energy&fuels

Article

pubs.acs.org/EF

Petroleum Biodegradation Effects on Polar Acidic Compounds and Correlation with Their Corresponded Hydrocarbon Fractions

Célio F. F. Angolini,[†] Ramsés Capilla,[‡] and Anita J. Marsaioli*[,†]

[†]Institute of Chemistry, University of Campinas − UNICAMP, Campinas, São Paulo, CP 6154, CEP 13083-97, Brazil

[‡]PETROBRAS/CENPES/PDGEO/GEOQ, Av. Horácio Macedo, 950, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, CEP 21941-915, Brazil

Supporting Information

ABSTRACT: A set of five oil samples from the same source rock, classified at different levels of biodegradation, was evaluated by high-resolution mass spectrometry (Orbitrap). O-containing acidic compounds from the acidic oil fractions were characterized by determining their molecular formulas. The effects of biodegradation were detected in all O classes and were consistent with the biodegradation of neutral compounds. Additionally, we found a slightly biodegraded sample that contained acidic compounds, biomarkers of a highly biodegraded sample. These results led us to propose a scale of biodegradation using acidic compounds, particularly those containing two oxygen atoms (carboxylic acids).

1. INTRODUCTION

Microorganisms may degrade petroleum in a low entropy process via different biological pathways[1,2] at rates that depend on the availability of inorganic and organic electron acceptors.[3,4] Biodegradation commonly occurs in low-temperature reservoirs (<80 °C) with low water salinity (<100 ppt),[5] by means of depleting first the saturated and second the aromatic hydrocarbons,[6] in processes that can be aerobic, anaerobic, or both.[7]

These biological transformations lead to a systematic alteration of the chemical and physical properties of crude oil which have significant negative impact on petroleum value by increasing its specific gravity, viscosity, and the abundance of heteroatom-containing compounds.[5,8] Therefore, microorganisms are responsible for large economic losses in petroleum industries and are involved in hydrocarbon biodegradation, as well as equipment and pipeline corrosion, which have significant impacts on petroleum exploration, production, and refining.[9,10]

Generally, heteroatom-containing compounds are dead-end products in microbial catabolism, and their abundance increase during biodegradation processes,[6,11] especially carboxylic acids,[12] prompted us to use them as markers of biodegradation levels in petroleum samples.

2. EXPERIMENTAL SECTION

2.1. Geological Settings.[13] The Brazilian marginal basins are directly related to the rupture of the African/South American plates. The origin of the basin is related to the Early Cretaceous breakup of the Gondwana Supercontinent. The northern and southern boundaries of the Basin are Vitória and Cabo Frio Arcs, respectively, encompassing an area of approximately 100 000 km^2.[12,13]

Tectonic evolution of the area can be divided into three main phases: Rift, Post-Rift, and Drift. The Rift phase was divided into three depositional sequences from the Hauterivian, Barremian (Andar Aratu), and Eoaptian ages (Andar Jiquiá). These sequences are characterized by the predominance of lacustrine sedimentation, which produced most of the source rocks. The Post-Rift sequence lies unconformable on top of the Rift sequence and is predominantly lacustrine. The Drift sequence is characterized by marine sedimentation and was subjected to a thermal subsidence regime.[12]

The source rocks and reservoir rocks were formed during the tectono-sedimentary evolution of the Campos Basin, which is one of the most prolific basins in Brazil and is responsible for approximately 95% of Brazilian oil production. The relatively late secondary migration allowed this petroleum to be trapped in a wide age range of reservoirs at different depths and temperatures. Such temperature variation has been interpreted as the most important controlling factor for oil preservation from biodegradation. Bulk, carbon isotope ($d^{13}C$) and molecular analyses, medium pressure liquid chromatography (MPLC), gas chromatography (GC), and biomarker gas chromatography−mass spectrometry (GC−MS) of the studied oils confirmed one common source and a similar level of maturity and distinct biodegradation levels. Therefore, the samples were considered appropriate to investigate biodegradation (Table 1).

2.2. Acidic Fraction Extraction. The acidic fractions of all oil samples (about 1 g) were extracted with silica modified by KOH (2.5 g) in a Soxhlet apparatus with refluxing ether (6 h) followed by ether/formic acid (20% v/v) for 2 h (reflux). The method was previously described,[12] and the modified silica was prepared by adding silicagel 60 (Merck 0.063−0.100 μm, 200 g) into a hot isopropanol solution (80 mL) containing KOH (25 g). Evaporation of the ether/formic acid mixture yielded the crude acidic fraction as a thick oil.

2.3. LTQ Orbitrap Fourier Transform Mass Spectrometry (FTMS) Analysis. All samples were prepared by dissolving 1 mg of the acidic fraction in toluene/methanol (1:1, v/v) with 0.5% NH_4OH and were analyzed with a LTQ Orbitrap XL mass spectrometer from Thermo Scientific with an external ESI source. We used a negative ionization mode with a spray voltage of 3.8 kV, a capillary voltage of 48 V and a capillary temperature of 250 °C. The ion optics was tuned to provide an optimal signal for the m/z 200−1000 range, an AGC target of 1×10^6. A resolution of 100 000@400 was used in all experiments, and 100 microscans were averaged.

