

Yang, S., Horsfield, B., Mangelsdorf, K., di Primio, R., Rinna, J., Hartwig, A., Martín-Monge, A., Tocco, R. A., Elias, R. (2024): Pristine biomarker compositions recovered from biodegraded oil samples using MSSV-Hy. - Marine and Petroleum Geology, 162, 106713.

<https://doi.org/10.1016/j.marpetgeo.2024.106713>

Pristine biomarker compositions recovered from biodegraded oil samples using MSSV-Hy

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Keywords: Bound biomarkers, biodegradation, asphaltene, Barents Sea, North Sea

Abstract:

Oil-source and oil-oil correlations are challenging when the primary chemical composition of petroleum was altered or destroyed by biodegradation. Biomarkers that are bound to macromolecular matrices rather than being in a free form are largely protected from alteration and therefore offer a solution to the problem. Using Microscale Sealed Vessel Catalytic Hydrogenation (MSSV-Hy), the bound biomarkers of two North Sea and two Barents Sea oil samples, with varying levels of biodegradation (ranging from 1 to 8 on the Peters and Moldowan scale), were released from their respective asphaltenes. In spite of the fact that biodegradation has changed the relative concentrations of steranes (C_{27} - C_{30} steranes) and hopanes (C_{29} - C_{30} hopanes) in the free biomarker fraction to varying degrees, the asphaltene-bound biomarkers were protected and show pristine ratios unaffected by biodegradation, e.g., the relative content of C_{27} - C_{29} steranes, the C_{30}/C_{29} sterane ratio, and the C_{29}/C_{30} hopane ratio. Empirical calibration then allows these bound biomarkers to be converted into regular biomarker ratios. Thus, the MSSV-Hy technique has been shown to be an efficient and effective tool for releasing asphaltene-bound biomarkers from biodegraded oil and revealing genetic information when free biomarkers are altered by biodegradation.

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1. Introduction

The microbial alteration of crude oil in subsurface reservoirs is referred to as biodegradation (Bailey et al., 1973; Connan, 1984). Biodegradation leads to an increase in density, viscosity, and the content of organic acids, sulphur, and metals. Biodegraded petroleum is consequently more difficult to produce and process. The degree of microbial degradation is determined by geological and geochemical factors. As an empirical observation, biodegradation is usually detected in reservoirs with temperatures <80 °C, though palaeopasteurisation can preclude biodegradation even at shallow present-day depths (Wilhelms et al., 2001). The most important factor governing biodegradation is probably the limited availability of electron acceptors for the microbial respiration processes (Head et al., 2003). Biodegradation has an adverse effect on the physical properties of oil because it irrevocably alters its chemical composition. As far as oil-oil and oil-source correlations are concerned, biodegradation at the molecular level hampers the genetic analysis of petroleum. Biodegraded oil is frequently featured by a baseline “hump” of unresolved complex mixture (UCM) in gas chromatographic data that becomes more prominent as biodegradation progresses. At increasing levels of biodegradation, a sequential removal of molecular groups occurs in a typical order: *n*-alkanes; *iso*- and *anteiso*-alkanes; acyclic isoprenoids; bicyclic alkanes; C₂₇–C₂₉ regular steranes; C₃₀–C₃₅ hopanes; diasteranes; C₂₇–C₂₉ hopanes; C₂₁–C₂₂ regular steranes; tricyclic terpanes; aromatics (Peters and Moldowan, 1991; Seifert and Moldowan, 1979). As a result, it becomes increasingly difficult to make accurate oil-source and oil-oil correlations, maturity assessments and reconstructions of the depositional environment using biodegraded oil samples.

Biomarkers occur as both free phase, which is solvent extractable, and bound phase, which is covalently bound to the kerogen or asphaltene macrostructures (Love et al., 1995). In addition, there are biomarkers adsorbed on the surface of minerals or physically trapped within the macromolecules (Snowdon et al., 2016). Pyrolytic methods have been previously applied in samples containing biodegraded free biomarkers to release kerogen- or asphaltene-bound biomarkers, which are expected to be less affected and thus contain still intact genetic

