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$\delta^{13}C$ and δD Values of *n*-Alkanes from In-Reservoir Biodegraded Oils: Implications for Understanding the Mechanisms of Biodegradation and for Petroleum Exploration

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Abstract: This study investigates the magnitude and direction of stable C and H isotope shifts of *n*-$C_{15–30}$ alkanes from biodegraded oils sourced from Type II (Oil suite S) and Type II/III (Oil suite H) kerogens. Compound-specific isotope data show a 2.0‰ ^{13}C-enrichment and no D-enrichment of *n*-alkanes in the most biodegraded oil from sample suite S. Similarly, there is a 1.5–2.5‰ ^{13}C-enrichment and no D-enrichment in Oil suite H. Overall, there is a <2.5‰ $\delta^{13}C$ and <20‰ δD variability among individual *n*-alkanes in the whole sequence of biodegradation. *N*-alkanes from the least biodegraded Oil H samples are 2–4‰ ^{13}C-enriched in comparison with the least biodegraded Oil S. However, there are no differences in the δD values of *n*-alkanes in these samples. Our indirect isotopic evidence suggests (1) a site-specific biodegradation process, most likely at position C-2 and/or C-3 or another site-specific process, and (2) a significant D/H exchange between organic compounds in the source rock and isotopically similar marine formation waters. We conclude that, unlike δD methodology, investigation of $\delta^{13}C$ composition of *n*-alkanes has strong potential as a supplementary tool for oil–oil and oil–source-rock correlation even in biodegraded oils when *n*-alkanes are present.

Keywords: oil biodegradation; *n*-alkanes; carbon; hydrogen; isotopes; correlation

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1. Introduction

Understanding the biodegradation processes that lead to changes in molecular composition of petroleum hydrocarbons is crucial for successful exploration for higher quality petroleum accumulations and, avoiding the highest carbon content, most biodegraded oils. Biodegradation of *n*-alkanes, the major components of the saturated hydrocarbon fraction in petroleum, has been the subject of intensive research by numerous investigators [1]. It has been recognized since the late 1950s that anaerobic biodegradation is the primary process that affects hydrocarbons in deep (below several hundred meters) petroleum reservoirs with methanogenic alkane degradation being the common and dominant process [2–5]. Based on laboratory studies, there are at least two described mechanisms of *n*-alkane activation during anaerobic biodegradation: fumarate addition at the C-2 position [6,7] and carboxylation at the C-3 position [8]. Biodegradation mechanisms affecting *n*-alkanes in deep petroleum reservoirs, however, remain still somewhat mysterious, and it is likely that undiscovered mechanisms, including potentially hydroxylation, may dominate processes in reservoirs [4]. In-reservoir biodegradation proceeds on completely different time scales than in laboratory studies [4], and the microorganisms involved are poorly known. Unlike in the laboratory, the researcher studying biodegradation in deep reservoirs has no control over biodegradation processes and has access only to the end products of microbial activity. Conversely, in the laboratory, the rates of anaerobic alkane degradation are so slow that it

is difficult to do an experiment on the common timescale of academic research projects as very long timelines are usually needed. According to the above mechanisms, i.e., fumarate addition at the C-2 position and carboxylation at the C-3 position, the initial *n*-alkane activation is an irreversible process that proceeds by breaking C-H bonds. The smaller amount of energy needed to break bonds with ^{12}C and H atoms would result in a kinetic isotope effect leading to an isotopic ^{13}C- and D-enrichment of *n*-alkanes in the residual fraction. The extent and character of this enrichment, however, depends on the mechanisms of microbial attack. Monitoring compound-specific δD and $\delta^{13}C$ values of *n*-alkanes at different stages of degradation could shed light on the role of these mechanisms in petroleum reservoirs.