2.4. Data Processing. All acquired data were submitted to a postacquisition mass spectra recalibration procedure using RecalOff-

Received: May 19, 2015
Revised: July 23, 2015
Published: July 27, 2015

DOI: 10.1021/acs.energyfuels.5b01116
Energy Fuels 2015, 29, 4886−4892

Table 1. Characteristics of Petroleum Reservoirs and Geochemical Properties of Oil Samples

				diagnostic features of source[b], biodegradation[c] and maturity[d] of oils						
sample	biodegradation level[a]	T (°C)	depth (m)	21/23 TRI	26/25 TRI	TET/26 TRI	NOR25/H30	*n*C17/Pr	*n*C18/Ph	Ts/Ts + Tm
P1	light (0−1)	82	2405−2588	0.97	1.30	0.39	0.05	0.76	1.61	0.20
P2	heavy (5−6)	71	1988−2222	0.97	1.18	0.41	0.05	n.d.	n.d.	0.30
P3	moderate (3−4)	62	3023	0.92	1.20	0.39	0.04	n.d.	1.83	0.29
P4	severe (6)	62	2066	0.93	1.19	0.41	0.88	n.d.	n.d.	0.39
P5	light (0−1)	85	3070−3240	0.97	1.23	0.44	0.04	0.79	1.78	0.37

[a]Peters and Moldowan biodegradation level. [b]m/z 191 for 21/23TRI (C21 tricyclic terpane/C23 tricyclic terpane), 26/25TRI (C26 tricyclic terpane/C25 tricyclic. terpane) and TET/26TRI (C24 tetracyclic terpane/C26 tricyclic). [c]m/z 191 for NOR25/H30 (C29 25-nor-17a(H) hopane/C30 17a,21b (H) hopane). m/z 71 for *n*C17/Pr (linear C17 hydrocarbon/pristane), *n*C18/Ph (linear C18 hydrocarbon/phytane) and Pr/Ph (pristine/phytane). [d]m/z 191 for Ts/Ts+Tm (C27 18a(H)-trisnorneohopane/C27 18a(H)-trisnorneohopane + C27 17a(H)-trisnorhopane).

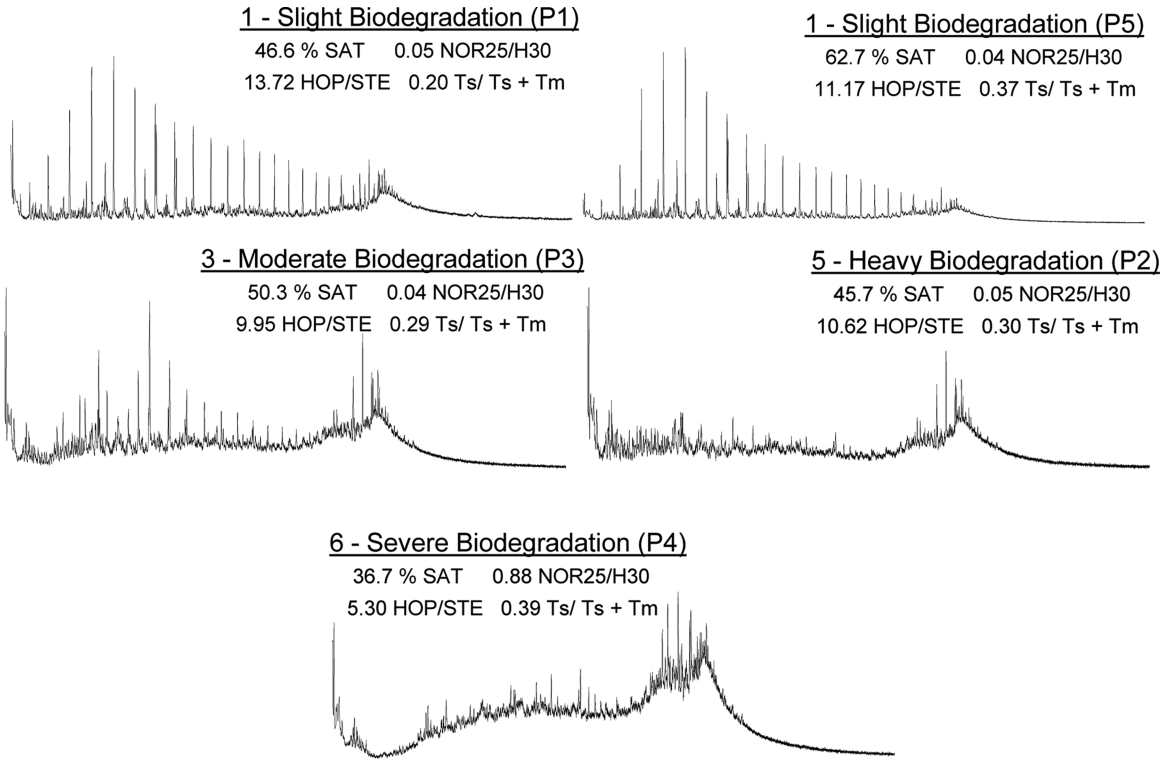

Figure 1. GC-MS chromatograms of five oil samples with different levels of biodegradation.[17] % Saturated hydrocarbons (SAT) from MPLC. For abbreviation, see Table 1.

line, a tool from Xcalibur software. The molecular formula was also attributed using Xcalibur with elemental composition restricted to $C_{100}{}^{13}C_2H_{200}O_4N_2$, and only formulas with errors below 2 ppm were accepted. All the graphics and data pretreatment were made using Microsoft Excel 2010 (for data reorganization) and Origin 8.0 (for graphical building).