1 information of the original oil. Bonnamy et al. (1987) carried out anhydrous pyrolysis
2 experiments on the asphaltenes of biodegraded oil samples but found the release of biomarkers
3 to be extremely low and the bound biomarkers to be significantly different from the free
4 biomarkers. Later, the catalytic hydrolysis (HyPy) was applied as an effective technique to
5 release bound biomarkers which resemble their free counterparts (Bishop et al., 1998; Love et
6 al., 1995), and has been applied to unravel the original biological signatures of biodegraded oil
7 samples (Meredith et al., 2008; Meredith et al., 2014). In recent years, a new method, called
8 Microscale Sealed Vessel Catalytic Hydrogenation (MSSV-Hy), was developed (Wu and
9 Horsfield, 2019). The technique is characterized by small sample requirement, low
10 experimental cost, and high reproducibility. Using only 1 mg of asphaltene for each MSSV-Hy
11 measurement, Yang et al. (2022) systematically compared the asphaltene-bound and free
12 biomarkers in fresh oil samples, finding that the asphaltene-bound biomarkers can provide key
13 genetic- and maturity-related information. Significant correlations of several source-related
14 parameters, including C_{27}/C_{29} sterane, regular C_{30}/C_{29} sterane, C_{29}/C_{30} hopane ($\alpha\beta + \beta\alpha$), and
15 C_{35}/C_{34} homohopane ratios, were found between the free biomarkers and the MSSV-Hy
16 released bound biomarkers (Yang et al., 2022).

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In this study, the MSSV-Hy technique has been applied to four oil samples with various levels of biodegradation. By comparing the free and bound biomarker fractions, this research aims to explore the applicability of MSSV-Hy to uncover the pristine biomarker characteristics of biodegraded oil samples.

2. Materials and methods

The Mesozoic reservoirs in the Norwegian waters are prolific for petroleum, but biodegraded oil can also be encountered where petroleum migrated into cool ($< 80\text{ }^{\circ}\text{C}$) reservoirs or where uplift had occurred (Horstad et al., 1992; Killups et al., 2019). Two oil samples recovered from Jurassic/Triassic sandstone reservoir in the Norwegian North Sea (named as North Sea-1 and North Sea-2, respectively) and another two oil samples produced

1 from early Cretaceous sandstone reservoir in the Barents Sea (named as Barents Sea-1 and
2 Barents Sea-2, respectively) were selected for this research.
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4 The two North Sea samples were taken from the 16/4-6 S and 16/4-8 S wells of the Solveig
5 field, respectively. The Solveig field comprises a series of Late Triassic half-grabens along the
6 basement of the south-western part of the Utsira High. The primary reservoir are sandstones
7 and conglomerates of Triassic and Devonian age which are compartmentalized by basement
8 highs and fault zones. Details to the structural and sedimentological evolution of the area can
9 be found in Rønnevik et al. (2017) and Coward et al. (2003). Petroleum migration occurred in
10 at least two episodes during the early to late Tertiary. An early low-mature oil migrated into the
11 trap and was subsequently biodegraded, followed by a later higher maturity oil charge from a
12 deeper source. The reservoir compartmentalization led to different degrees of mixing and
13 alteration effects of the reservoir oils (Georgiev et al., 2021; Matapour and Karlsen, 2018).
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26 Both the two Barents Sea samples were taken from oil discoveries along the Western edge
27 of the Loppa High in the Norwegian Barents Sea. The Loppa High is a Paleozoic aged basement
28 high that has undergone several episodes of periodic rifting, tilting, uplift, and erosion
29 throughout the Mesozoic and Cenozoic resulting in a complex petroleum charge history. Details
30 to the geologic evolution of the area can be found in Ktenas et al. (2019) and Faleide et al.
31 (2008) and references therein. The petroleum in the discoveries was sourced by the Late
32 Jurassic aged Hekkingen Formation in the Tromsø and Bjørnøya Basin kitchen areas, which
33 underwent rapid subsidence during the Cretaceous to Early Cenozoic. The trap locations
34 experienced several episodes of uplift and erosion since the Early Cenozoic to present-day,
35 resulting in a complex charge and re-migration history of petroleum in the area (Ktenas et al.,
36 2019; Lerch et al., 2016b).
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51 Whole-oil gas chromatography (GC) was carried out using an Agilent 6890 GC with a
52 flame ionisation detector (FID). The GC–FID system was equipped with a HP Ultra 1 capillary
53 column using helium as carrier gas. The oven temperature is programmed from 40 °C to 300 °C
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Asphaltenes were precipitated from the oil samples using the method developed by Theuerkorn et al. (2008). Briefly, the procedure is as follows: (1) the oil is dissolved with a v/v 99:1 mixture of dichloromethane/methanol (DCM/MeOH); (2) a 40-fold excess of *n*-hexane is added; (3) the sample is settled for 4 hours; (4) asphaltenes are removed by vacuum filtration and subsequently purged with *n*-hexane; and (5) asphaltenes are redissolved by extracting the filter with DCM/MeOH (v/v 99:1) in the accelerated solvent extractor. This protocol is repeated three times to maximize the yield of pure asphaltenes from the oil. The residual de-asphalted bitumen is further separated into aliphatic, aromatic, and polar fractions via medium pressure liquid chromatography (MPLC) as described by Radke et al. (1980).