In spite of its significance to petroleum exploration, particularly in the context of unconventional petroleum systems, only a few published studies have used the combined compound-specific approach to explore biodegradation of *n*-alkanes in the field [9]. One of the most relevant studies on the subject [10] reported the $\delta^{13}C$ and δD values of mid- to long-chain *n*-alkanes from a series of in-reservoir biodegraded oils. The authors found a significant (up to 4‰) ^{13}C-enrichment of $nC_{15–18}$ alkanes and an absence of any significant shifts in $\delta^{13}C$ values of longer chain *n*-alkanes. The study also showed a major positive shift (up to 35‰) in the δD values. These results, however, are difficult to explain in light of the mechanisms mentioned above. Neither fumarate addition at the C-2 position, nor carboxylation at the C-3 position would lead to *n*-alkane shortening, i.e., the mechanism that was proposed to explain the pattern of *n*-alkanes $\delta^{13}C$ values. Additionally, neither of these two mechanisms nor other putative alkane activation routes would account for *n*-alkane D-enrichment.

In this study, we extend the combined $\delta^{13}C/\delta D$ approach by investigating in-reservoir biodegraded oils derived from Type II and Type II/III kerogens. We address two main questions. Do the $\delta^{13}C$ and δD values of $n\text{-}C_{15–30}$ alkanes respond to anaerobic biodegradation in deep petroleum reservoirs? What could these $\delta^{13}C$ and δD data tell us about the mechanisms of biodegradation and petroleum generation?

2. Materials and Methods

Two suites of in-reservoir biodegraded oils were investigated in this study. Oil S was derived from Type II kerogen bearing marine source rocks charging into several clastic reservoirs. The oil composition ranges from a near pristine oil (sample #1a, Peters and Moldowan Biodegradation Level 0 (PM0), [11]) characterized by an intact distribution of *n*-alkanes through to samples with different levels of biodegradation where only traces of *n*-alkanes remain (sample #1h, PM3) (Figure 1).

25-Norhopanes, compounds that form during severe biodegradation of petroleum [12], were identified in some of the biodegraded oils, and they, together with declining concentrations of *n*-alkanes (Figure 2), suggest that a significant component of the oils was severely biodegraded and that mixtures of both fresh and degraded oil are present in the reservoirs. These mixed oil scenarios are essentially ubiquitous in biodegraded oil settings [13]. The most pristine oil (sample #1a), however, contains no 25-norhopanes and differs substantially with respect to qualitative and quantitative molecular parameters, indicating that this oil might represent the latest episode of charging into the reservoir that received no early charge. Oil suite H was derived from Type II/III organic matter that accumulated in a deltaic/marine depositional environment. The degradation levels range from PM0 to PM4.5 (isoprenoid alkanes were present but being degraded). 25-Norhopanes were identified in all biodegraded oils and as trace compounds in the nondegraded oils.