2.5. Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. GC-MS analysis was performed using a HP6890 instrument coupled to a mass detector HP5970-MSD and equipped with a HP-5-MS fused silica column (30 m, 0.25 mm, 0.25 μm film thickness). Helium was the carrier gas (approximately 1 mL min^{-1}), and the oven temperature programs began at 80 °C (held for 2 min), increased from 80 to 270 °C at 4 °C min^{-1} and to 300 °C at 10 °C min^{-1} (holding for 25 min) for linear hydrocarbon; and started at 70 °C (holding for 2 min), increased from 70 to 190 °C at 30 °C min^{-1}, to 250 °C at 1 °C min^{-1}, to 300 °C at 2 °C min^{-1} (holding for 20 min) for hopanoic, tricyclic and bicyclic hydrocarbons. The instrument was operated in the SCAN and SIM mode over a range of m/z 50−700. The injector (240 °C) was operated in the split mode (10:1).

3. RESULTS AND DISCUSSION

The relationship between carboxylic acid content and biodegradation is a long-standing interest in our group.[12] With the advent of petroleomics,[14−16] these analyses have become easier, covering a wider range of compounds. We have therefore selected five different oil samples from the same source rock and similar levels of maturity but different levels of biodegradation (Figure 1), addressing the oxygen-containing compounds.

However, the Campos Basin oils possess high levels of nitrogen- and sulfur-containing compounds (Figure 2, C) requiring purification prior to the analyses of the acidic fraction (Figure 2B), which is a problem in accurate FTMS analysis using standard Orbitrap.[16] This purification step improved the MS analyses by avoiding unresolved compounds due to limited resolution of standard Orbitrap, for instance, the doublet SH_4 vs C_3, requires a 135 000@400 resolution to be fully resolved.

Oxygen is the second most abundant element in organisms and the fourth in petroleum samples (0.05−1.5%),[18] and its