The release of bound biomarkers from asphaltene was carried out using the MSSV-Hy procedure described by Yang et al. (2021). About 1 mg asphaltene is mixed with 4 μ L tetralin and 0.5 mg PtO₂ catalyst, and sealed in a 3 cm-long glass tube (Horsfield et al., 2015). The tube was heated from 200 °C to 390 °C at a heating rate of 0.7 °C/min in a purpose-built high-precision oven. After cracking the tube in a vial, the newly released bound biomarkers were dissolved in dichloromethane and recovered after filtration over a Pasteur pipette column filled with sodium sulphate. The gas chromatography–mass spectrometry (GC–MS) analyses of both the free and bound aliphatic biomarkers, separated by MPLC, were conducted using a Trace GC Ultra gas chromatograph coupled with a DSQ-mass spectrometer from Thermo Fisher Scientific as described in detail by Yang et al. (2021). The extent of biodegradation in the samples studied here is assessed using the empirical Peters and Moldowan (PM) scale (Peters and Moldowan, 1991), which accounts for the effects of various levels of biodegradation on the composition of typical mature oil, ranked from 0 to 10.

3. Results and discussion

3.1 Biodegradation levels

A first insight into the level of biodegradation of the investigated oils can be obtained from the inspection of the GC–FID chromatograms, which show the distribution and abundance of *n*-alkanes and isoprenoids, and reveal the development of the UCM (Fig. 1). No significant

1 depletion of neither normal nor isoprenoid alkanes is observed in the North Sea-1 sample,
2 indicating the oil was not biodegraded, or only very slightly biodegraded. In comparison, the
3 relative content of pristane and phytane over normal alkanes is elevated in the North Sea-2
4 sample which indicates that the biodegradation rank is at least 2 in the PM scale. Furthermore,
5 this sample shows a small UCM. The aliphatic compound distributions in the Barents Sea
6 samples are more complex. The Barents Sea-1 sample displays non-smooth distributions of *n*-
7 alkanes where the C₁₃-C₁₇ alkanes are abnormally abundant compared to the longer chain *n*-
8 alkanes and a small UCM hump. It is possible that this sample is a mixture of biodegraded oil
9 and a non-biodegraded, fresh oil charge, which is a common occurrence in the Barents Sea
10 according to Lerch et al. (2016b). Both the normal and isoprenoid alkanes in the Barents Sea-2
11 sample are clearly biodegraded with a considerable UCM hump (Fig. 1). To determine the exact
12 biodegradation levels sterane and hopane biomarkers need to be examined.

26 Both the free sterane and free hopane distributions of the two North Sea samples are highly
27 similar (Fig. 2), and there is no indication that these two types of biomarkers have been
28 significantly affected by biodegradation in these two samples. In contrast, the free biomarkers
29 of the two Barents Sea samples have been significantly altered. The signal to noise ratios of the
30 steranes, especially the C₂₈ and C₂₉ steranes, are low. The relative content of diasteranes over
31 regular steranes is high considering the thermal maturity levels. These maturity levels can be
32 inferred from other biomarker parameters such as the 18 α (H)-22,29,30-
33 trisnorneohopane/17 α (H)-22,29,30-trisnorhopane (Ts/Tm) ratio and the 20S/R ratio of the
34 5 α ,14 α ,17 α (H)-C₂₇ steranes. Thus, the overall sterane distribution pattern suggests that the
35 steranes in these samples have been strongly biodegraded, and that the PM scale biodegradation
36 level is at least 6. Diasteranes, more resistant against biodegradation than steranes (Seifert and
37 Moldowan, 1979), are still very prominent compared to the sterane signal. In the free hopanes,
38 the relative content of Ts, Tm, and C₂₉ hopanes over the C₃₀₊ hopanes in the Barents Sea-2
39 sample is higher than in the Barents Sea-1 sample, indicating that the hopanes in the Barents
40 Sea-2 sample are altered by biodegradation and thus a PM scale biodegradation rank of 8.