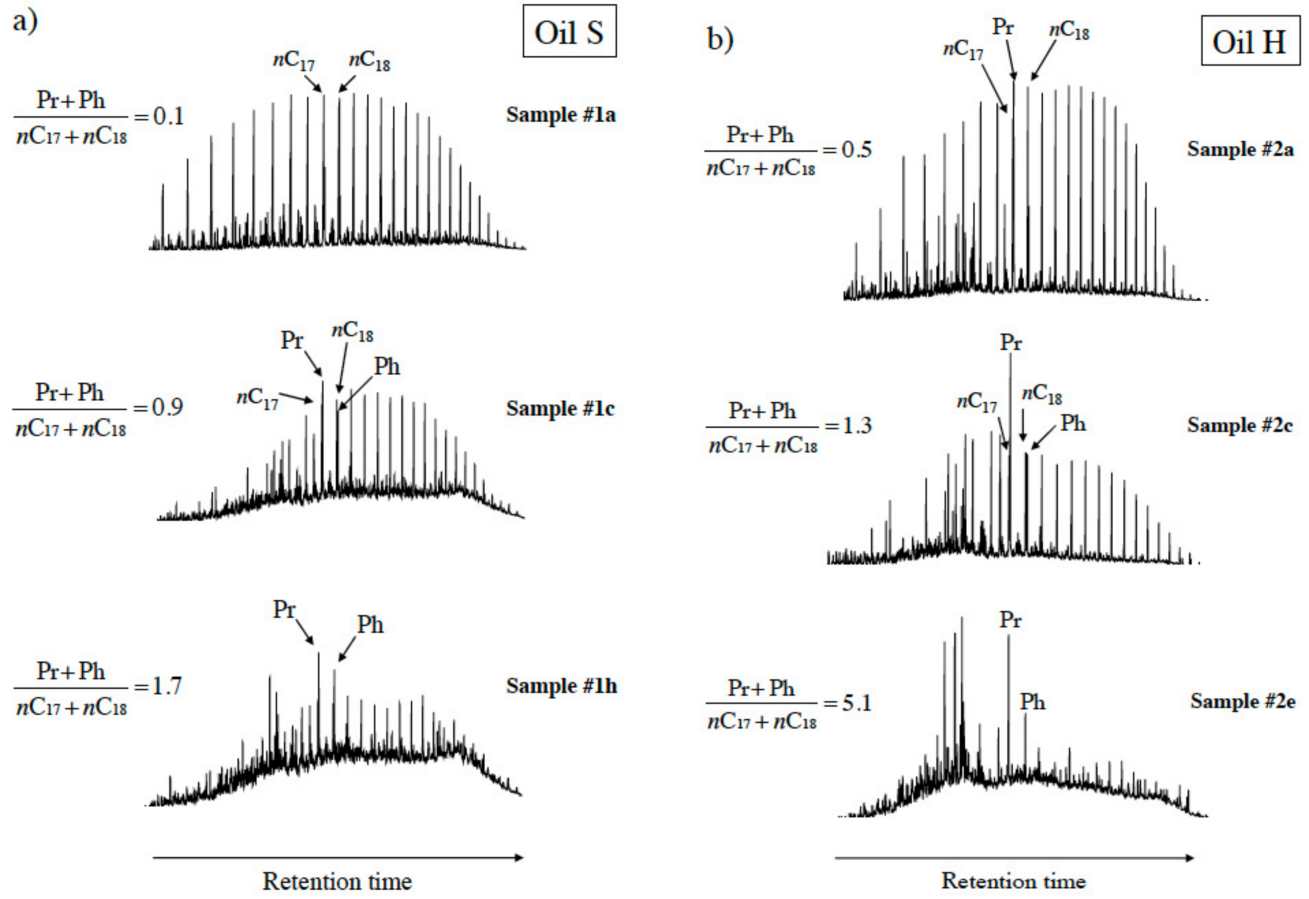

Figure 1. Total ion current traces of the saturate fraction from oils representing 3 stages of biodegradation of two sets of oil families (**a**) Oil S and (**b**) Oil H.

