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Geochimica et Cosmochimica Acta 70 (2006) 651–665
Geochimica
www.elsevier.com/locate/gca

Deciphering biodegradation effects on light hydrocarbons in crude oils using their stable carbon isotopic composition: A case study from the Gullfaks oil field, offshore Norway

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Received 10 March 2005; accepted in revised form 18 August 2005

Abstract

Compound-specific isotope analysis has become an important tool in environmental studies and is an especially powerful way to evaluate biodegradation of hydrocarbons. Here, carbon isotope ratios of light hydrocarbons were used to characterise in-reservoir biodegradation in the Gullfaks oil field, offshore Norway. Increasing biodegradation, as characterised, for example, by increasing concentration ratios of Pr/*n*-C_{17} and Ph/*n*-C_{18}, and decreasing concentrations of individual light hydrocarbons were correlated to ^{13}C-enrichment of the light hydrocarbons. The $\delta^{13}C$ values of C_4 to C_9 *n*-alkanes increase by 7–3‰ within the six oil samples from the Brent Group of the Gullfaks oil field, slight changes (1–3‰) being observed for several branched alkanes and benzene, whereas no change (<1‰) in $\delta^{13}C$ occurs for cyclohexane, methylcyclohexane, and toluene. Application of the Rayleigh equation demonstrated high to fair correlation of concentration and isotope data of *i*- and *n*-pentane, *n*-hexane, and *n*-heptane, documenting that biodegradation in reservoirs can be described by the Rayleigh model. Using the appropriate isotope fractionation factor of *n*-hexane, derived from laboratory experiments, quantification of the loss of this petroleum constituent due to biodegradation is possible. Toluene, which is known to be highly susceptible to biodegradation, is not degraded within the Gullfaks oil field, implying that the local microbial community exhibits rather pronounced substrate specificities. The evaluation of combined molecular and isotopic data expands our understanding of the anaerobic degradation processes within this oil field and provides insight into the degradative capabilities of the microorganisms. Additionally, isotope analysis of unbiodegraded to slightly biodegraded crude oils from several oil fields surrounding Gullfaks illustrates the heterogeneity in isotopic composition of the light hydrocarbons due to source effects. This indicates that both source and also maturity effects have to be well constrained when using compound-specific isotope analysis for the assessment of biodegradation.

1. Introduction

Anaerobic biodegradation of light hydrocarbons has often been reported and is now widely accepted as a significant process in natural environments (Edwards and Grbić-Galić, 1992; Rabus and Widdel, 1995; Rueter et al., 1994; Widdel and Rabus, 2001). An important example is the biodegradation of hydrocarbons within petroleum reservoirs, leading to substantial change in petroleum composition and an associated decrease in the economic value of the crude oil (Connan, 1984). The recent discovery of anaerobic biodegradation mechanisms (e.g., Rabus et al., 2001; Wilkes et al., 2002, 2003) and the detection of anaerobic microorganisms in oil fields suggest that anaerobic degradation of light hydrocarbons is possible within the reservoirs (Aitken et al., 2004). However, to date no microorganisms have been enriched or isolated from petroleum reservoirs that can degrade light hydrocarbons anaerobically.

There is still some debate on the exact order in which different compounds are removed from crude oil during biodegradation, but most studies present similar degradation sequences where short-chain *n*-alkanes tend to be removed faster than longer-chain *n*-alkanes, the latter

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doi:10.1016/j.gca.2005.08.022

being removed faster than branched and isoprenoid hydrocarbons (Peters and Moldowan, 1993; Volkman et al., 1984; Wenger et al., 2001). The strongest deterioration of petroleum quality occurs from slight to moderate biodegradation (Wenger et al., 2001) or between biodegradation levels 1 and 4 (Peters et al., 2005). To characterise and understand this very important aspect of biodegradation, scientific studies are needed that focus on light hydrocarbons.

Most recently, the determination of compound-specific carbon and hydrogen isotopic compositions has become an effective tool in oil–oil and oil–source rock correlations (Li et al., 2001; Odden et al., 2002; Schimmelmann et al., 2004). Carbon and hydrogen isotopic composition was also used to trace biodegradation of aromatic hydrocarbons or chlorinated compounds in laboratory experiments (Mancini et al., 2003; Meckenstock et al., 1999; Slater et al., 2001; Wilkes et al., 2000) and studies on contaminated aquifers (Hunkeler et al., 1999; Mancini et al., 2002; Meckenstock et al., 2004; Sherwood Lollar et al., 2001). Biodegradation leads to an enrichment of the heavier isotopes in the residual fraction of an organic compound. This change in isotopic composition can be related to the decrease in concentration by the Rayleigh equation, allowing the quantification of the extent of biodegradation in both laboratory and field studies (Meckenstock et al., 1999; Richnow et al., 2003a; Vieth et al., 2005).

Carbon isotope fractionation of the residual substrate occurs in the initial reaction step, which mechanistically is localised at one specific carbon atom of the substrate in most known cases. Therefore, the overall isotope effect will become less with increasing number of carbon atoms in the molecule (Boreham et al., 1995). For biodegradation of, for example, aromatic hydrocarbons, starting with an enzyme reaction of the benzylsuccinate-type, it was calculated that hydrocarbons with more than 13 carbon atoms will not show a detectable change in the carbon isotopic composition, even when more than 90% of the substrate was degraded (Morasch et al., 2004). Thus, it can be assumed that biodegradation of hydrocarbons with more than 15 carbon atoms will not lead to a significant change in their isotopic composition. In support of this, Boreham et al. (1995) observed no enrichments in ^{13}C in n-C_{12+} alkanes from biodegraded oils. Therefore, the carbon isotopic compositions of such hydrocarbons are not compromised and can still be used as specific indicators of the origin and maturity of the oil sample.

It is also known that other in-reservoir processes such as water washing and evaporation have little or no effect on the isotopic composition of petroleum hydrocarbons (Harrington et al., 1999; Mansuy et al., 1997; Smallwood et al., 2002). Thus, it can be expected that the carbon isotopic composition of petroleum hydrocarbons within a certain reservoir, regardless of differences in isotopic composition due to source and/or maturity, might only be influenced by the kinetic isotope effect caused by biodegradation processes. This is important with respect to the differentiation of various alteration processes, that all may influence the concentration of the petroleum components.

To date, only few studies have used carbon isotope ratios of light hydrocarbons in crude oils as an indicator of biodegradation. In general, the enrichment of ^{13}C of certain hydrocarbons in the residual oil served as a qualitative indicator of the biological process (George et al., 2002; Masterson et al., 2001; Rooney et al., 1998). The present study pays particular attention to the quantitative evaluation of the biodegradation process in reservoired oils using carbon isotope fractionation of light hydrocarbons. As described in more detail below, crude oils from the Gullfaks field, offshore Norway, provide a unique opportunity to study the effects of biodegradation for two reasons. First, as has been shown previously, the oil column in the Brent Group reservoir at Gullfaks is vertically homogeneous (Horstad et al., 1990a). Therefore, relatively few oil samples from different wells representing the lateral heterogeneity in the petroleum composition are sufficient for characterisation of the principal biodegradation effects. Second, the oils are characterised by a high similarity with respect to source facies and maturity, giving rise to the assumption that changes in the molecular and isotopic composition are predominantly due to in-reservoir biodegradation.

2. Geological background

In this study, 12 crude oil samples from the Gullfaks and surrounding oil fields were investigated. The location of all sampled wells within the Tampen Spur area is given in Fig. 1 and a detailed description of each sample can be obtained from data in Table 1. The main source rock in the Tampen Spur area is the Upper Jurassic Draupne Formation (Gormly et al., 1994). Also the underlying Heather Formation locally represents a source rock with high oil potential (Gormly et al., 1994; Horstad et al., 1995; see Section 4.1 for details). The organic rich shales (5–10% total organic carbon) of the Draupne Formation are the regional Kimmeridge Clay equivalents. The underlying Heather Formation contains type II kerogen with high oil potential, but usually of a much lower kerogen quality than the Draupne Formation (Horstad et al., 1995).