1 To summarize, based on the GC–FID and GC–MS analyses of the *n*-alkanes, isoprenoids
2 and the free biomarker fraction, the PM biodegradation level ranks of the North Sea-1, North
3 Sea-2, Barents Sea-1, and Barents Sea-2 samples are roughly assessed to be 0–1, 2, 6, and 8,
4 respectively (Peters and Moldowan, 1991).
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7 3.2 Comparison between free and bound biomarkers 8 9

10 We have previously shown that both similarities and differences can be observed in the
11 biomarker distributions between the free and bound fractions of non-biodegraded oil samples
12 (Yang et al., 2022). The similarities are also present in the slightly biodegraded North Sea
13 samples of this study (Fig. 2). These North Sea samples also exhibit differences in the
14 biomarker distributions of the free and bound fractions reported for non-biodegraded oils by
15 Yang et al. (2022), including the absence of rearranged biomarkers (e.g., diasteranes,
16 trisnorneohopane (Ts)), and the relatively high trisnorhopane (Tm) content in the bound
17 fraction (Fig. 2). It is worth mentioning that these systematic differences are common traits not
18 only for bound biomarkers released by MSSV-Hy (Wu and Horsfield, 2019; Yang et al., 2022),
19 but also for those released by HyPy (Chen et al., 2020; Meredith et al., 2019). Traces of
20 rearranged biomarkers in the bound fraction might be remnants from free biomarkers that are
21 still physically occluded in the asphaltene macromolecular structure after asphaltene
22 purification (Orea et al., 2021; Snowdon et al., 2016), that were later released upon heating.
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24 Apart from the differences that can be attributed to the pyrolysis methods, there is a striking
25 similarity on the regular steranes and C₃₀₊ hopanes between the free and bound biomarker
26 fractions of the North Sea samples, which clearly corroborates the bound biomarkers to carry
27 key genetic information.
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30 In terms of the more biodegraded Barents Sea samples, the MSSV-Hy released bound
31 biomarkers also show the expected dominance of Ts and the absence of rearranged biomarkers
32 (Fig. 2). Steranes, strongly degraded in the free biomarker fraction, are akin to what is expected
33 in a mature, non-degraded oil in the bound fraction. Moreover, in the Barents Sea-1 sample the
34 17 α (H),21 β (H)-C₂₉ and C₃₀ hopanes are well preserved in the bound fraction. Also, the severely
35 biodegraded Barents Sea-2 sample (PM rank of 8) displays a C₂₉/C₃₀ hopane ratio of >1 in its
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1 free biomarker fraction, compared to a more reasonable value in the bound fraction (non-
2 carbonate generated oil typically show C_{29}/C_{30} hopane ratios <1) (Fig. 2B).
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4 3.3 The applications of bound biomarkers 5