DOI: 10.1021/acs.energyfuels.5b01116
Energy Fuels 2015, 29, 4886−4892

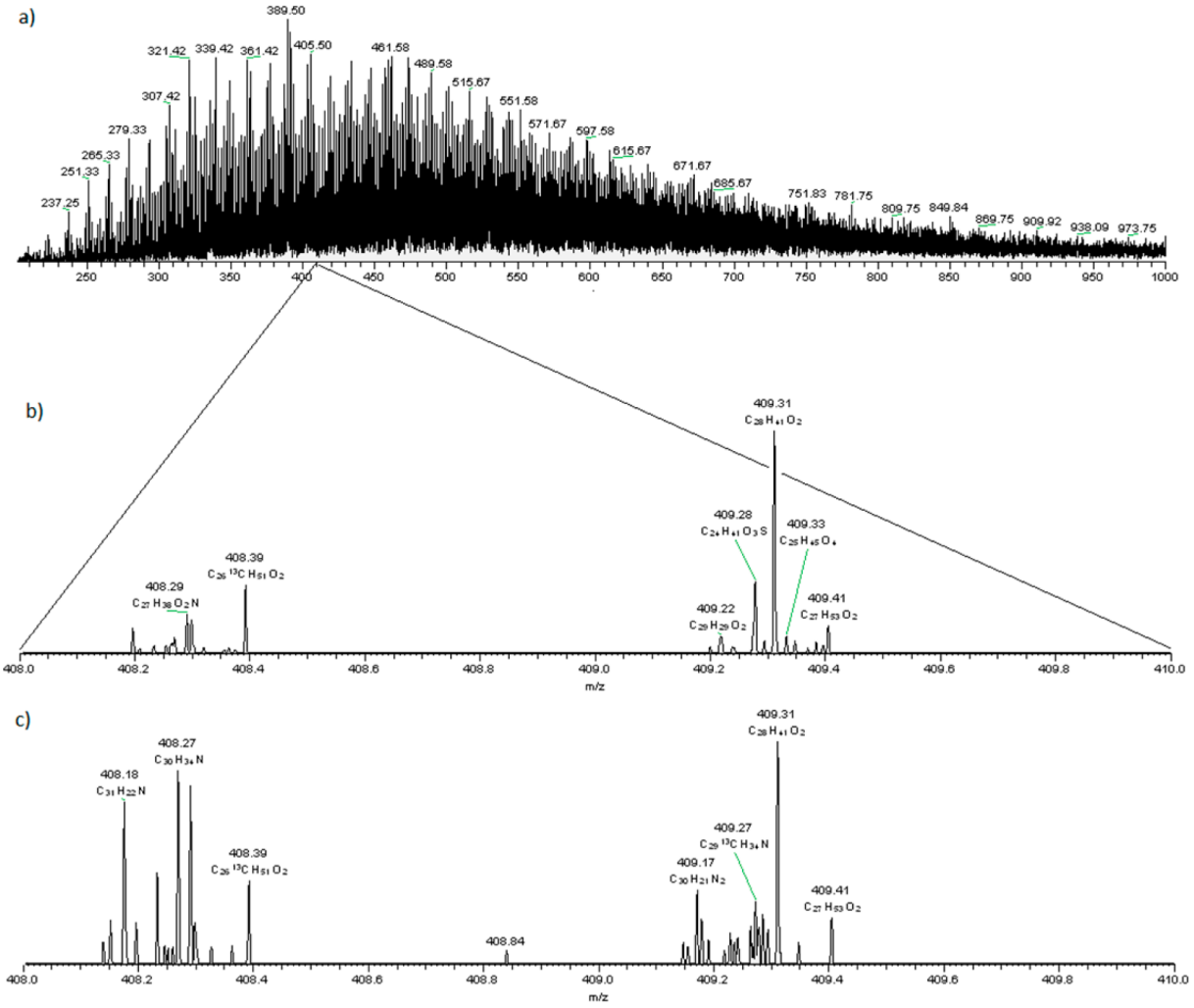

Figure 2. (a) HRFT-MS chromatograms of sample P5; their expanded view from m/z 408 to 410: (b) for the acidic fraction and (c) for the crude oil.

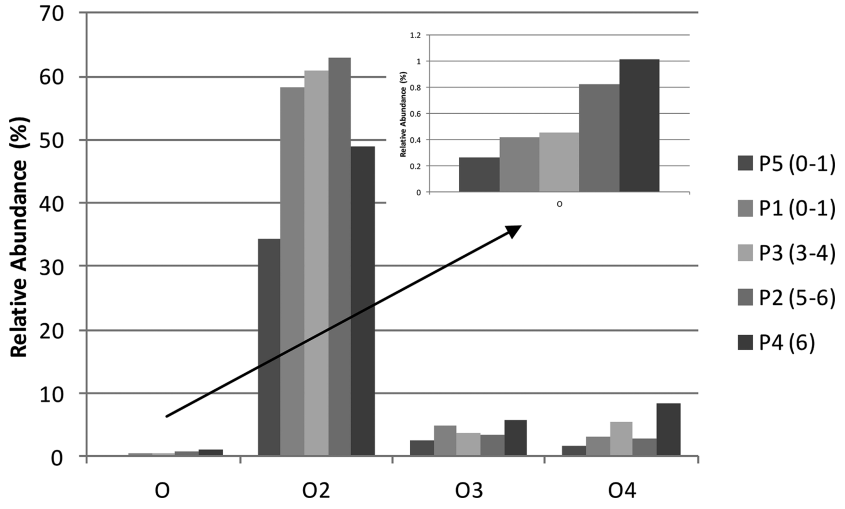

Figure 3. Relative abundance of O_x classes in samples P1 to P5. Biodegradation levels in parentheses.

concentration depends on the biodegradation levels with bulk effects similar to those observed in aromatic hydrocarbons; that is, they become more recalcitrant to biodegradation with an increase in ring-number and aromaticity.[19,20] In general, the O and O_2 classes increased with biodegradation, while the remaining O_x classes did not match to the biodegradation levels (Figure 3).

Among O-containing compounds, the O_1 class comprises approximately 1%; however, its relative abundance and its DBE range distributions increase with biodegradation (Figure 4),