Biodegradation effects were evaluated using seven oil samples from the Gullfaks oil field. The Gullfaks oil field is located in the northeastern part of Block 34/10 in the Norwegian sector of the North Sea (Carlsen and Nygaard, 1990). It represents the shallowest structural element of the Tampen Spur and was formed during the Late Jurassic to Early Cretaceous. A detailed description of this field and its oil reservoirs is given in Horstad et al. (1992) and Olaussen et al. (1992). The reservoir sandstones comprised of the Middle Jurassic Brent Group, the Lower Jurassic Cook Formation, and the Lower Jurassic Statfjord Formation (Carlsen and Nygaard, 1990). It was suggested that the Gullfaks field was filled from two different source kitchens. The Cook and Statfjord Formations were filled from the

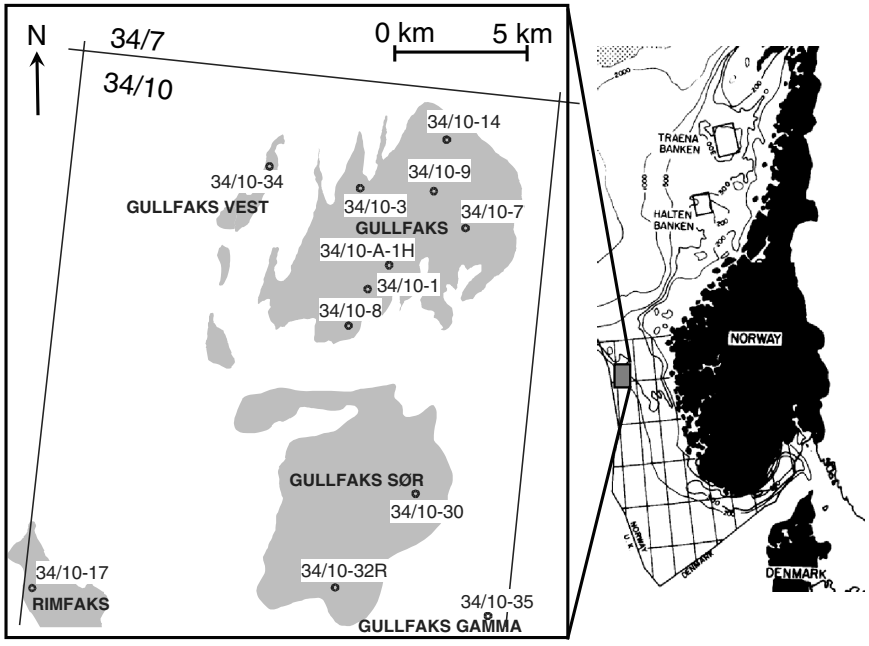

Fig. 1. Location of the boreholes within the Gullfaks and surrounding oil fields, offshore Norway.

Table 1
Origin and characteristics of the investigated oil samples

Well	Oil field	Reservoir formation	Depth (m)	API[a]	Biodegradation level[b]	Pr/n-C_{17}[c]	Ph/n-C_{18}[c]	Pr/Ph[c]	i-C_5/n-C_5	MCH/n-C_7	DNH/NH
34/10-1	Gullfaks	Brent	1839–1844	28.6	3	6.2	4.8	1.5	2.2	5.9	0.81
34/10-3	Gullfaks	Brent	1895–1900	28.8	3	11.0	7.9	1.5	2.4	5.7	0.80
34/10-7	Gullfaks	Cook	1835–1865	37.2	0-1	0.8	0.6	1.7	0.8	1.7	0.62
34/10-8	Gullfaks	Brent	1848–1845	28.6	3	12.3	9.0	1.5	2.9	8.5	0.81
34/10-9	Gullfaks	Brent	1910–1906	31.1	2	1.9	1.4	1.5	1.1	2.6	0.75
34/10-14	Gullfaks	Brent	n.a.	28.9	3	4.7	3.8	1.5	1.7	4.6	0.76
34/10-A1H	Gullfaks	Brent	n.a.	30.9	3	6.5	5.2	1.5	2.3	6.7	0.83
34/10-17	Rimfaks	Brent	2880–2890	36.1	0	0.6	0.3	2.1	0.9	1.6	0.31
34/10-30	Gullfaks Sør	Statfjord	3297–3318	31.7	0-1	0.6	0.3	1.9	0.2	1.9	0.39
34/10-32R	Gullfaks Sør	Statfjord	3345–3351	31.5	0-1	0.6	0.2	2.2	0.6	2.2	0.29
34/10-34	Gullfaks Vest	n.a.	n.a.	31.7	2	1.0	0.8	1.4	0.9	1.5	0.77
34/10-35	Gullfaks Gamma	Cook	4015–4025	43.5	0	0.5	0.3	1.9	0.6	1.9	n.a.

n.a., not available.
[a] Data taken from Oldenburg (2004).
[b] According to Peters et al. (2005).
[c] Ratios calculated with results of saturated fractions.

north and/or east, while the Brent Group was filled from the west (Horstad et al., 1995). It is well documented that the oil within the Gullfaks field is compositionally altered by in-reservoir biodegradation (Horstad et al., 1990b, 1992). The most severe biodegradation took place within the uneroded Brent Group sediments in the western part of the field. The Cook Formation is an important reservoir in the eastern part of the Gullfaks field (wells 34/10-7 and 34/10-9) and is proposed to represent a different oil population with respect to filling direction and source kitchen (Horstad et al., 1990a; Olaussen et al., 1992). Based on both sampling depths and geochemical characteristics (Table 1, Section 4.1), the oil from well 34/10-7 investigated in this study is reservoired in the Cook Formation, whereas the sample from well 34/10-9, like all other studied oils from Gullfaks, originates from the Brent Group.

For comparison, and to better assess effects not related to biodegradation, five additional oil samples were analysed. One of these oils originates from the Gullfaks Vest oil field that was discovered in 1991. Geochemical investigations by Horstad et al. (1995) showed that the oil from Gullfaks Vest is very similar to the oil population found in the Brent Group of the Gullfaks oil field. Two further samples originate from Gullfaks Sør, which is located south of the Gullfaks oil field (Fig. 1). In this structure, petroleum has been found in the Lunde Formation, the Statfjord Formation, the Cook Formation, and the Brent Group (Horstad et al., 1995). During the final stages of

Late Jurassic deformation, the Gullfaks Sør field was displaced one kilometre down with respect to the Gullfaks field along the main fault bounding the Gullfaks field to the east and south. During the subsidence that occurred in the Cretaceous and Tertiary, the temperatures increased more rapidly within the Gullfaks Sør reservoirs, and today, the reservoir temperature is above 120 °C (Hesthammer et al., 2002). Additional samples were obtained from the Gullfaks Gamma oil field, located southeast of Gullfaks Sør, and from the Rimfaks oil field, southwest of Gullfaks Sør (Fig. 1).

3. Materials and methods

3.1. Abbreviations

The abbreviations used in the text, figures, and tables for the different hydrocarbons are listed in Table 2 with their respective compound names.

3.2. Whole oil analysis (GC)

The amount of individual light hydrocarbons of the oil samples was quantified by gas chromatography (GC)-FID-measurements. The crude oil (0.5 μl) was injected into the GC (6890 Series, Agilent Technology, USA), equipped with a programmable temperature vaporisation inlet (PTV, Agilent Technology, USA) with a septumless head, working in split/splitless mode. The injector was held at a split ratio of 1:50 and an initial temperature of 40 °C. With injection, the injector was heated to 300 °C at a programmed rate of 700 °C min^{-1} and held at this temperature for the rest of the analysis time. Helium was set at a flow rate of 2 ml min^{-1}. Petroleum components were separated on a HP Ultra 1 fused silica capillary column (50 m × 0.32 mm i.d. × 0.52 μm film thickness, Agilent Technology, USA). The temperature of the GC oven was initially held at 40 °C for 2 min, followed by a 3 °C min^{-1} ramp to 140 °C, then at a rate of 5 °C min^{-1} to 300 °C and held there for a further 25 min. Quantification of the light hydrocarbons was done with reference to the externally quantified concentrations of phytane without using response factors. Phytane concentrations were determined by GC-FID with reference to an internal standard (5α-androstane) using aliphatic hydrocarbon fractions obtained by medium pressure liquid chromatography (MPLC) (Radke et al., 1980).

Table 2
List of hydrocarbons and their abbreviations

Abbreviation	Compound name
n-C_4	*n*-Butane
i-C_5	*i*-Pentane
n-C_5	*n*-Pentane
3MP	3-Methylpentane
n-C_6	*n*-Hexane
B	Benzene
CH	Cyclohexane
3MH	3-Methylhexane
n-C_7	*n*-Heptane
MCH	Methylcylohexane
T	Toluene
26DMHe	2,6-Dimethylheptane
n-C_9	*n*-Nonane
n-C_{10}	*n*-Decane
n-C_{11}	*n*-Undecane
n-C_{12}	*n*-Dodecane
n-C_{13}	*n*-Tridecane
n-C_{14}	*n*-Tetradecane
n-C_{15}	*n*-Pentadecane
Pr	Pristane
Ph	Phytane
DNH	17α(H),21β(H)-28,30-Dinorhopane
NH	17α(H),21β(H)-30-Norhopane

3.3. Stable carbon isotope analysis

The carbon isotopic composition of petroleum components was measured by gas chromatography/combustion/isotope-ratio mass spectrometry (GC–C–IRMS). The GC–C–IRMS system consisted of a GC unit (6890 Series, Agilent Technology, USA) connected to a Finnigan MAT GC III combustion device coupled via open split to a Finnigan MAT 253 mass spectrometer (ThermoElectron, Germany). The organic components of the GC effluent stream were oxidised to CO_2 in the combustion furnace held at 940 °C on a CuO/Ni/Pt catalyst. CO_2 was transferred on-line to the mass spectrometer to determine carbon isotope ratios. Crude oil (0.5 μl) was injected to the programmable temperature vaporisation inlet (PTV, Agilent Technology, USA) with a septumless head, working in split/splitless mode. The injector was held at a split ratio of 1:30 and an initial temperature of 50 °C. With injection, the injector was heated to 300 °C at a programmed rate of 700 °C min^{-1} and held at this temperature for the rest of the analysis time. Helium was set at a flow rate of 1 ml min^{-1}. Petroleum components were separated on a HP Ultra 1 fused silica capillary column (50 m × 0.32 mm i.d. × 0.52 μm film thickness, Agilent Technology, USA). The temperature of the GC oven was initially held at 40 °C for 3 min, followed by a 2 °C min^{-1} ramp to 100 °C, then at a rate of 4 °C min^{-1} to 310 °C and held there for a further 30 min. All oil samples were measured in triplicate with a standard deviation of $\leqslant 0.5‰$ for most of the compounds and samples (all standard deviations are provided in Table 3).