6 The relative content of C_{27} - C_{29} steranes offers key information about the depositional
7 environment and precursor types (Huang and Meinschein, 1979). Although all the free steranes
8 of the four investigated samples are plotted in the area of Open Marine depositional
9 environment (Fig. 3A), the severely biodegraded Barents Sea samples are shifted closer to the
10 Plankton and Algae area, which is due to the strong degradation of the C_{29} steranes in the free
11 fraction. Although the samples still plot in the field of Open Marine, the extent of the shift is
12 significant and could lead to a different assessment of the depositional environment or source
13 area in other cases. In contrast, the bound steranes of the Barents Sea samples show strikingly
14 similar relative sterane compositions compared to the free counterparts and bound steranes of
15 the only minor degraded North Sea samples (Fig. 3). Their compositions are typical for oil
16 produced from the Mesozoic reservoirs on the Norwegian continental shelf (Hughes et al., 1985;
17 Pedersen et al., 2006). The free and bound steranes of the less biodegraded North Sea samples
18 themselves, plot quite close to each other, confirming the potential of the bound biomarkers to
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37 Sterane-based maturity assessment is usually conducted using the regular C_{29} steranes
38 (Seifert and Moldowan, 1978). Although the sterane distributions are significantly altered in
39 the free biomarkers of the severely biodegraded Barents Sea samples (Fig. 2), the bound
40 fractions still contain the C_{29} steranes allowing the assessment of the thermal maturity after
41 calibration of the bound biomarker to the free biomarker ratios as outlined in Yang et al. (2022).
42 For instance, the $20S/(20S+20R)-5\alpha,14\alpha,17\alpha$ C_{29} sterane ratios for the bound biomarkers of
43 the Barents Sea-1 and Barents Sea-2 samples are 0.18 and 0.21, respectively (Fig. 2A),
44 indicating these samples were generated in an early oil window maturity.
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55 The presence of regular C_{30} steranes is suggested to be an indicator of marine-derived
56 organic matter (Moldowan et al., 1985). According to Peters et al. (1989), the relative content
57 of C_{30} steranes can be used to distinguish oil sourced from different shales in the North Sea, i.e.,
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1 oil generated from the Kimmeridge Clay has high C₃₀ sterane content, whereas oil derived from
2 the Devonian shale lacks C₃₀ steranes. According to previous MSSV-Hy measurements of
3 source rock and reservoir oil from different areas of the world (Yang et al., 2022), the C₃₀/C₂₉
4 sterane ratios are almost 1:1 correlated in the free and bound fractions. In this study, the lightly
5 biodegraded North Sea samples (only alkanes affected, biomarkers unaltered) also fall into this
6 trend line (Fig. 3B) which further verify the established correlation presented in Yang et al.
7 (2022). In contrast, the C₃₀/C₂₉ sterane ratios in the free biomarker fraction of the Barents Sea
8 oils are highly disturbed by biodegradation and plot far away from the trend line. However, the
9 bound biomarkers show similar values as those in the North Sea oils. Tentative extrapolation
10 of the free biomarker ratio to the free-bound trend roughly reveals the range of original free
11 C₃₀/C₂₉ sterane ratio, and provide a more accurate evaluations of the marine input. Similar ratio
12 values can be obtained for other fresh Barents Sea oils (Lerch et al., 2016a).

26 In the hopane regime, the C₂₉ and C₃₀ hopanes are typically the most abundant ones, and
27 their relative content is indicative for depositional environments. For example, a higher C₂₉ vs.
28 C₃₀ hopane content is indicative for oils generated from anoxic carbonate or marl source rocks
29 (Sharaf, 2003). It is a common feature that the C₂₉/C₃₀ ratio (considering both the αβ and βα
30 isomers) from the bound biomarkers is higher than that from its free counterpart (Fig. 3C) as
31 shown by a correlation plot created in a previous investigation (Yang et al., 2022) and used as
32 a background in Figure 3C. Except for the most severely biodegraded Barents Sea-2 sample
33 (blue diamond), all the other three investigated samples also fall on the previously established
34 trend line. This indicates that the empirical conversion formula (Fig. 3C) for free and bound
35 hopanes is valid. In contrast, the free biomarker hopane ratio in the Barents Sea-2 sample is
36 completely off the trend, which is most likely caused by the severe biodegradation. However,
37 the bound biomarker value is similar to those of the North Sea and Barents Sea-1 samples. Thus,
38 the bound biomarker value could be used to project the sample back to the trend line to obtain
39 a meaningful value for the free biomarker ratio. Hopane maturity parameters indicators for all
40 the samples obviously seem not to be influenced by biodegradation (Fig. 2).

4. Conclusions

The four investigated oil samples have experienced varying levels of biodegradation, ranging from very light (PM scale rank of 0–1) to severe biodegradation (PM scale rank of 8). Accordingly, the free sterane and hopane biomarkers were altered, making the oil-oil and oil-source correlation, maturity assessment and depositional environment reconstruction challenging. In contrast, the relative content of C₂₇-C₃₀ steranes and C₂₉-C₃₀ hopanes in the bound biomarkers released by MSSV-Hy is still intact, enabling maturity level and source facies assessment. MSSV-Hy requires only small amounts of sample material, is inexpensive and has proven to be a viable means for conducting regional geochemical assessments, even where secondary microbial degradation of petroleum has occurred. However, additional analyses on more samples with known geological background will help to better constrain the free vs bound correlations and to verify the applications of MSSV-Hy for biodegraded samples.

