). The brighter end of the colour scale (towards red), indicates an increase in relative abundance of species intensity at high degree of biodegradation whereas points shown as shades of grey indicate species show depletion in the concentration of specific pseudohomologs as biodegradation advances.

Figure 20. ESI-P FTICR-MS data for oils from the Chinese petroleum system case history. The pseudohomolog carbon number distribution of the single N1 heterocyclic compound group with DBE 9 from FT-MS data is shown (A), as is the correspondence between the relative concentration of pyridinohopanoids determined by GC-MS and the trend of ion intensity for N1 DBE 9 species from ESI-P FTICR-MS data (this study) (B). [Sample labels: PM# means level on Peters and Moldowan biodegradation scale (1993)]

Figure 21. ESI-P FTICR-MS data for heterocyclic compounds with an NO₂ speciation, in oils from the Chinese case history (main plot). The small embedded diagram at top right, compares the DBE group distribution of the pyridinic N1 class of the most severely biodegraded sample (PM 8) with the same samples, pyridinic NO₂ class DBE group distribution. [Sample labels: PM# means level on Peters and Moldowan biodegradation scale (1993); structure in the top left corner is a speculative illustration of a possible amphoteric species]

Figure 22. A comparison of the relative percentage of the pyridinic N1 DBE 9 group heterocyclic compounds, to the NO₂ heterocyclic compounds, DBE 10 group (plotted on two axes as a relative percentage of the N1 DBE 9, NO DBE 9 and NO₂ DBE 10 species as a summed percentage of the total abundance of the 3 compound groups (total abundance equals 100 percent), for the entire sample set of the oils from the Chinese ES1 and ES3 reservoirs. [Sample labels: PM# means level on Peters and Moldowan biodegradation scale (1993)]. The blue text in the figure relates to speculations on the possible relationships between biodegradation level and the enrichment of, or formation of new species.

Figure 23. The main plot illustrates the changes in the distribution pattern of the O₂ DBE groups with increasing level of biodegradation sorted as groups of species with common double bond equivalents (DBE), and plotted versus the fraction of the total heteroatom class ion response (sum of intensities of all DBE groups of the heteroatom class is 1), for each DBE compound group, measured in ESI negative ion mode. [Sample labels: PM# means level on Peters and

Moldowan biodegradation scale (1993)]. The embedded diagram in the top right corner illustrates a subtraction modified Kendrick plot of the most severe (PM 8) minus the least biodegraded sample (PM 1) using ESI-N FTICR-MS data from the Chinese case history. These plots compare the relative intensities of each individual pseudohomolog in a compound class in the least biodegraded end member sample (PM 1) and the most severely biodegraded end member sample (PM 8). These are plotted as the ion intensity for the component in the most degraded sample, minus the ion intensity for the same pseudohomolog in the least degraded sample, plotted as a function of pseudohomolog C# and DBE group for heterocyclic compounds with two oxygen atoms. The brighter end of the colour scale (towards red), indicates an increase in relative abundance of species intensity at high degrees of biodegradation whereas points shown as shades of grey, indicate species showing depletion in the concentration of specific pseudohomologs as biodegradation advances.

Figure 24. A comparison of the concentrations of C_{30} - C_{33} $\alpha\beta$ (S)hopanoic acids determined by GC-MS (Oldenburg et al., 2004) with intensities of the analog pseudohomologs with the same carbon number of the FTICR-MS determined analog O2 class DBE 6 group, at different levels of biodegradation in the Chinese case history.

Figure 25. ESI-P FT-MS data from the oils derived from several petroleum systems at different levels of biodegradation. The triangular plot shows biodegradation resistant source markers, tentatively identified as pyridine derivatives- N1_DBE8, sulfur containing pyridine derivatives- NS_DBE10 and NS2_DBE12, for lacustrine oils from N.E. China (PM level range 1 to 8); two

sample suites of marine Type II kerogen sourced, sulfur-rich oils (S content: 4-5%) from W. Canada (PM level 5 to 8); one sample suite marine Type IIS kerogen sourced high sulfur oils (S content: 8%) from W. Canada (PM level 3 to 5); and a suite of marine Type II kerogen sourced low sulfur oils from N. Europe which are all non-biodegraded but cover a broad range of source maturity from 0.68% to 1.1% vitrinite reflectance equivalent (%Re).

Figure 26. The nitrogen isotope composition of polar compound fractions in the two reservoirs of the Liaohe Basin, the shallower ES1 reservoir in the upper part [biodegradation level PM 5 to 8] and 9 samples from the less biodegraded, deeper ES3 reservoir in the lower part of the graph [biodegradation level PM 1 to 5]. The polar fractions shown here are pyridine-type base constituents (red) and low polar neutral/acidic pyrrole-type compounds (blue). The functional groups of the high polar fraction (orange) are not defined. The highlighted circled two samples indicate unfinished separation during liquid extraction (verified by FTICR-MS analysis). [Liquid chromatography separation and nitrogen isotope analysis methods are described in Oldenburg et al., 2007].