3.4. Calculations

The carbon isotope composition is reported in δ-notation (per mill) relative to Vienna Pee Dee Belemnite standard (V-PDB) according to

$$\delta^{13}C[‰] = \left(\frac{(^{13}C/^{12}C)_{Sample}}{(^{13}C/^{12}C)_{Standard}} - 1 \right) \times 1000. \quad (1)$$

Table 3
Carbon isotope ratios (with standard deviation) of selected hydrocarbons in crude oils from the Gullfaks and surrounding oil fields

	n-C_4	*i*-C_5	*n*-C_5	3MP	*n*-C_6	B	CH	3MH	*n*-C_7	MCH	T	26DMHe	*n*-C_9	*n*-C_{10}	*n*-C_{11}	*n*-C_{12}	*n*-C_{13}	*n*-C_{14}	*n*-C_{15}	Pr	Ph
34/10-1																					
$\delta^{13}C$	−23.3	−27.3	−25.4	−27.8	−28.2	−24.6	−26.1	−29.2	−26.0	−26.7	−26.4	−31.7	−25.7	−25.7	n.d.	n.d.	n.d.	−31.1	n.d.	−31.6	−32.0
SD	0.1	0.2	0.3	0.1	0.5	0.3	0.3	0[a]	0.2	0.2	0.4	0.3	0.4	0[a]	n.d.	n.d.	n.d.	0.3	n.d.	0.6	0.4
34/10-3																					
$\delta^{13}C$	−20.3	−26.8	−24.5	−27.7	−26.2	−25.7	−26.9	n.d.	−25.1	−27.2	−27.0	−31.3	−25.1	n.d.	−29.1	n.d.	n.d.	−28.5	−29.6	−31.0	−31.7
SD	0.5	0.2	0.1	0.2	0.3	0.3	0.5	n.d.	0.3	0.1	0.2	0.6	0.1	n.d.	0.4	n.d.	n.d.	0.3	0.2	0.3	0.5
34/10-7																					
$\delta^{13}C$	−27.3	−28.5	−28.5	−28.7	−29.0	−26.4	−26.8	−29.2	−29.5	−27.5	−26.6	−31.0	−28.5	−28.9	−29.8	−29.5	−29.9	−29.6	−30.0	−31.8	−31.8
SD	0.2	0.3	0.3	0.4	0.3	0[a]	0.5	0[a]	0[a]	0.3	0.5	0.1	0.4	0.4	0.2	0.4	0.3	0.3	0.2	0.4	0.8
34/10-8																					
$\delta^{13}C$	−21.5	−26.9	−23.9	−27.5	−25.9	−24.5	−26.5	−29.1	−25.8	−27.2	−27.3	−31.8	−26.1	−28.5	−30.1	n.d.	n.d.	−28.7	−29.5	−31.3	−31.8
SD	0.2	0.2	0.3	0.2	0[a]	0.4	0.2	0.2	0.3	0.4	0.3	0.2	0.2	0.4	0.3	n.d.	n.d.	0.1	0.2	0.3	0.4
34/10-9																					
$\delta^{13}C$	−26.5	−28.1	−28.0	−28.5	−28.5	−25.4	−26.9	−29.6	−28.5	−27.5	−26.9	−31.7	−27.3	−27.6	−29.1	−28.0	−28.9	−28.7	−29.2	−31.5	−31.5
SD	0.1	0.1	0.2	0.2	0.5	0.4	0.4	0.2	0.3	0.4	0.4	0.2	0.8	0.6	0.5	0.3	0.6	0.1	0.3	0.5	0.7
34/10-14																					
$\delta^{13}C$	−24.1	−27.6	−26.6	−28.2	−28.4	−25.6	−26.9	−29.6	−27.2	−27.3	−27.2	n.d.	−27.7	−28.8	−29.5	n.d.	n.d.	−28.6	n.d.	−31.8	−32.0
SD	0[a]	0.3	0.4	0.3	0.5	0.4	0.1	0[a]	0.1	0.4	0.4	n.d.	0[a]	0.4	0.4	n.d.	n.d.	0[a]	n.d.	0.2	0.3
34/10-A-1H																					
$\delta^{13}C$	−22.3	−26.5	−24.5	−27.7	−28.4	−25.3	−26.3	−29.3	−26.1	−26.8	−26.9	−31.4	−27.3	−28.2	−28.9	−28.9	n.d.	−29.4	−29.8	−31.0	−31.1
SD	0.1	0.3	0.2	0.2	0.1	0.9	0[a]	0.1	0.3	0.4	0.4	0.5	0[a]	0.1	0.3	0.1	n.d.	0.5	0[a]	0.1	0.1
34/10-17																					
$\delta^{13}C$	−25.1	−26.0	−26.0	−26.4	−26.6	−27.6	−25.2	−27.6	−27.7	−26.5	n.d.	−29.4	−29.4	−29.9	−30.2	−30.2	−30.1	−29.8	−29.9	−30.3	−30.3
SD	0.2	0.1	0.2	0.1	0[a]	1.2	0.1	0.4	0[a]	0.4	n.d.	0.4	0.2	0.5	0.5	0.5	0.6	0.4	0[a]	0.1	0.6
34/10-30																					
$\delta^{13}C$	n.d.	−24.1	−25.1	−26.3	−26.8	−25.1	−25.6	−26.9	−27.8	−26.5	n.d.	−29.8	n.d.	n.d.	−28.4	−28.5	−28.7	−28.8	−29.1	−29.8	−29.9
SD	n.d.	0.4	0.2	0.7	0.3	1.1	0.6	0.8	0.5	0.8	n.d.	0.1	n.d.	n.d.	0.5	0.4	0.5	0.9	0.2	0.3	0.6
34/10-32R																					
$\delta^{13}C$	−24.8	−25.5	−26.2	−26.6	−27.4	−26.0	−26.5	−27.1	−28.3	−27.0	−28.0	−29.2	n.d.	−29.9	−30.4	−30.0	−29.7	n.d.	−29.6	−29.4	−29.8
SD	0.5	0.2	0.2	0.2	0.4	0.3	0.4	0.5	0.5	0.3	0.7	0.4	n.d.	0.9	0.8	0.8	0.7	n.d.	0.3	0.4	0.6
34/10-34																					
$\delta^{13}C$	−27.8	−28.8	−29.3	−28.9	−30.4	n.d.	−28.4	−29.8	−30.5	−28.6	n.d.	n.d.	−30.2	−31.2	−31.6	−32.2	−31.8	−32.1	−31.5	−32.4	−32.8
SD	0.3	0.1	0.1	0.4	0.4	n.d.	0.6	0.2	0.6	0.5	n.d.	n.d.	0.2	0.6	1.0	1.0	0.6	0.4	0.2	0.5	0.4
34/10-35																					
$\delta^{13}C$	−25.3	−27.1	−26.6	−27.7	−27.7	−28.4	−25.5	n.d.	−29.2	−28.2	−29.3	−29.8	−29.0	−29.4	−30.0	−30.2	−29.8	−29.8	−30.2	−30.8	−30.4
SD	0.1	0.1	0.1	0.2	0.1	0.4	0.3	n.d.	0.3	0.3	0.4	0.6	0[a]	0[a]	0.1	0.3	0.3	0.5	0.2	0.2	0.5

n.d., not determined.
[a] $n = 1$.

The isotope fractionation associated with microbially mediated degradation of light hydrocarbons can be described by the Rayleigh equation (Eq. (2))

$$\frac{R}{R_i} = F^{\left(\frac{1}{\alpha}-1\right)}, \tag{2}$$

where F is the fraction of the light hydrocarbon remaining (C/C_i), R is the isotopic composition of the light hydrocarbon at a particular F, and R_i gives the initial isotopic composition of the light hydrocarbon. The isotope fractionation factor (α) relates the changes in the isotope composition to changes in concentration of the residual fraction during the transformation.

The Rayleigh equation can be applied to describe biodegradation processes in oil samples when assuming that the investigated oil samples are equal in terms of maturity and facies, and that the differences between the oil samples are only due to in-reservoir biodegradation. The sample that shows the lowest influence of biodegradation (highest concentration and lightest isotopic composition of the light hydrocarbon of concern) is assumed to represent the initial oil composition (giving C_i and R_i) and will be used as the local end-member of the biodegradation sequence. According to the Rayleigh equation (Eq. (2)) any concentration and isotopic signature of an individual hydrocarbon is suitable as an end-member as long as it indeed represents the least degraded sample in a sequence of samples.

To test if the concentration and isotope data fulfil the Rayleigh equation and to calculate the isotope fractionation factor (α) that describes the specific reaction, the following equation (Eq. (3)) can be used

$$\ln\left(\frac{R}{R_i}\right) = \left(\frac{1}{\alpha} - 1\right) \times \ln F. \tag{3}$$

For quantification of biodegradation using the Rayleigh equation it is necessary to have an appropriate isotope fractionation factor of the individual compound and the degradation pathway. This factor can only be obtained from laboratory experiments with pure cultures. Fractionation factors determined in field studies may give hints on the actual mechanism active for a specific compound in this reservoir (Vieth et al., 2005).