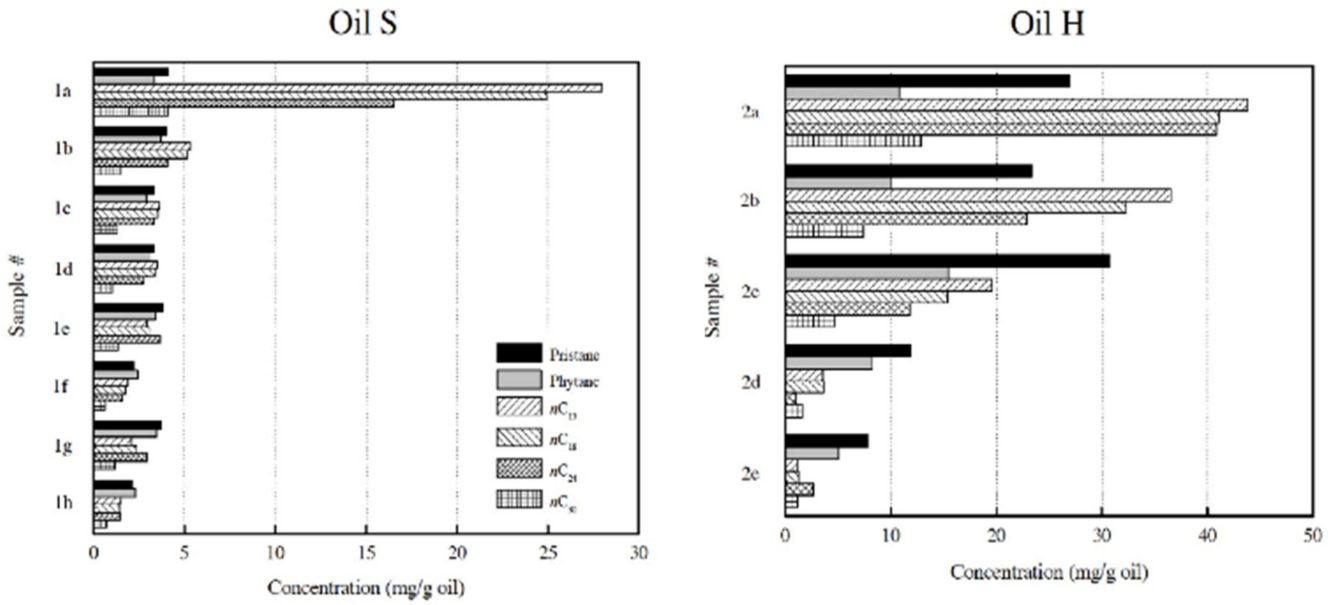

Figure 2. Concentrations of selected compounds (pristane, phytane, *n*-C_{17}, *n*-C_{18}, *n*-C_{24} and *n*-C_{30} alkanes) in the saturate fraction from oil samples analyzed in this study (Table S1). Calculations were performed based on areas in the m/z 85 mass chromatogram, with m/z 183 for squalane as an internal standard.

The aliphatic hydrocarbon fraction was isolated from the crude oil samples by solid-phase extraction (SPE) methods following the procedure described in [14]. Mass spectral characterization of compounds in this fraction was carried out using combined gas chromatography-mass spectrometry (GC-MS) on a Hewlett Packard 5890 GC interfaced to a HP 5970B quadrupole mass selective detector. The aliphatic hydrocarbons were analyzed using a J&W Scientific DB-5 fused silica GC column. The GC program was from 40 °C (2 min) to 300 °C at 4 °C/min and held at 300 °C (20 min). A mixture of the internal standards 5β(H)-cholane and squalane (5 µL from a standard stock solution) was added to the SPE cartridge placed on the balance to weigh out (dropwise by pipette) the 50 mg of oil. Peak area integration during GC-MS analysis was by MASS LAB. The relative response factor for quantification of individual saturated hydrocarbons versus the internal standards was assumed to be 1. Prior to irm-GC/MS analyses, cyclic and branched alkanes in the aliphatic hydrocarbon fraction were separated from normal and iso-alkanes by adduction with urea [15].

The adducted fraction was analyzed for $\delta^{13}C$ and δD compositions on a Thermo Electron Delta V Plus mass spectrometer. Individual *n*-alkanes were separated using a J&W Scientific DB-5 GC column. The GC was programmed from 60 °C (1 min) at 6 °C/min to 320 °C (35 min). The analytical accuracy and precision of the irm-GC/MS system during $\delta^{13}C$ measurements were ±0.5‰ (n = 180) (RMS error) based on *n*-$C_{15–30}$ alkane standards. The error of sample *n*-alkane $\delta^{13}C$ measurements was ≤0.6‰ based on duplicate runs. Analytical accuracy and precision of the system during D/H measurements were determined using standard mixtures "*n*$C_{16–30}$ alkanes", 5α-androstane, and squalane (A. Schimmelmann, Indiana University). The RMS error for δD measurements of these compounds was 6.3‰ (n = 255). The error of sample *n*-alkane δD measurements, based on triplicate runs, was generally better than ±10‰, reaching ±12‰ only in a few cases.