Acknowledgement

The authors thank Aker BP ASA, Lundin Norway AS, Repsol Exploración S.A., and Total S.A. for sponsoring the multiclient research project Quick Bound Biomarkers (QBB), a joint initiative of GEOS4 GmbH and the Helmholtz Centre Potsdam GFZ German Research Centre for Geosciences. Lundin Norway is acknowledged for allowing the publication of the investigated samples. S. Yang is also supported by the Young Taishan Scholar and the Youth Foundation of Shandong NSFC (ZR2021QD092) programs. The excellent technical support from Ferdinand Perssen and Cornelia Karger, both of GFZ, is greatly appreciated.

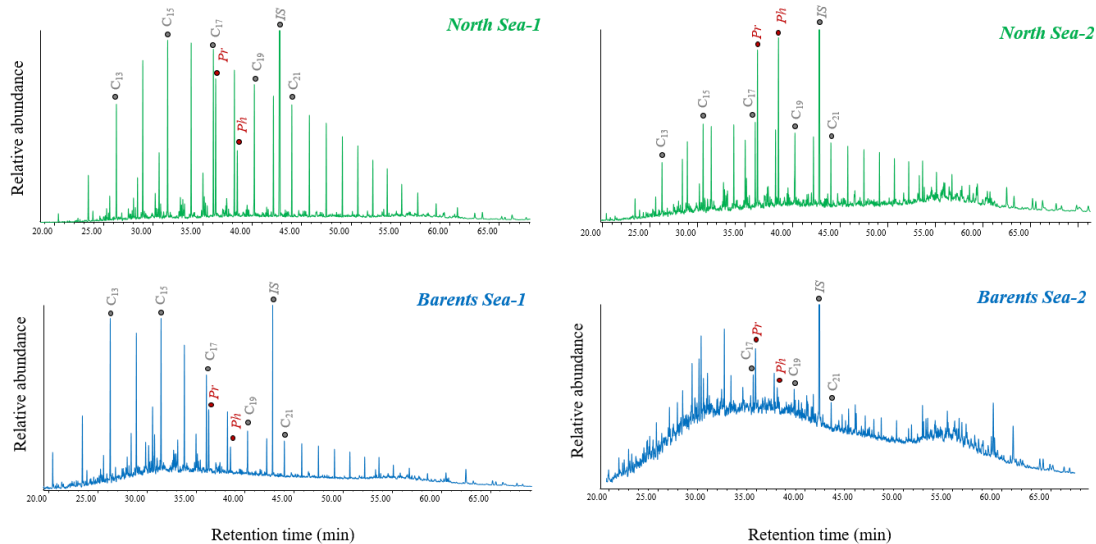
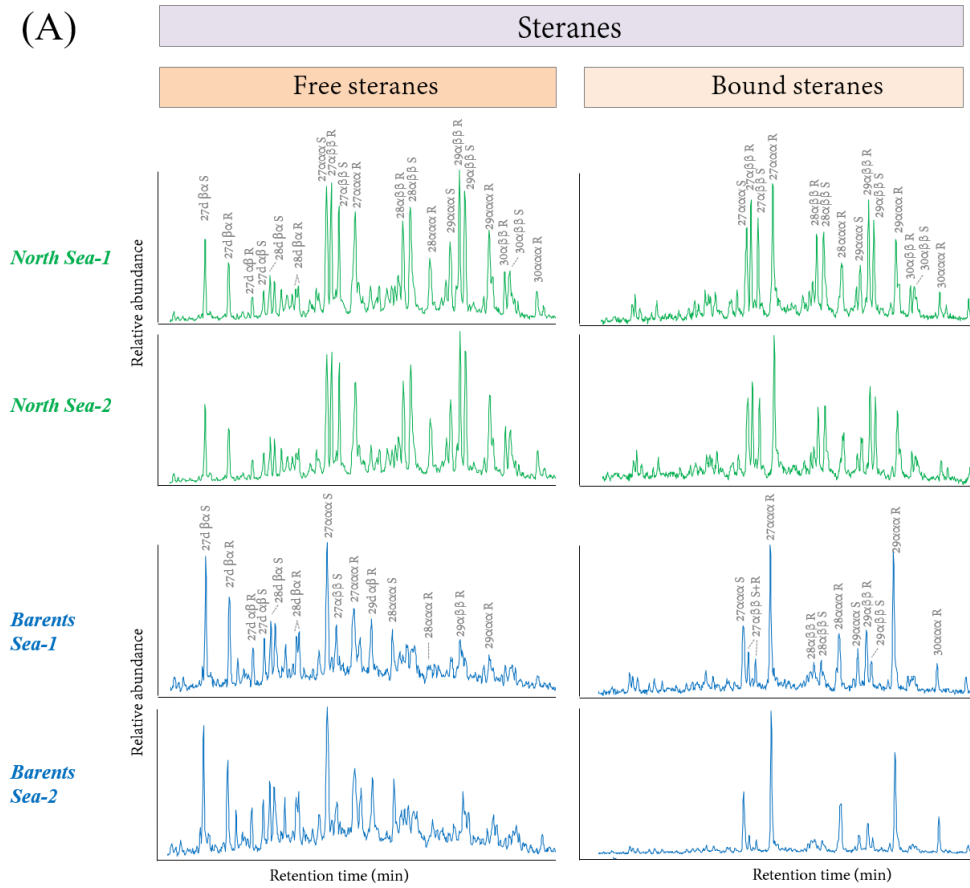
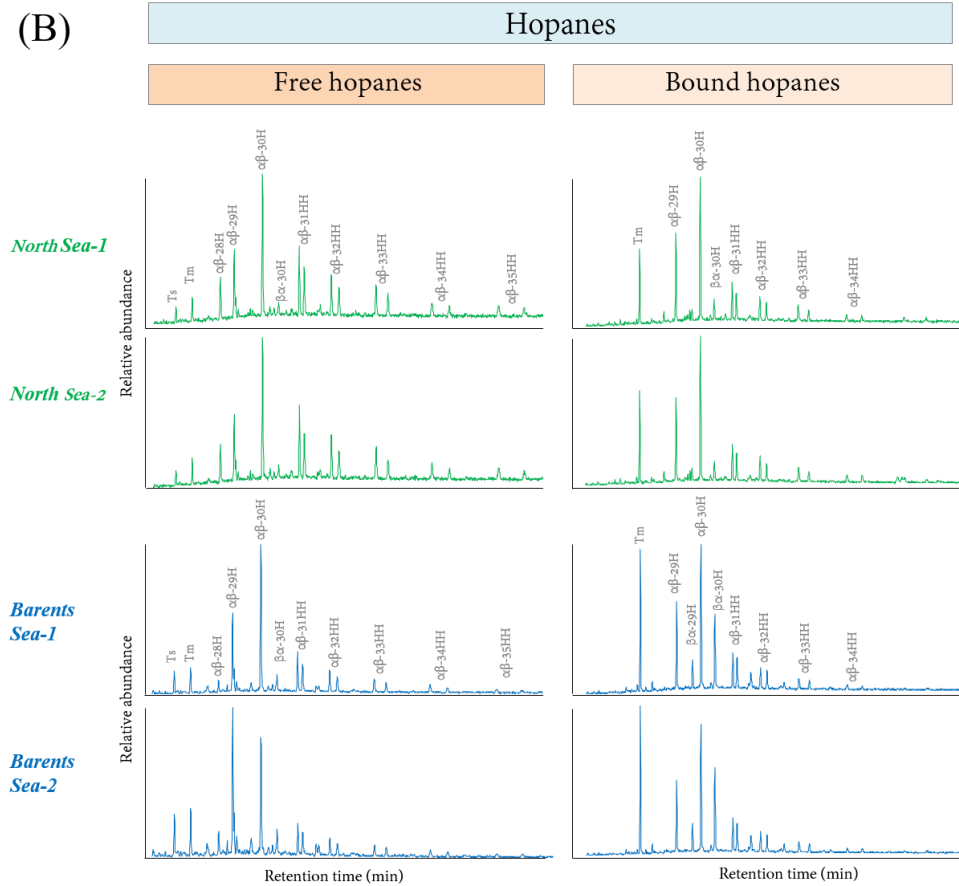