To quantify the amount of a hydrocarbon that is lost by biodegradation, the percentage of biodegradation (B_c) is calculated by Eq. (4)

$$B_c = (1 - F) \times 100. \tag{4}$$

Additionally, the percentage of biodegradation (B_i) of the residual substrate fraction is calculated using a known isotope fractionation factor (α) and the isotopic composition of a substrate at the beginning of the reaction (R_i) and the change in the isotopic composition during the transformation reaction (Eq. (5); Richnow et al., 2003b)

$$B_i = \left[1 - \left(\frac{R}{R_i}\right)^{\left(\frac{1}{\frac{1}{\alpha}-1}\right)}\right] \times 100. \tag{5}$$

This calculation of the percentage of biodegradation is independent of the concentrations and gives the amount of biodegradation that is necessary to lead to the measured change in isotopic composition of the substrate.

4. Results and discussion

4.1. Origin of the investigated oils

Crude oils in the North Viking Graben are either sourced from the Draupne or the Heather Formation, or represent mixed oils originating in variable proportions from both of these two source rocks (Gormly et al., 1994; Horstad et al., 1995). Gormly et al. (1994) demonstrated that pristane/phytane (Pr/Ph) ratios and whole oil carbon isotopic signatures are diagnostic for oil–oil and oil–source correlations in the North Viking Graben. Draupne-sourced oils were shown to have lower Pr/Ph ratios (1.0–1.5) and lower $\delta^{13}C$ values (−31 to −28‰) in comparison to Heather-sourced oils (Pr/Ph ratios 2.15 to 4.0; whole oil $\delta^{13}C$ −28 to −25‰). Oils having values between these ranges were assessed as being of mixed origin. It has also been shown that within the Tampen spur area the 17α(H),21β(H)-28,30-dinorhopane/17α(H),21β(H)-30-norhopane (DNH/NH) ratio is one of the most powerful biomarker parameters to discriminate between oils (Horstad et al., 1995). 28,30-Dinorhopane apparently is more abundant in the Draupne than in the Heather Formation, and its abundance in an oil seems to be more dependent upon source rock facies than on maturity (Horstad et al., 1995).

Representative whole oil gas chromatograms are shown in Fig. 2. According to the above mentioned Pr/Ph classification, the oil from Gullfaks Vest (34/10-34) and the oils from the Brent Group of the Gullfaks field (Table 1) can be classified as exclusively sourced from the Draupne Formation, while only one oil from Gullfaks Sør (well 34/10-32R) can be classified as exclusively Heather-sourced (Fig. 3). The oil from the Cook Formation of the Gullfaks field (well 34/10-7) as well as the oils from Gullfaks Gamma and Rimfaks would be regarded as mixtures. Accordingly, the samples from Gullfaks Vest and Gullfaks, besides sample 34/10-7, have high DNH/NH ratios, while this parameter has the lowest value in sample 34/10-32R from Gullfaks Sør (Table 1).

In principal agreement with the observed variability of the Pr/Ph ratios, we found that these isoprenoids showed the lightest carbon isotope signature for the oil from Gullfaks Vest and the heaviest for that from Gullfaks Sør (Fig. 3). A comparison of our $\delta^{13}C$ data of pristane and phytane with those for the whole oils (Gormly et al., 1994) reveals depletion in ^{13}C of ~3‰ for these isoprenoids.

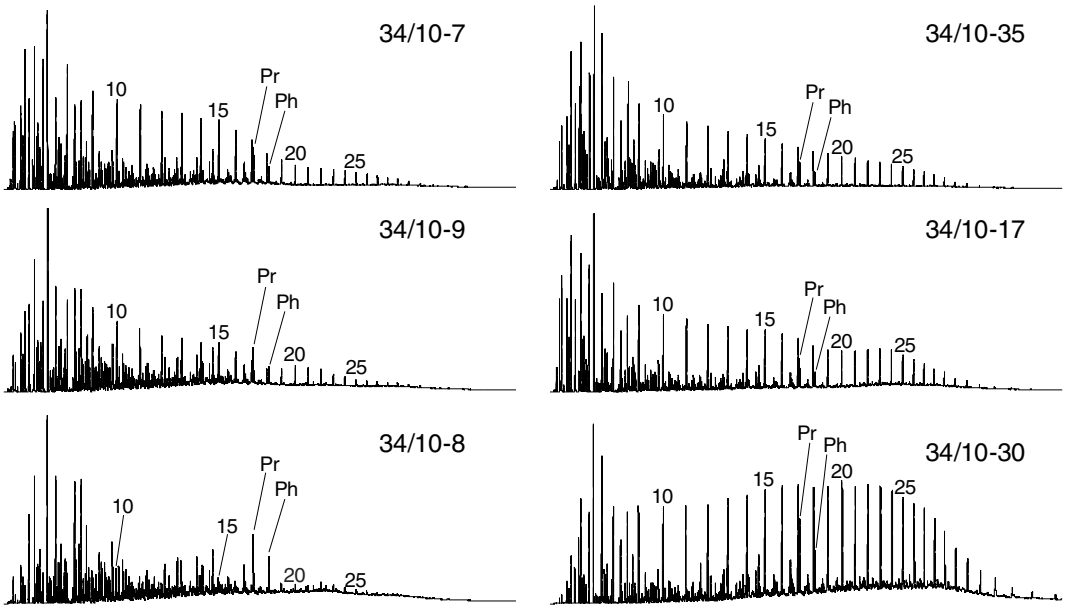

Fig. 2. GC traces of whole oil analysis of selected oil samples from Gullfaks and surrounding oil fields.

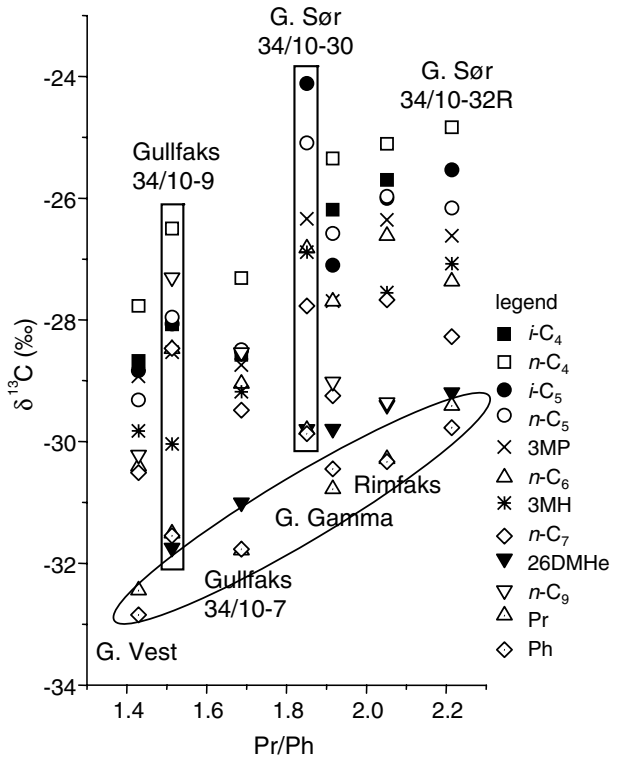

Fig. 3. Carbon isotope ratios of selected hydrocarbons in crude oils from the Tampen Spur area plotted as a function of the Pr/Ph ratios.

The maximum isotopic difference between $\delta^{13}C_{pristane}$ and $\delta^{13}C_{phytane}$ for a given oil sample is 0.7‰ (sample 34/10-3), which is well within the standard deviation of the measurements. This similarity in the carbon isotopic composition is consistent with the hypothesis that both isoprenoid biomarkers are derived predominantly from the same source, i.e., group of primary producers (Collister et al., 1992).

In addition to the carbon isotope signatures of pristane and phytane, the $\delta^{13}C$ values of various light hydrocarbons also show a good positive correlation with the Pr/Ph ratios (Fig. 3). Moreover, we observe a pronounced shift to heavier $\delta^{13}C$ values for compounds of decreasing molecular weight, with some apparent differences between linear and branched isomeric compounds. Surprisingly, no similarly pronounced correlation of $\delta^{13}C$ values of the C_{9+} *n*-alkanes with the Pr/Ph ratios can be observed (plot not shown; data are provided in Table 3). These results suggest that the carbon isotope signatures of light hydrocarbons can have a high potential for assessment of source and for correlation, provided these compounds are not or only marginally affected by biodegradation (see detailed discussion below). It becomes clear, on the other hand, that possible differences regarding source and maturity must be well constrained before applying carbon isotope signatures of light hydrocarbons as indicators for biodegradation.

With reference to Gormly et al. (1994), oils from Gullfaks Gamma were derived from the Heather Formation, while oils from Gullfaks Sør would be expected to be a mixture derived from both source rocks. This is not completely in agreement with our results, which suggest the opposite at least for the two particular samples from Gullfaks Gamma and Gullfaks Sør analysed here. Furthermore, the oil in the Cook Formation reservoir of the Gullfaks field (34/10-7) should have obtained at least some contribution from the Heather Formation according to our results. These inferences are in agreement with the abovementioned observations on the DNH/NH ratio (Table 1).

Both oils from Gullfaks Sør have a similar isotopic signature of pristane and phytane (Table 3), but the sample from well 34/10-30 shows a small, but significantly lower, Pr/Ph ratio. Most of the compounds reported in Table 3 have slightly heavier carbon isotope signatures for sample

34/10-30 in comparison to sample 34/10-32R. We interpret this as a slightly higher impact of secondary alteration on the sample from well 34/10-30. Therefore, the carbon isotope signatures of pristane and phytane might be more suitable as source indicators for sample 34/10-30 than the Pr/Ph ratio. Both oils from Gullfaks Sør have low values of the DNH/NH ratio.