DOI: 10.1021/acs.energyfuels.5b01116
Energy Fuels 2015, 29, 4886−4892

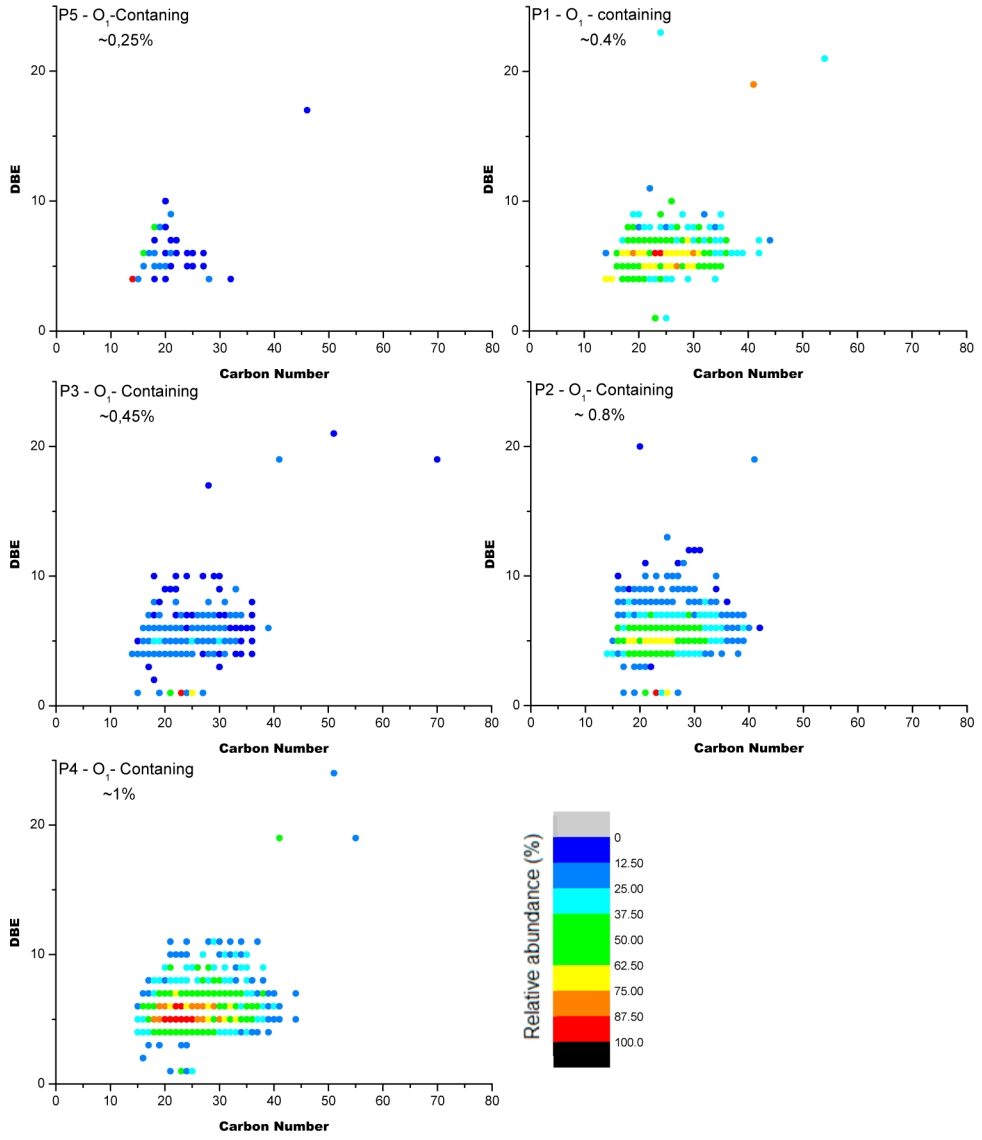

Figure 4. DBE vs Carbon Number distribution for the O_1 class of samples P1 to P5.

indicating that these compounds are being generated during the biodegradation process. These results differ from those observed by Kim and co-workers[5] and are more consistent with biodegradation preferences, as their precursors (aromatic compounds) are more recalcitrant than aliphatic hydrocarbons. Therefore, their abundance should decrease later in more biodegraded samples. Taking into consideration the use of electrospray ionization (ESI), the O_1 class is mainly composed of phenols that rise from the degradation of aromatic compounds.

During the first stages of the biodegradation, there is an increase of the O_2 compounds abundance and a decrease when more advanced levels are reached (Figure 3). In general, the abundance of each DBE class increases at the beginning and decreases at higher biodegradation levels (Figure 5), which overlaps with the depletion of the corresponding hydrocarbon class in the neutral fraction.

For example, the acyclic fatty acids (DBE 1) produced from the biodegradation of acyclic hydrocarbons decreases from sample P3 to P2 (Figure 5), where we also observed the depletion of these precursors in the neutral fraction (Figure 6). The same pattern was observed in the DBE 2 class with the

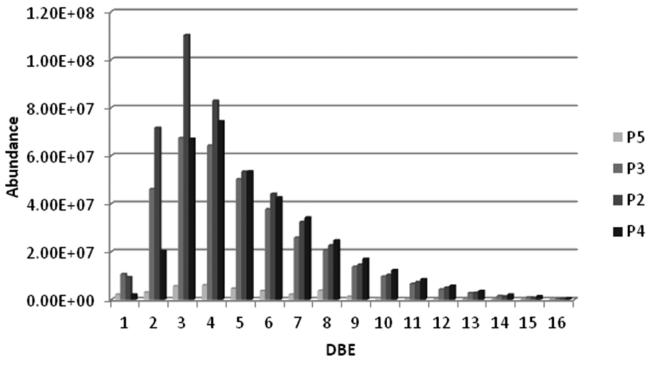

Figure 5. Abundance of O_2 classes in samples P5, P3, P2, and P4.

depletion of alkyl-cyclohexenes in the neutral fraction (Figure S1). This same trend repeats for compounds of DBE 3 to 6; however, these are more difficult to correlate to hydrocarbons due to the larger complexity of the biodegradation pathways, which generates carboxylic acids of different DBE numbers.[6] However, the consumption of these molecules in the neutral fraction was observed (Figure S2). Additionally, 25-norhopanes