Fig. 1. Whole oil GC–FID chromatograms for the samples from the North Sea and the Barents Sea. Pr and Ph stand for pristane and phytane, respectively. IS = internal standard. C_x = individual *n*-alkanes from the homologue series, with *x* the number of carbon atoms.

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Fig. 2. GC–MS trace chromatograms of free and bound (A) sterane ($m/z = 217 + 218$) and (B) hopane ($m/z = 191$) biomarkers for the four samples from the North Sea and Barents Sea investigated in this study. $\alpha\alpha\alpha S$ or $R = 5\alpha(H), 14\alpha(H), 17\alpha(H)-20S$ or $20R$ steric C_x -sterane configuration, $X\alpha\beta\beta S$ or $R = 5\alpha(H), 14\beta(H), 17\beta(H)-20S$ or $20R$ steric C_x -sterane configuration. $T_s = 18\alpha(H)-22,29,30$ -trisinorneohopane, $T_m = 17\alpha(H)-22,29,30$ -trisinorhopane, $H =$ hopane, $HH =$ homo-hopane. $\alpha\beta = 17\alpha(H), 21\beta(H)$ steric hopane configuration, $\beta\alpha = 17\beta(H), 21\alpha(H)$ steric hopane configuration, S and $R =$ steric homohopane configuration at $C-22$.

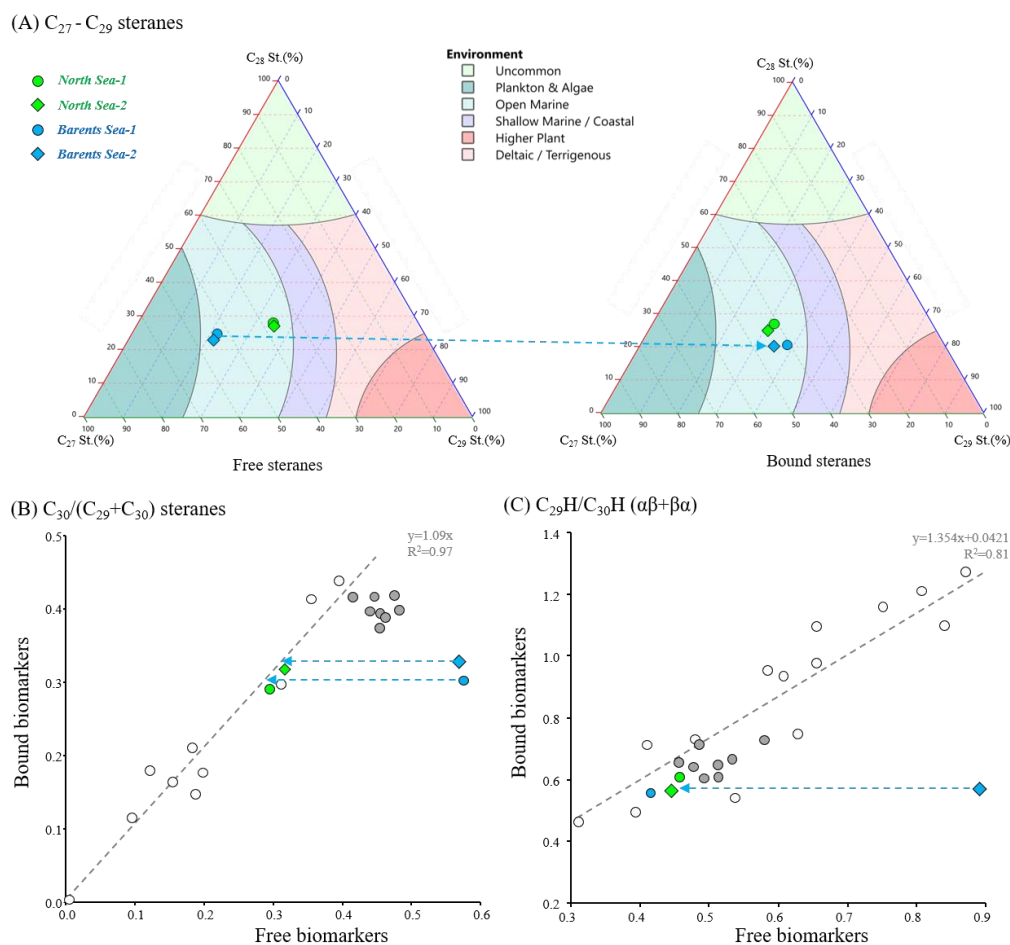


Fig. 3. Comparisons between free and bound biomarker parameters from two North Sea and two Barents Sea samples, including (A) the regular $C_{27}-C_{29}$ sterane [$\alpha\alpha\alpha$ ($20S + 20R$) and $\alpha\beta\beta$ ($20S + 20R$)] distributions and (B) the $C_{30}/(C_{29} + C_{30})$ sterane as well as (C) the C_{29}/C_{30} hopane ratios. White dots in (B) and (C) represent data measured on source rock samples and oil samples for different areas in the world using the same technique (Yang et al., 2022), and fresh oil samples produced from North Sea wells drilled in the Greater Alvheim area Viking Graben are marked in grey.

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