Fig. 3 includes the Pr/Ph ratio and the $\delta^{13}C$ values of pristane and phytane for the least degraded of the six oils from the Brent Group of the Gullfaks oil field (34/10-9). The data plot slightly above a trend line defined by the Pr/Ph ratio and the $\delta^{13}C$ values of the unaltered oils in our sample set, i.e., the Pr/Ph ratio is too low or the $\delta^{13}C$ value is too heavy. The Pr/Ph ratios of all six Brent Group-derived oils from the Gullfaks field show only very low variability (Table 1). We therefore assume that the Pr/Ph ratio in this case is an appropriate indicator of a common origin of these six oils, which is well in agreement with assumptions on the origin of these oils and the filling history of the reservoir (Horstad et al., 1995). The Pr/Ph ratio of the Cook Formation derived oil from Gullfaks (34/10-7) is significantly higher, supporting the hypothesis that it represents a different oil population. This again is confirmed by the DNH/NH ratio, which is lower for sample 34/10-7 than for the six oil samples of the Gullfaks field that were derived from the Brent Formation. The $\delta^{13}C$ values of pristane and phytane of sample 34/10-9 are $-31.5 \pm 0.5‰$ and $-31.5 \pm 0.7‰$. Isotopic values for the other oils are essentially the same (from -32.0 to $-31.0‰$; Table 3) falling within the experimental error. We assume that the small isotopic differences represent the natural variability for closely related oil populations, i.e., we would expect a similar scatter for the other oil fields if the number of analysed samples would be higher than 1 or 2.

4.2. Characterisation of biodegradation

A quite common indicator of microbial alteration in crude oil samples is the increase in the pristane/*n*-heptadecane (Pr/*n*-C_{17}) and phytane/*n*-octadecane (Ph/*n*-C_{18}) ratios (Table 1). The data of the 12 oil samples are shown in Fig. 4, indicating a strong increase in biodegradation from well 34/10-7 to well 34/10-3, that can be described by a linear regression with the slope $b = 1.34$ and a coefficient of determination $R^2 = 0.99$. The samples from the Gullfaks oil field show a wide range in Pr/*n*-C_{17} and Ph/*n*-C_{18} ratios, from 0.8 to 12.3 and 0.6 to 9.0, respectively. The samples from the other oil fields show a quite narrow range in Pr/*n*-C_{17} and Ph/*n*-C_{18} ratios from 0.5 to 1.0 and 0.2 to 0.8, respectively. Also the higher ratios for methylcyclohexane/*n*-heptane (MCH/*n*-C_7) and *i*-pentane/*n*-pentane (*i*-C_5/*n*-C_5) within the Gullfaks oil samples compared to the lower ratios of the unbiodegraded oil samples from the surrounding oil fields clearly demonstrate the increasing levels of biodegradation in the former (Table 1, Fig. 4). Therefore, the following considerations on biodegradation are limited to the six Brent Group oil samples from the

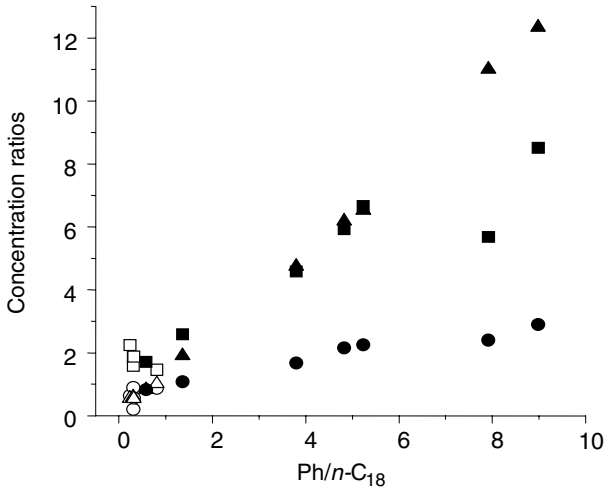

Fig. 4. Ratios of Pr/*n*-C_{17} (triangles), *i*-C_5/*n*-C_5 (circles), and MCH/*n*-C_7 (squares) of crude oil samples from the Gullfaks field (filled symbols) and surrounding oil fields (open symbols) plotted versus the Ph/*n*-C_{18} ratios.

Gullfaks field because: (I) the samples from the other oil fields show no or little (Gullfaks Vest) indication of biodegradation, (II) only samples from the Gullfaks field were available that represent an in-reservoir gradient in biodegradation, and (III) only these six samples derive from the Brent Group and thus represent the same origin, filling history, and also more or less the same maturity.

The carbon isotope ratios of the short-chain *n*-alkanes (*n*-C_4 to *n*-C_7) show a decreasing variability with increasing carbon number in the Brent Group oils from the Gullfaks field (Fig. 5). The carbon isotope ratios of *n*-butane are between $-26.5 \pm 0.1‰$ (34/10-9) and $-20.3 \pm 0.5‰$ (34/10-3), whereas the carbon isotope ratios of *n*-pentane, *n*-hexane, and *n*-heptane become only enriched by 4.0, 2.5, and 3.4‰, with sample 34/10-9 always showing the lowest $\delta^{13}C$ value (lightest isotopic composition) (Fig. 5). This indicates that the oil from well 34/10-9 can be regarded as the least biodegraded sample within the investigated

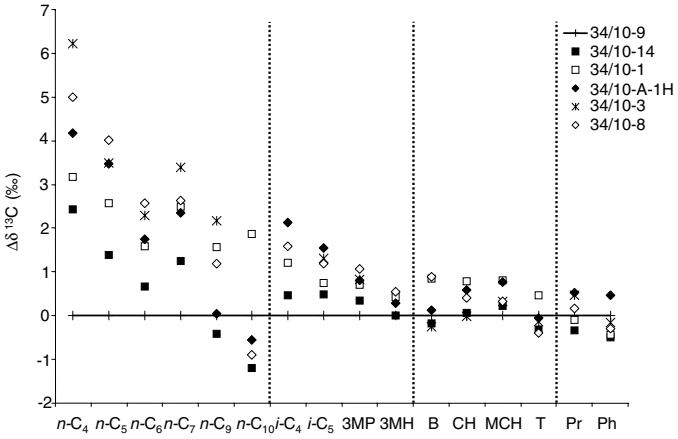

Fig. 5. Carbon isotope ratios of selected hydrocarbons (given as difference to the proposed end-member (sample 34/10-9)) in crude oils from the Gullfaks field.

sample set, which is corroborated by this sample having the highest concentrations for most evaluated light hydrocarbons (Table 4). Therefore, this sample is used as the local end-member in our further evaluations. The fact that the enrichment in ^{13}C is higher for *n*-butane than for *n*-pentane, *n*-hexane, and *n*-heptane may indicate that *n*-butane is more susceptible to biodegradation than the other *n*-alkanes, as also observed in natural gases (Boreham et al., 2001), also that the already mentioned "dilution effect" leads to a lesser overall isotope fractionation with increasing number of carbon atoms in the molecule.

Due to the lack of baseline separation from dimethylcyclohexanes, no reliable carbon isotope ratios are available for *n*-octane. Carbon isotope ratios of *n*-nonane are between $-27.8 \pm 0.1‰$ (34/10-14) and $-25.1 \pm 0.1‰$ (34/10-3). Here, the carbon isotope ratio of *n*-nonane in sample 34/10-9 is slightly higher ($-27.3 \pm 0.8‰$), but because of the high standard deviation, this difference in isotopic composition is not significant. For *n*-decane, carbon isotope ratios in five samples are between -28.0 and $-27.6‰$, but for sample 34/10-1 *n*-decane is unusually enriched in ^{13}C ($\delta^{13}C = -25.7 \pm 0.4‰$). No significant change with degree of biodegradation in the carbon isotopic composition of *n*-undecane can be observed, and the $\delta^{13}C$ values are between -29.1 and $-30.1‰$. This is consistent with the $<1‰$ enrichments in the C_7 to C_{11} *n*-alkanes from biodegraded Permian oils (Boreham et al., 1995). Considering these carbon isotope ratios, it can be concluded that (I) susceptibility of *n*-alkanes to biodegradation decreases with increasing carbon number and/or (II) biodegradation of *n*-alkanes is related to a decreasing overall isotope effect with increasing carbon number of the molecule.

For *i*-butane, *i*-pentane, 3-methylpentane, and 3-methylhexane the lightest isotopic compositions were observed in sample 34/10-9, also supporting that this sample represents the local end-member (Fig. 5). The total enrichment in ^{13}C within the six biodegraded oil samples is 2.1, 1.6, 1.1, and 0.5‰ for *i*-butane, *i*-pentane, 3-methylpentane, and 3-methylhexane, respectively, generally indicating that biodegradation also has an influence on the isotopic composition of these branched alkanes. The total change in carbon isotopic composition of 3-methylpentane and 3-methylhexane is not significant; however, it is obvious that this enrichment is in accordance with the biodegradation trend as given by the increasing Ph/*n*-C_{18} ratios.