3. Results and Discussion

Microbial degradation of *n*-alkanes in deep reservoirs might proceed through non-site-specific and/or site-specific mechanisms. The $\delta^{13}C$ and δD values of *n*-alkanes in progressively degraded oils represent isotopic compositions of compounds that were not solely part of the metabolic cycle but also experienced pathogenic impacts and subsequent biodegradation. However, a comparative analysis of the isotopic compositions of a series of biodegraded oils such as here does allow us to examine the degradation process in some degree.

If biodegradation proceeds through a non-site-specific mechanism, no significant shift in the $\delta^{13}C$ values of remaining *n*-$C_{15–30}$ alkanes would be expected. During a microbial attack, a weaker ^{12}C-^{12}C bond is targeted, no matter where within the molecule this bond is located. Even if an alkane is ^{13}C-enriched overall and/or contains ^{13}C atom at C-2 or C-3 sites, it may still not survive because it would be attacked at another site with the ^{12}C-^{12}C bond. As a result, there would not be any preferential accumulation of ^{13}C-enriched *n*-C_{15-30} alkanes as biodegradation proceeds.

Alternatively, if *n*-alkane activation takes place at a particular site, e.g., at C-2 or C-3, ^{13}C-enrichment of remaining *n*-alkanes would be expected because there is only a single site available for attack. Thus, molecules with the weaker ^{12}C-^{12}C bond at these sites would be targeted preferentially, leaving *n*-$C_{15–30}$ *n*-alkanes with the stronger ^{12}C-^{13}C bond at these sites in the oil.

In contrast to the expected carbon isotope trends, the δD values of *n*-$C_{15–30}$ alkanes are unlikely to be affected by microbiological degradation. Even if fumarate addition at the C-2 position and/or carboxylation at the C-3 position leads to a D-enrichment of nonbiodegraded *n*-$C_{15–30}$ alkanes at these sites, it would hardly influence the δD value of the whole molecule because of a very small proportion of D atoms at a single site relative to the total number of H atoms in an alkane with 15 or more C atoms. This rationale contrasts with the observation of an approximately 35‰ D-enrichment of *n*-alkanes in a series of biodegraded oils in the Liaohe Basin, China [10]. The authors of that study, however, did

not provide a purely microbiological explanation for the observed phenomenon. It is likely that in the case of their study, there were other nonbiological processes (e.g., hydrogen isotope exchange between *n*-alkanes and porewaters and/or physical in-reservoir processes such as hydrocarbon washing, migration, phase change and mixing) that resulted in the observed D-enrichment.

Our compound-specific stable isotope measurements revealed that there is a *c.* 2.0‰ ^{13}C-enrichment and no D-enrichment of *n*-alkanes in the most biodegraded Oils S (Figure 3). As indicated above, there is a possibility that sample #1a in this series contains a later mixed oil charge scenario with contributions from a slightly different facies source rock. However, the similar overall concave shape in the distribution of δ^{13}C values of *n*-alkanes from all 8 Oil S samples suggests a common petroleum system framework.