DOI: 10.1021/acs.energyfuels.5b01116
Energy Fuels 2015, 29, 4886−4892

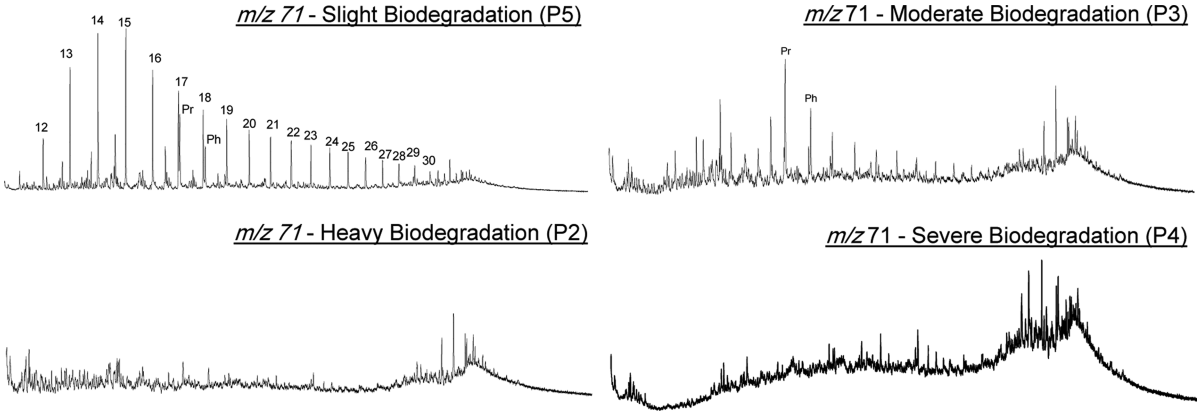

Figure 6. GC-MS reconstructed ion chromatograms (m/z 71) of oil samples with different levels of biodegradation showing linear hydrocarbons composition. The number refers to the hydrocarbon length chain.

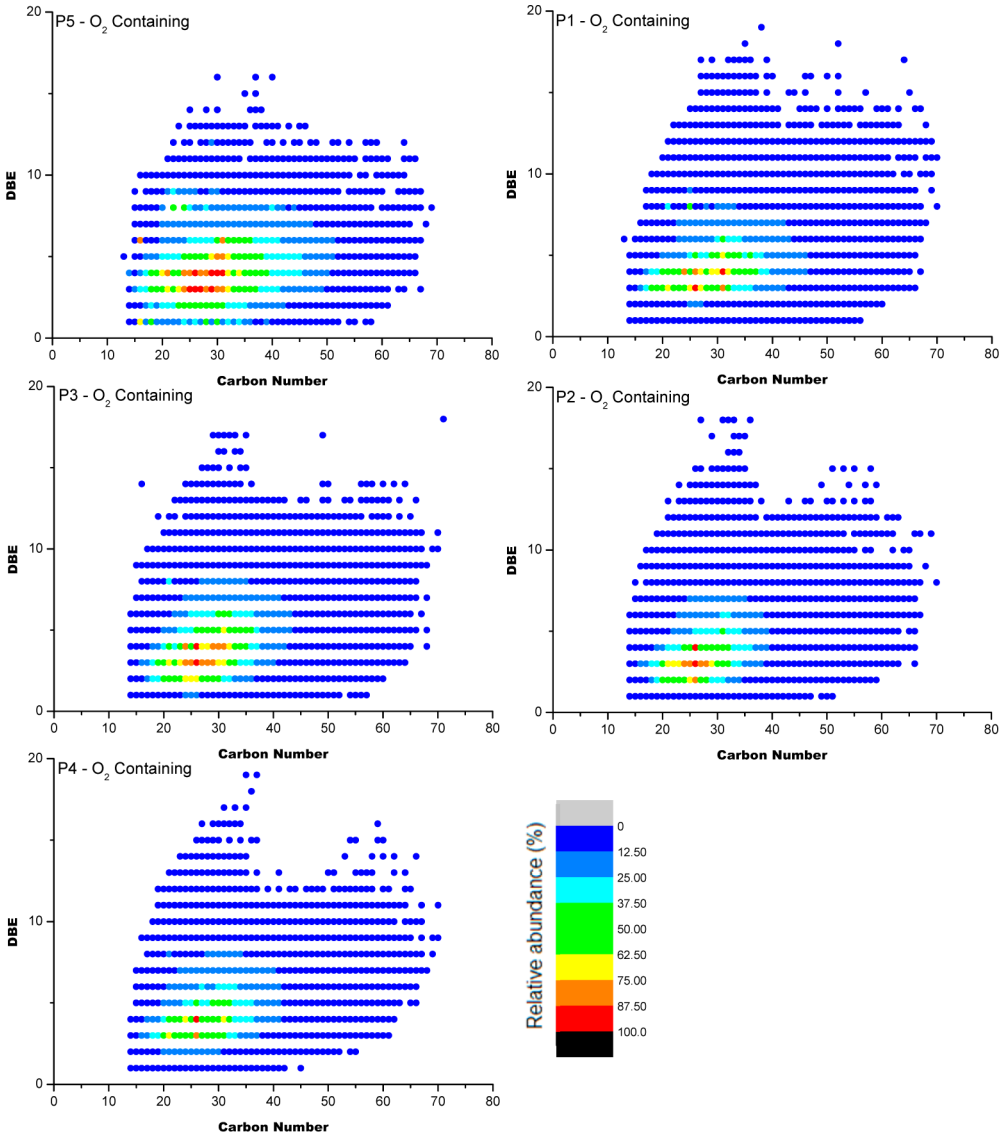

Figure 7. DBE vs Carbon Number distribution for the O2 class of samples P1 to P5.

can be seen in sample P4 (m/z 177, Figure S2), which attest to its high biodegradation level.