4.3. Carbon isotope constraints on the evolution of the *i*-C_5/*n*-C_5 and MCH/*n*-C_7 ratios

Considering biodegradation as a relevant process within a reservoir, a change in the concentration ratio of *i*-pentane and *n*-pentane will give valuable information. It is assumed that the branched hydrocarbon is less susceptible to microbial attack than the *n*-alkane, hence the increase in *i*-C_5/*n*-C_5-ratio is a robust and widely applied indicator of biodegradation (Welte et al., 1982). Within the samples from the Brent Group of the Gullfaks field, the *i*-C_5/*n*-C_5-ratio

Table 4
Concentrations of selected hydrocarbons in crude oils samples (mg g^{-1} oil) from the Gullfaks and surrounding oil fields

	n-C_4	*i*-C_5	*n*-C_5	3MP	*n*-C_6	B	CH	3MH	*n*-C_7	MCH	T	26DMHe	*n*-C_9	*n*-C_{10}	*n*-C_{11}	*n*-C_{12}	*n*-C_{13}	*n*-C_{14}	*n*-C_{15}	Pr	Ph
34/10-1	0.5	1.5	0.7	0.5	0.7	0.2	2.5	0.5	0.8	4.7	2.3	0.5	1.9	1.6	1.0	1.0	1.1	0.6	0.7	3.5	2.2
34/10-3	0.2	1.0	0.4	0.8	0.7	0.2	3.2	0.8	1.1	6.2	3.6	0.4	1.9	1.4	0.7	0.7	0.8	0.7	0.4	3.5	2.3
34/10-7	2.9	4.8	5.7	2.3	6.5	1.8	6.2	2.1	6.1	10.4	4.2	0.4	8.3	8.2	7.4	7.2	6.7	5.8	5.9	3.9	2.1
34/10-8	0.4	1.1	0.4	0.4	0.7	0.2	2.6	0.5	0.5	4.6	2.8	0.5	1.5	1.1	0.6	0.6	0.8	0.3	0.4	4.1	2.6
34/10-9	1.3	2.2	2.1	0.9	2.6	0.7	3.4	0.9	2.2	5.7	3.1	0.5	4.7	4.0	3.1	2.9	2.8	2.2	2.3	3.3	2.0
34/10-14	0.5	1.3	0.8	0.6	1.0	0.4	2.8	0.7	1.2	5.3	3.4	0.7	2.8	2.4	1.6	1.4	1.4	0.9	1.0	3.6	2.3
34/10-A-1H	0.1	0.8	0.4	0.6	0.7	0.2	2.6	0.6	0.8	5.6	3.0	n.d.	2.0	1.7	1.0	1.0	1.0	0.6	0.7	3.5	2.2
34/10-17	2.4	3.3	3.7	1.8	6.0	0.8	5.6	0.8	6.3	10.0	5.4	1.8	9.5	7.9	6.6	5.9	5.8	6.2	5.2	2.5	1.3
34/10-30	0.2	0.3	1.5	0.2	2.0	2.9	1.8	0.2	2.6	4.9	5.1	1.2	6.2	5.7	5.7	6.0	6.7	8.6	7.9	4.9	2.7
34/10-32R	1.3	1.2	1.9	0.9	2.8	3.7	2.8	0.3	3.9	8.8	8.4	0.9	5.5	4.9	4.7	4.8	5.3	7.1	6.4	3.9	1.6
34/10-34	1.7	1.9	2.2	1.0	8.3	0.1	2.3	0.7	4.0	5.8	2.0	2.9	7.1	5.6	4.4	4.2	4.3	4.5	3.8	3.3	2.3
34/10-35	0.8	2.0	3.6	2.0	8.9	8.5	8.7	3.2	15.2	28.6	31.5	4.8	21.4	16.1	13.1	11.3	10.5	8.3	8.7	3.7	2.0

n.d., not determined.

increases from 1.1 (34/10-9) to 2.9 (34/10-8) (Table 1 and Fig. 4). Highest concentrations of *i*-pentane and *n*-pentane were found in the sample from well 34/10-9. In all samples from the Brent Group of the Gullfaks oil field, the *i*-pentane concentration is higher than the *n*-pentane concentration (Fig. 6). For both compounds, the decrease in concentration is related to an enrichment in ^{13}C in the residual fractions. For *n*-pentane, a change in carbon isotope ratio from −28.0 to −23.9‰ was observed, whereas the isotopic composition of *i*-pentane only changes from −28.1 to −26.5‰ (Fig. 6). This observation is in agreement with data from George et al. (2002) who investigated the molecular and isotopic composition of the light hydrocarbons in oil samples from the Barrow Island oil field, Australia.

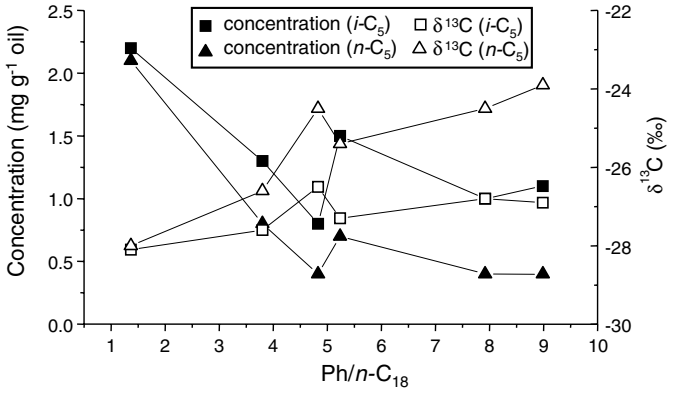

Fig. 6. Concentrations (filled symbols) and carbon isotope ratios (open symbols) of *i*-pentane (squares) and *n*-pentane (triangles) in the six oil samples from the Gullfaks Field (Brent Group oils), plotted as a function of the Ph/*n*-C_{18} ratios.

For application of the Rayleigh equation the carbon isotopic compositions of *n*-pentane and *i*-pentane of sample 34/10-9, the local end-member, give the R_i for the calculations, and the concentrations of *n*-pentane and *i*-pentane give the C_i (see Section 3.4). The other samples are obviously more influenced by biodegradation, giving the data for *R* and *C*. The coefficients of determination of the linear regression lines, when natural logarithms of R/R_i are plotted over natural logarithms of *F*, are 0.93 and 0.90 for *n*-pentane and *i*-pentane, respectively (Figs. 7A and B). This high correlation demonstrates that the data fit the Rayleigh model. We therefore conclude that the decrease in concentration and the enrichment in ^{13}C for both *n*-pentane and *i*-pentane are in fact caused by microbial degradation. From the slope of the linear regression lines, the isotope fractionation factors (α) can be calculated. The fractionation factor is a measure of the overall kinetic isotope fractionation that will go along with biodegradation of a substrate. Here, the isotope fractionation factors that were calculated from the field data are significantly different for *n*-pentane ($\alpha = 1.0023$) and *i*-pentane ($\alpha = 1.0016$). Both compounds are composed of the same number of carbon atoms, therefore our results point to mechanistic differences in the microbial utilisation of these two substrates in the Gullfaks field, which result in the different fractionation factors. It is well known that the fractionation factor may not only differ for different substrates but also for different enzymatic reactions (Morasch et al., 2004; Zwank et al., 2005). Although the actual mechanisms active in Gullfaks are not known, it becomes evident that the higher enrichment of ^{13}C in the residual *n*-pentane is not only due to more efficient utilisation of this compound in comparison to *i*-pentane (George et al., 2002), but also

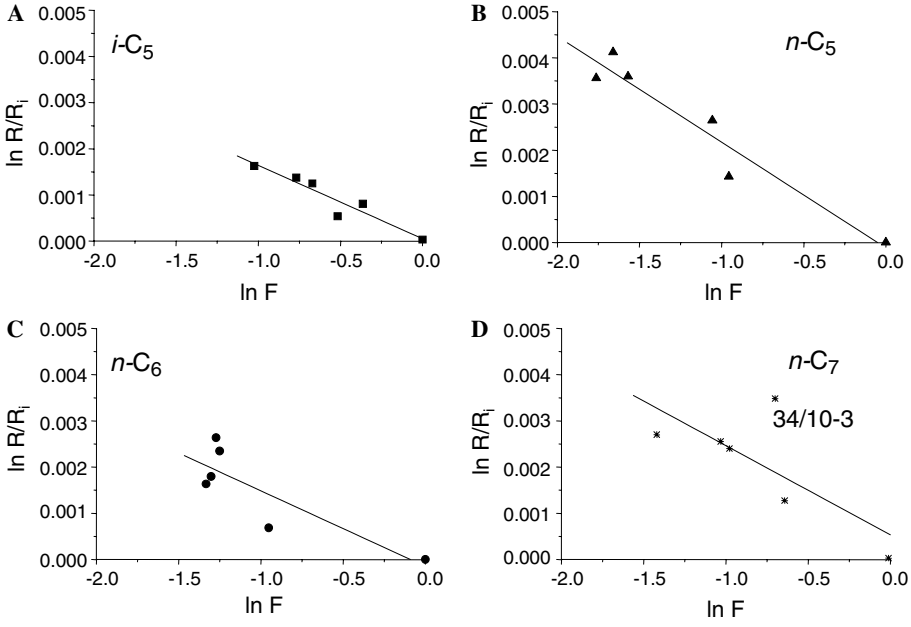

Fig. 7. Concentration and carbon isotope data of (A) *i*-pentane, (B) *n*-pentane, (C) *n*-hexane, and (D) *n*-heptane for six oil samples from the Gullfaks field (Brent Group oils), plotted according to the Rayleigh equation as $\ln R/R_i$ over $\ln F$.

to a different isotope effect of the initial activation reaction. In Gullfaks, the evolution of the *i*-C_5/*n*-C_5 ratio involves concomitant degradation of both compounds, however, at different rates.