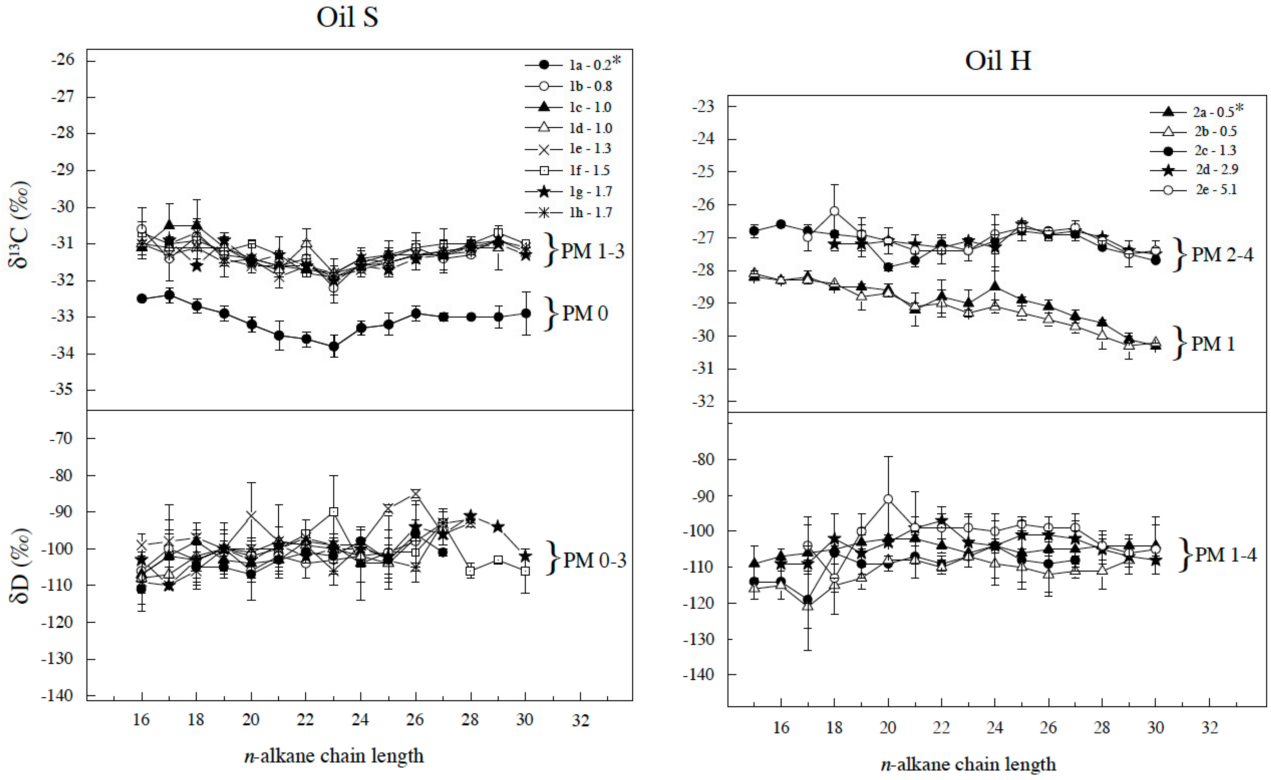

Figure 3. Carbon (based on V-PDB scale) and hydrogen (based on V-SMOW) isotopic composition of *n*-alkanes from Oils S and Oils H (Tables S2 and S3). * sample identification numbers and the ratio of pristane + phytane over nC_{17} + nC_{18} alkanes. The least biodegraded sample is characterized by the lowest ("lightest") ratio. PM refers to biodegradation levels according to [11].

Similarly, there is a *c.* 1.5–2.5‰ ^{13}C-enrichment and no D-enrichment in Oil suite H. Overall, there is < 2.5‰ δ^{13}C and < 20‰ δD variability among individual *n*-alkanes throughout the whole sequence of biodegradation (Figure 3). There are differences in the general level of enrichments between the two oil sample suites. Normal alkanes from the least biodegraded Oil H samples are 2–4‰ ^{13}C-enriched in comparison with the least biodegraded Oil S sample (Figure 4). The same difference in δ^{13}C values of *n*-alkanes is maintained between the biodegraded Oil suite H and Oil suite S. However, we observed no difference among the δD values of *n*-alkanes at different levels of biodegradation between the two series of oils.