An alternate overview of the O_2 class can be seen in Figure 7, where the dispersion of DBE versus carbon number indicates that biodegradation causes a shift in the maximum abundance toward higher DBE. Additionally, a correlation between biodegradation and the A/C ratio [acyclic (DBE 1)/cyclic (DBE 2−4)][21] was observed (Figure 8), in which A/C values

DOI: 10.1021/acs.energyfuels.5b01116
Energy Fuels 2015, 29, 4886−4892

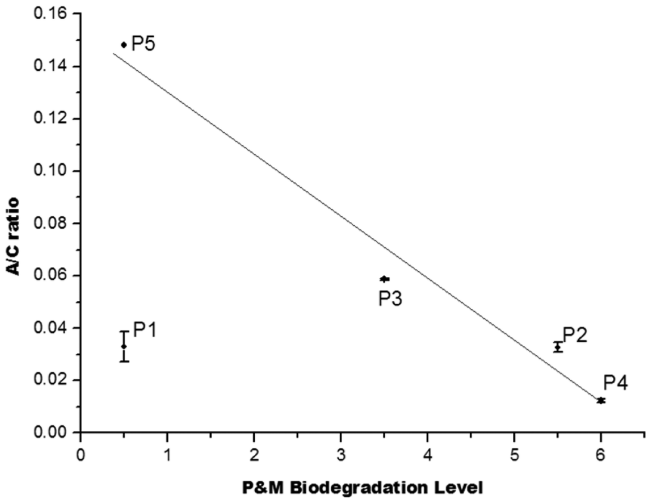

Figure 8. Correlation between the A/C ratio and the biodegradation level (P&M). P1 was excluded for linear regression.

decreased with increasing biodegradation. However, the A/C ratio has to be validated using highly biodegraded samples, once samples with depleted linear carboxylic acids can preclude the A/C ratio calculation.

The outlier result of sample P1 may be due to the mixture of biodegraded oil with a nondegraded oil, leading to its misclassification as a slightly biodegraded oil based on hydrocarbon evaluation. However, the acidic compounds, and especially carboxylic acids, reveal this sample as a moderately biodegraded sample, such as P3. Based on this assumption, and according to all observed trends, a complemented biodegradation scale similar to the Peter & Moldowan scale was suggested (Figure 9), but now using carboxylic acid compounds. This

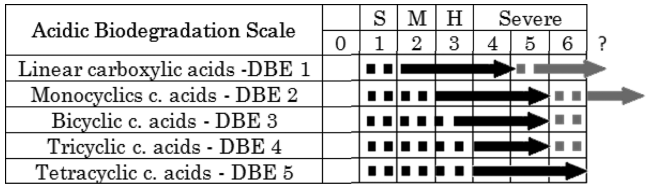

Figure 9. Proposed petroleum biodegradation scale using O_2 compounds, with the carboxylic acid production/removal at increasing levels of biodegradation. Black arrows indicate the beginning of the carboxylic acid production (dashed lines) until maximum concentration (solid). Gray arrows indicate were the carboxylic acid depletion overcome the production (dashed lines) until total depletion (solid).

could extend the classification of oil samples to higher biodegradation levels, which was limited when most hydrocarbons were biodegraded.

4. CONCLUSIONS

We observed that carboxylic acid production is related to the consumption of their neutral precursors, providing evidence for their biodegradation origin. Similar to the hydrocarbon fraction, we understand that the production and biodegradation of O-containing compounds follow a preferential order. Compounds with less complex structures (lower DBE) are more depleted than those with more complex structures (higher DBE). Additionally, O-containing classes are more recalcitrant to biodegradation than their hydrocarbon precursors, but these are biodegraded when hydrocarbons are absent.

Consequently, these analyses can reveal further biodegradation levels, unpredicted by previously reported ratio (A/C ratio) due the depletion of acyclic acids. It is thus appropriate to propose a biodegradation level scale based on all carboxylic compounds. Moreover, we speculated that P1 was a mixture of degraded and nondegraded oils based on polar and neutral compound analyses, showing the extent of polar compound analyses in the characterization of recharge phenomena in petroleum reservoirs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.energyfuels.5b01116.

GC-MS reconstructed ion chromatograms of alkylcyclohexanes (m/z 82, Figure S1) hopanes and steranes (m/z 123, 177 and 191, Figure S2). (−) ESI-FTMS Orbitrap spectra of P1−P5 (Figure S3) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: anita@iqm.unicamp.br.

Author Contributions

All authors contributed equally to this paper and have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Petrobras, CAPES, and São Paulo Research Foundation (FAPESP, grant 2010/06557-9) for financial support.

■ REFERENCES

(1) Widdel, F.; Rabus, R. Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Curr. Opin. Biotechnol.* **2001**, *12* (3), 259−276.

(2) Bitton, G. *Encyclopedia of Environmental Microbiology*; Wiley: New York, 2002.

(3) Head, I. M.; Jones, D. M.; Larter, S. R. Biological activity in the deep subsurface and the origin of heavy oil. *Nature* **2003**, *426* (6964), 344−52.

(4) Larter, S.; Wilhelms, A.; Head, I.; Koopmans, M.; Aplin, A.; Di Primio, R.; Zwach, C.; Erdmann, M.; Telnaes, N. The controls on the composition of biodegraded oils in the deep subsurface—part 1: biodegradation rates in petroleum reservoirs. *Org. Geochem.* **2003**, *34* (4), 601−613.