An increase in the ratio of MCH/*n*-C_7, the inverse of the paraffinicity ratio (Thompson, 1983), also indicates increasing extent of biodegradation. With cyclic compounds being less susceptible to microbial attack, the linear *n*-heptane will be degraded preferentially (George et al., 2002; Wenger et al., 2001). The observed MCH/*n*-C_7 ratios are in a range from 2.6 to 8.5, with the lowest value calculated for the oil from well 34/10-9 and the highest for the oil from well 34/10-8 (Table 1 and Fig. 4). The concentration of methylcyclohexane reaches a maximum value of 6.2 mg g^{-1} oil in the sample from well 34/10-3, and the concentrations in the other oils are lower, with a minimum value of 4.6 mg g^{-1} oil in the sample from well 34/10-8 (Fig. 8). The differences in the carbon isotope ratio of methylcylohexane for all samples are lower than 1‰, indicating that the difference in concentration was not due to biodegradation, or that the low level of biodegradation did not cause a significant change in the carbon isotopic composition of methylcyclohexane. This observation is in contrast to results from George et al. (2002) and Masterson et al. (2001) who measured large isotope enrichments for methylcyclohexane in biodegraded oil samples from the Barrow Island oil field, Australia, and the West Sak oil field, Alaska, respectively. Also, the carbon isotope ratios determined for cyclohexane in the six biodegraded oil samples only vary from −26.9 to −26.1‰ (Table 3; Fig. 5), showing no significant change along the biodegradation trend.

The concentration of *n*-heptane decreases from 2.2 mg g^{-1} oil (well 34/10-9) to 0.5 mg g^{-1} oil (well 34/10-8) (Table 4). The stable carbon isotopic composition of the residual *n*-heptane becomes enriched in ^{13}C, with $\delta^{13}C$ changing from −28.5 to −25.1‰ (Fig. 8). If these data from the oil samples of the Brent Group were considered and evaluated according to the Rayleigh equation (Eq. (2)), the coefficient of determination of the linear regression line is 0.56 (Fig. 7D). This correlation is much lower than for other fractionation factors determined in this study, although fair considering that field data were evaluated. From Fig. 7D it is obvious that the data for sample 34/10-3 cause this relatively low coefficient of determination, because the $\delta^{13}C$ value indicates a high degree of biodegradation, whereas the concentration is still high; reasons for this apparent deviation of *n*-heptane are unknown. If this data point is not considered, the coefficient of determination of the linear regression line is as good as $R^2 = 0.94$ and the isotope fractionation factor has only slightly changed from $\alpha = 1.0019$ to $\alpha = 1.0021$.

It is obvious that methylcyclohexane is not biodegraded and therefore that the evolution of the MCH/*n*-C_7 ratio depends exclusively on the selective removal of *n*-heptane due to in-reservoir biodegradation. This observation is in contrast to the *i*-C_5/*n*-C_5 ratio, where both components are biodegraded but at different rates.

4.4. Quantification of n-hexane biodegradation

The concentration of *n*-hexane decreases from 2.6 (34/10-9) to 0.7 mg g^{-1} oil (34/10-3; 34/10-8; 34/10-A-1H; 34/10-1) (Table 4 and Fig. 9) and the $\delta^{13}C$ values of *n*-hexane increased from -28.5 ± 0.5‰ (34/10-9) to -25.9 ± 0.1‰ (34/10-8) (Table 3 and Fig. 9). To check the correlation between the decrease in concentration and the enrichment in ^{13}C, the natural logarithms of R/R_i are plotted versus the natural logarithms of F (Fig. 7C). The data of the oil sample from well 34/10-9 again served as local end-member because of the highest concentration and lightest isotopic composition. The linear regression line has a coefficient of determination of $R^2 = 0.71$, indicating the fair correlation between concentration and carbon isotopic composition of *n*-hexane. From the slope of the linear regression line, the value of the isotope fractionation factor was calculated to be $\alpha = 1.0016$. In the laboratory, an isotope fractionation factor of $\alpha = 1.0023$ was determined for

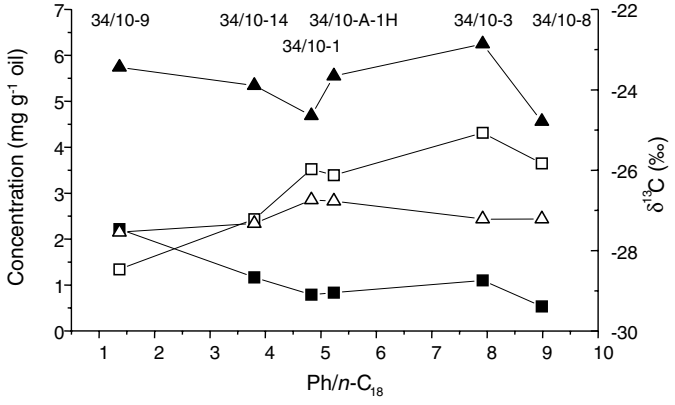

Fig. 8. Concentrations (filled symbols) and carbon isotope ratios (open symbols) of methylcyclohexane (triangles) and *n*-heptane (squares) in six oil samples from the Gullfaks field (Brent Group oils), plotted as a function of the Ph/*n*-C_{18} ratios.

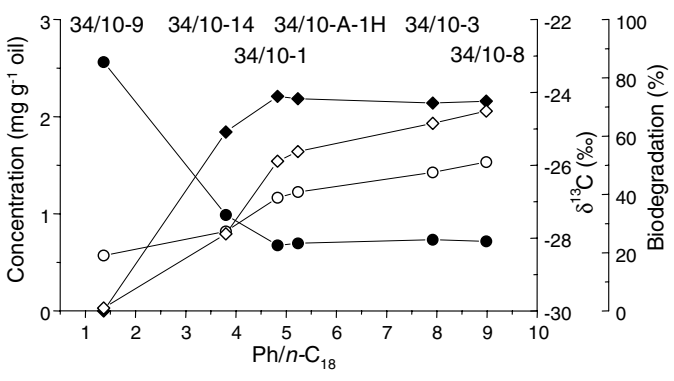

Fig. 9. Concentrations (filled circles) and carbon isotope ratios (open circles) of *n*-hexane, given as a function of the Ph/*n*-C_{18} ratios for six oil samples from the Gullfaks field (Brent Group oils). Calculated values of B_c (closed diamonds) and B_i (open diamonds) are also shown (see text for explanation).

the degradation of *n*-hexane by the denitrifying bacterium strain HxN1 (Vieth et al., unpublished results). This difference in isotope fractionation factors may be due to the fact that under the defined conditions of the laboratory experiment, concentration and carbon isotopic composition of *n*-hexane are only influenced by biodegradation, whereas within the reservoir the concentration of *n*-hexane may also be influenced by other factors. It is also possible that mechanistic differences in the initial reaction of the degradation pathway are responsible for this difference. This leads to the conclusion that appropriate laboratory-derived isotope fractionation factors are necessary for the valid quantification of biodegradation using isotope analysis.

Assuming that the decrease in concentration and change in the isotopic composition of *n*-hexane are due to microbial degradation, the percentage of biodegradation was calculated. The sample of well 34/10-9 was set to represent 0% of biodegradation, giving the end-member in the following calculations. This neglects that 34/10-9 has suffered some biodegradation as well, but does not invalidate the comparison of results obtained from isotope ratios versus those obtained from concentrations, since both approaches use the same end-member. First, the amount of biodegradation was calculated in the common way, using only the decrease in concentration (Eq. (4)), resulting in a loss of *n*-hexane of 61–74% (Fig. 9). The loss due to biodegradation was additionally calculated based on the carbon isotope data of *n*-hexane and the isotope fractionation factor determined in the laboratory experiment (Vieth et al., unpublished results), using Eq. (5) which yields losses of *n*-hexane of 26–69% (Fig. 9). For samples 34/10-3 and 34/10-8, characterised by Ph/*n*-C_{18} ratios of 7.9 and 9.0, both calculations give similar results. For the other samples, characterised by Ph/*n*-C_{18} ratios between 3.8 and 5.2, the values of B_c are much higher than the values of B_i. This implies that in these samples, the concentration may be influenced by processes that have no effect on isotopic composition. When only the concentrations were used, this would have led to an overestimation of in-reservoir biodegradation.