Appendix

Here we provide a brief summary of typical heteroatom classes and more speculatively, component functional groups detectable with the different ionization techniques.

- The ESI-N (negative ion mode), technique allows the optimal detection and monitoring of acidic compounds, i.e. compounds which are able to deprotonate, such as compounds, including carboxylic acids, alcohols, pyrroles (Qian et al. 2001a).

- The ESI-P (positive ion mode), technique allows the detection of basic compounds, i.e. compounds which can be readily protonated- for example compounds with pyridinic nitrogen groups (Qian et al. 2001b).
- The APPI-P technique allows the detection of analyte components as protonated (PRO) and/or radical (RAD) ion species. What ions are formed depends on the proton and electron affinities of the dopant relative to the analyte. With a toluene dopant, if the proton affinity of the analyte is higher than the proton affinity of the benzyl radical, a protonated molecule can form. If the electron affinity of the toluene radical cation is higher than the electron affinity of the analyte (lower or equal ionization energy than toluene) a radical molecular ion can form (Purcell et al., 2006).

Data illustrations

Definition of oil constituent species:

A (heteroatom or hydrocarbon) class is defined here as the sum of all constituents containing the same heteroatoms (number and elements) independent of the degree of unsaturation (DBE). These would be measured in one ionization technique mode (ESI-N, ESI-P or APPI-P; radical ions (RAD) and protonated ions (PRO) of one heteroatom or hydrocarbon class in APPI-P are considered as two classes).

A DBE group of compounds is defined as the sum of all constituents containing the same heteroatoms (number and elements) and double bond equivalent number measured in one ionization technique mode.

A pseudohomolog at a specific carbon number (C#), of a species is defined as the sum of all compounds containing the same molecular formula measured in one ionization technique mode.

Note: A DBE group of compounds would be recognized by the interpreter as having an acceptable signal-to-noise ratio, within any ionization mode, if the pseudohomolog distribution at that DBE group number, showed a minimum of five consecutive pseudo-homologues with Gaussian shaped peak distribution easily recognizable above the noise.

The different plot types used in this article are briefly explained below.

❖ Bar and Line plots:

➤ *'Ion Intensity' versus pseudohomolog (C#), DBE group or distribution of heteroatom and hydrocarbon classes plots:*

An ion intensity plot represents the absolute ion intensity, measured in a single ionization mode, of either the response of a pseudohomolog at one carbon number: the response of a DBE group of compounds within one heteroatom (or hydrocarbon) class: or the distribution of a heteroatom (or hydrocarbon) class in total. Commonly, we plot the absolute ion intensity of all pseudohomologs; all DBE groups; or all heteroatom (or hydrocarbon) classes on one diagram against absolute ion intensity measured in that single ionization mode.

This plot allows a tentative estimation of the relative concentration and variation between samples, of different constituents (acidic moieties in ESI-N; basic moieties in ESI-P; low polarity species to non-polar species in APPI-P) in the oil sample, as in all cases, and for comparative

purposes, the whole oil samples (without any prior separation) are analyzed under identical conditions.

- *'Fraction of Total Ions' versus distribution of a compound class, DBE group or pseudohomolog (C#) plots:*

A *'Fraction of Total Ions'* plot is an ion intensity plot, for a heteroatom (or hydrocarbon) class, a DBE group of compounds, or for pseudohomologs at one or more carbon numbers, where the ion counts for each species group are now normalized to the total ion response of the entire sample analyzed in one ionization mode.

- *'Fraction of Class' versus distribution of compound class, DBE or pseudohomolog C# plots:*

A *'Fraction of Class'* plot is a heteroatom (or hydrocarbon) class specific plot, whereby the ion intensities for a DBE group of compounds or for pseudohomologs at one or more carbon numbers, are now normalized to the total ion response of the heteroatom (or hydrocarbon) class analyzed in one ionization mode.

- ❖ **Modified Kendrick plot:**

The *'Modified Kendrick plot'* illustrates the distribution of all components within a heteroatom compound class (analyzed with any ionization technique), as a plot of C# versus DBE with the intensity of the component being reflected by a color code.

The colors reflect the peak intensity of individual carbon number components, relative to the most intense peak within the overall compound class.

❖ Average Molecular Mass:

The Average Molecular Mass (AMM) plot shows the averaged molecular mass of all components in an individual DBE group of compounds from a heteroatom (or hydrocarbon) class, plotted as a function of the DBE number. The AMM is defined as: $AMM = \sum \text{intensity of each individual pseudohomolog} \times \text{accurate mass of the pseudohomolog} / \text{total ion intensity for all pseudohomologs within a DBE group of compounds}$.

Highlights Oldenburg et al.

- Destruction and production of compounds occurs during in-reservoir biodegradation
- Oxidation of species impact transport, interfacial and corrosion properties of oils
- Oil species with MW at least up to m/z 600 (C# 44) are affected by biodegradation
- Biodegradation resistant, parameter set was developed for biodegraded case studies
- Nitrogen-containing oil compounds are nitrogen and carbon source for microorganisms