Even though we do not expect a large intramolecular variation in carbon isotope composition of *n*-alkanes in mature crude oils from a single suite, little variability in *n*-alkane δ^{13}C values among *n*-alkanes of different chain lengths suggests that they were attacked simultaneously, which is consistent with other interpretations of the anaerobic biodegradation process [4]. Furthermore, carbon isotope results are not consistent with

a random attack on C atoms in biodegraded *n*-alkanes in both types of oils, particularly at the early stages of biodegradation. Our indirect isotopic evidence, i.e., ^{13}C-enrichment of *n*-alkanes at stages PM 1–3 in Oil S and PM 2–4 in Oil H, points toward a site-specific process (es), that most likely takes place at positions C-2 or C-3 as discussed above.

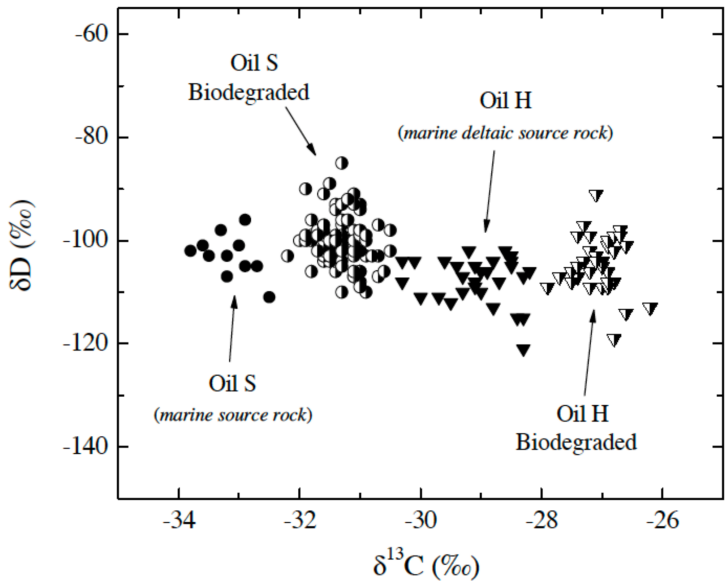

Figure 4. A cross plot of δ^{13}C and δD values of all *n*-alkanes for which both types of isotope data were acquired. Normal alkanes from Oil S correspond to sample #1a, *n*-alkanes from Oil S Biodegraded to samples #1b, 1c, 1d, 1e, 1f, 1g and 1h, respectively. Normal alkanes from Oil H correspond to samples #2a and 2b, and *n*-alkanes from Oil H Biodegraded to samples #2c, 2d and 2e, respectively.

Normal alkanes of all chain lengths from within Oil suites S and Oils H have approximately the same δD values. Furthermore, Oils S and Oils H have very similar δD values as well, even though they derived from different sources rocks (Figure 4). The source rocks generating Oils H would be expected to have more D-depleted kerogen because of a greater proportion of terrestrial-derived OM and thus produce relatively D-depleted hydrocarbons. However, this is not the case. Taken together, these observations imply that there was a significant D/H exchange between organic compounds in the source rocks corresponding to Oils S and Oils H and isotopically similar marine-derived formation waters during *n*-alkane generation.

4. Conclusions

We conclude that the relatively homogeneous and sustained nature of δ^{13}C composition of *n*-alkanes maintained during in-reservoir biodegradation indicates that these compounds have a good potential as a supplementary tool for oil–oil and oil–source-rock correlation even in biodegraded oils. However, labor-intensive cost-prohibitive δD methodology and a high probability of D/H exchange during individual hydrocarbon generation and potentially subsequent equilibration with petroleum system waters make the application of δD analysis far less attractive for these purposes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/geosciences11090365/s1, Table S1: Concentrations of selected compounds in the saturate fraction; Table S2: Carbon isotope compositions of C15–30 n-alkanes in the saturate fraction; Table S3: Hydrogen isotope compositions of C15–30 *n*-alkanes in the saturate fraction.

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Data Availability Statement: Data are contained within the Supplementary Material.

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