4.5. Evaluation of toluene and benzene

Several laboratory and field studies clearly demonstrate that bacteria can degrade toluene and benzene using nitrate, sulphate or CO_2 as terminal electron acceptor (for review, see Heider et al., 1999 and Coates et al., 2002). It is also known that anaerobic degradation of toluene seems to be favoured in comparison to degradation of benzene (Widdel and Rabus, 2001). Therefore, in environments where both benzene and toluene are present, toluene will be degraded preferentially and with higher rates (Wiedemeier et al., 1999). A multitude of laboratory experiments revealed that anaerobic degradation of toluene, regardless of sulphate-, iron- or nitrate-reducing conditions, caused an isotopic shift in the residual toluene that can be described with an isotope fractionation factor of

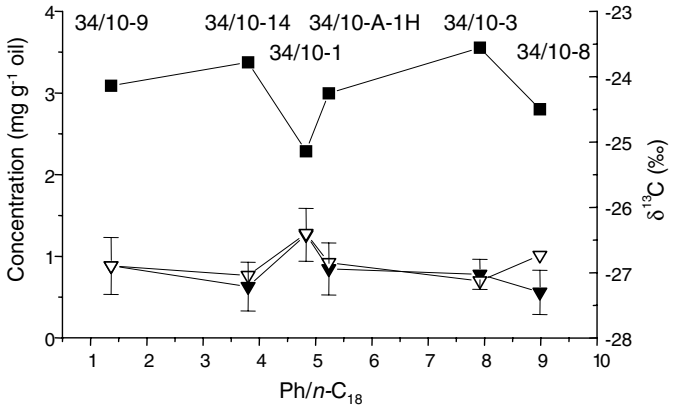

Fig. 10. Concentrations (squares) and measured carbon isotope ratios (filled triangles) of toluene, given as a function of the Ph/*n*-C_{18} ratios for six oil samples from the Gullfaks field (Brent Group oils). Theoretical $\delta^{13}C$ values (open triangles) were calculated based on the Rayleigh equation.

$\alpha = 1.0017$ (Meckenstock et al., 1999). These experiments, using different bacterial cultures and experimental conditions, resulted in very similar isotope fractionation factors because all toluene-degrading cultures used the benzylsuccinate synthase pathway.

Within the Brent Group of the Gullfaks oil field, the variation of toluene concentration (3.5–2.2 mg g^{-1} oil) is not correlated to the increase of Ph/*n*-C_{18} (Fig. 10). The $\delta^{13}C$ value of toluene remains relatively constant between $-27.3 \pm 0.3‰$ (34/10-8) and $-26.4 \pm 0.4‰$ (34/10-1) (Fig. 10). Assuming that the variation of toluene is completely due to microbial activity, it can be calculated (Eq. (2)) that the observed change in toluene concentration would theoretically result in an enrichment of the initial isotopic composition of only 0.8‰. This low change in the $\delta^{13}C$ values of toluene is within the standard deviation of the isotope measurements. Therefore, it can be concluded that toluene has not been affected by biodegradation in the Gullfaks oil field.

Laboratory studies with anaerobic benzene-degrading enrichment cultures showed pronounced carbon and hydrogen isotope fractionation under denitrifying, sulphate-reducing, and methanogenic conditions (Mancini et al., 2003). For benzene biodegradation, the estimated fractionation factors for the carbon isotopes were $\alpha = 1.0020$, $\alpha = 1.0023$, and $\alpha = 1.0036$ under methanogenic, denitrifying, and sulphate-reducing conditions, respectively. However, the full extent of biochemical pathways for anaerobic benzene degradation is currently unknown (Chakraborty and Coates, 2004), and it is uncertain which might have been active in the experiments performed by Mancini et al. (2003). Thus, the variability in isotope fractionation factors cannot be discussed in terms of differences in degradation mechanisms.

In the oil samples of the Brent Group, benzene concentrations were in the range from 0.7 mg g^{-1} oil (34/10-9) to 0.2 mg g^{-1} oil (34/10-8; 34/10-1; 34/10-3; 34/10-A-1H) (Table 4), and the carbon isotope ratios

varied from $-25.7 \pm 0.3‰$ (34/10-3) to $-24.5 \pm 0.4‰$ (34/10-8) (Table 3). Evaluation according to the Rayleigh equation is critical, because the sample with the highest benzene concentration (34/10-9) did not show the lightest isotopic composition of benzene. Thus, no sample fulfils the prerequisites to serve as local end-member of this biodegradation gradient. Assuming anaerobic benzene degradation as the reason for the decrease in concentration, this would have resulted in a change in the isotopic composition of the residual benzene by 2.4, 2.8 or 4.4‰ if the above-mentioned isotope fractionation factors of $\alpha = 1.0020$, $\alpha = 1.0023$ or $\alpha = 1.0036$ were used in the Rayleigh equation (Eq. (2)). However, with the measured change in the isotopic composition being lower than the theoretical ones it can be concluded that benzene was not biodegraded within this reservoir, and that the decrease in concentration has been caused by different processes.

4.6. Implications for in-reservoir biodegradation

The investigation of the molecular and isotopic composition of the light hydrocarbon fraction in six oil samples from the Gullfaks oil field provides detailed insights into the effects of in-reservoir biodegradation. Within the Gullfaks oil field, short-chain *n*-alkanes are very susceptible to biodegradation, whereas branched alkanes are degraded to a lesser extent. Biodegradation of the cycloalkanes such as cyclohexane and methylcyclohexane is at best marginal. It should be noted that mineralisation and co-metabolic transformation of cycloalkanes are possible under anoxic conditions (Rios-Hernandez et al., 2003; Townsend et al., 2004; Wilkes et al., 2003). The complete lack of biodegradation of toluene is surprising, because toluene is known to be easily degradable under anaerobic conditions. Benzene appears to be a poorer substrate for anaerobic bacteria, hence the lack of biodegradation of benzene is less critical. In summary, our results provide evidence for a rather specific and to some extent unusual degradation pattern of light hydrocarbons in the Gullfaks oil field. The observed limitations in biodegradation appear to contradict in several laboratory and environmental studies, and therefore may indicate the presence of a microbial consortium within the Gullfaks reservoir that has rather restricted degradative capabilities. To date, isolated anaerobic hydrocarbon-degrading bacteria exhibit rather pronounced substrate specificities, e.g., currently no aromatic hydrocarbon-utilising bacteria are known that could also utilise aliphatic hydrocabons and vice versa (for more details see, e.g., Widdel and Rabus, 2001). In other words, it will need the work of various specialised microorganisms acting in concert to achieve the complex compositional alteration of crude oil during in-reservoir biodegradation. It appears that the microbial community in the Gullfaks oil field lacks miroorganisms that may degrade certain types of hydrocarbons, in particular cycloalkanes and alkylbenzenes.

A situation as in Gullfaks, where benzene and toluene are not strongly affected either by biodegradation or by other alteration processes, while *n*-alkanes are rapidly depleted due to biodegradation, rules out water washing as a significant process. This may be used as an indication for restricted water flow through the reservoir, which, in turn, may have implications for the viability of microorganisms concerning the delivery of electron acceptors and water soluble nutrients, and also the removal of toxic waste products such as sulphide.

The observed limitation in toluene degradation can be used to illustrate that biodegradation of light hydrocarbons and therefore biodegradation, in general, in Gullfaks most likely proceeds under anaerobic conditions and not in the presence of oxygen. The aerobic biodegradation of 1 mol toluene requires 9 mol oxygen, as can be seen from the net reaction

$$C_7H_8 + 9O_2 \rightarrow 7CO_2 + 4H_2O.$$

The solubility of oxygen in water is very low (8.6 mg L^{-1} at 25 °C) and will decrease with increasing temperature and salinity. The water solubility of toluene is much higher (515 mg L^{-1} at 25 °C) and will decrease with increasing salinity but will increase with increasing temperature, and will furthermore be influenced by competing effects of other solutes present in the system. Neglecting these restrictions, the complete oxidation of 1 mol toluene ($T = 25$ °C; $S = 0$) would require equilibration with 33,500 L of oxygen-saturated water. This amount of water, in turn, would be able to dissolve as much as 187.5 mol toluene. Therefore, conditions providing sufficient amounts of oxygen for aerobic biodegradation should lead to significant water washing of toluene. This, however, is not seen in the Gullfaks field allowing the conclusion that biodegradation took place has to proceed under anoxic conditions.

5. Conclusions

Compound-specific carbon isotope analysis of light hydrocarbons has been shown to be a versatile tool for evaluating biodegradation processes in an oil reservoir. Importantly, our results document that the Rayleigh model is applicable to the biodegradation of light hydrocarbons in such reservoirs. Therefore, carbon isotope signatures can be used to quantify the mass loss of individual light hydrocarbons due to biodegradation, provided valid fractionation factors from laboratory studies are available. A particular strength of this approach comes from the combination of compositional and isotopic analysis, enabling the differentiation of physical and (bio)chemical alteration processes. These approaches are easily adaptable to any oil field where compositional alteration of oil is due to the initial stages of biodegradation. However, valid assessment of biodegradation requires that the potential impacts of source and maturity on the carbon isotope signatures of the light hydrocarbons have to be well constrained. If the oils are unbiodegraded or only marginally affected, the carbon

isotope signatures of the light hydrocarbons may be useful as source indicators. This is valuable in situations such as the North Viking Graben, where two different source rocks with isotopically distinct kerogen may contribute to the reservoired petroleums. Our results allow inferences about the peculiarities of biodegradation in Gullfaks, e.g., the observed alteration patterns point to a microbial community structure with restricted degradative capabilities. The results furthermore rule out any volumetrically significant aerobic biodegradation in the Gullfaks oil field.

Acknowledgments

We are grateful to Rouven Elias, Ann-Kathrin Scherf, and Kristin Günther for whole oil measurements and Michael Gabriel for his assistance in isotope analysis. We thank Dr. Arnd Wilhelms for oil samples and background information about the study area. Drs. Simon C. George, Christopher J. Boreham, and Roger E. Summons provided valuable comments on an earlier draft which helped to improve this publication.

Associate editor: Roger Summons

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