(5) Kim, S.; Stanford, L. A.; Rodgers, R. P.; Marshall, A. G.; Walters, C. C.; Qian, K.; Wenger, L. M.; Mankiewicz, P. Microbial alteration of the acidic and neutral polar NSO compounds revealed by Fourier transform ion cyclotron resonance mass spectrometry. *Org. Geochem.* **2005**, *36* (8), 1117−1134.

(6) Wentzel, A.; Ellingsen, T. E.; Kotlar, H. K.; Zotchev, S. B.; Throne-Holst, M. Bacterial metabolism of long-chain n-alkanes. *Appl. Microbiol. Biotechnol.* **2007**, *76* (6), 1209−1221.

(7) da Cruz, G. F.; de Vasconcellos, S. P.; Angolini, C. F.; Dellagnezze, B. M.; Garcia, I. N.; de Oliveira, V. M.; Dos Santos Neto, E. V.; Marsaioli, A. J. Could petroleum biodegradation be a joint achievement of aerobic and anaerobic microrganisms in deep sea reservoirs? *AMB Express* **2011**, *1*, 47.

(8) Qian, K. N.; Robbins, W. K.; Hughey, C. A.; Cooper, H. J.; Rodgers, R. P.; Marshall, A. G. Resolution and identification of elemental compositions for more than 3000 crude acids in heavy petroleum by negative-ion microelectrospray high-field Fourier

DOI: 10.1021/acs.energyfuels.5b01116
Energy Fuels 2015, 29, 4886−4892

transform ion cyclotron resonance mass spectrometry. *Energy Fuels* **2001**, *15* (6), 1505−1511.
(9) Magot, M.; Ollivier, B.; Patel, B. K. C. Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek* **2000**, *77* (2), 103−116.
(10) Dias, H. P.; Pereira, T. M. C.; Vanini, G.; Dixini, P. V.; Celante, V. G.; Castro, E. V. R.; Vaz, B. G.; Fleming, F. P.; Gomes, A. O.; Aquije, G. M. F. V.; Romao, W. Monitoring the degradation and the corrosion of naphthenic acids by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry and atomic force microscopy. *Fuel* **2014**, *126*, 85−95.
(11) Das, N.; Chandran, P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnol. Res. Int.* **2011**, *2011*, 941810.
(12) Nascimento, L. R.; Reboucas, L. M. C.; Koike, L.; Reis, F. D. M.; Soldan, A. L.; Cerqueira, J. R.; Marsaioli, A. J. Acidic biomarkers from Albacora oils, Campos Basin, Brazil. *Org. Geochem.* **1999**, *30* (9), 1175−1191.
(13) de Vasconcellos, S. P.; Crespim, E.; da Cruz, G. F.; Senatore, D. B.; Simioni, K. C. M.; Neto, E. V. D.; Marsaioli, A. J.; de Oliveira, V. M. Isolation, biodegradation ability and molecular detection of hydrocarbon degrading bacteria in petroleum samples from a Brazilian offshore basin. *Org. Geochem.* **2009**, *40* (5), 574−588.
(14) Rodgers, R. P.; Schaub, T. M.; Marshall, A. G. Petroleomics: MS returns to its roots. *Anal. Chem.* **2005**, *77* (1), 20A−27A.
(15) Zhurov, K. O.; Kozhinov, A. N.; Tsybin, Y. O. Evaluation of High-Field Orbitrap Fourier Transform Mass Spectrometer for Petroleomics. *Energy Fuels* **2013**, *27* (6), 2974−2983.
(16) Pomerantz, A. E.; Mullins, O. C.; Paul, G.; Ruzicka, J.; Sanders, M. Orbitrap Mass Spectrometry: A Proposal for Routine Analysis of Nonvolatile Components of Petroleum. *Energy Fuels* **2011**, *25* (7), 3077−3082.
(17) Peters, K. E.; Moldowan, J. M. *The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments*; Prentice Hall: Englewood Cliffs, NJ, 1993; p xvi, p 363 .
(18) Speight, J. G. *The Chemistry and Technology of Petroleum*, 4th ed.; CRC Press/Taylor & Francis: Boca Raton, FL, 2007; p 945
(19) Aitken, C. M.; Jones, D. M.; Larter, S. R. Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs. *Nature* **2004**, *431* (7006), 291−294.
(20) Hughey, C. A.; Minardi, C. S.; Galasso-Roth, S. A.; Paspalof, G. B.; Mapolelo, M. M.; Rodgers, R. P.; Marshall, A. G.; Ruderman, D. L. Naphthenic acids as indicators of crude oil biodegradation in soil, based on semi-quantitative electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Rapid Commun. Mass Spectrom.* **2008**, *22* (23), 3968−3976.
(21) Hughey, C. A.; Rodgers, R. P.; Marshall, A. G.; Qian, K. N.; Robbins, W. K. Identification of acidic NSO compounds in crude oils of different geochemical origins by negative ion electrospray Fourier transform ion cyclotron resonance mass spectrometry. *Org. Geochem.* **2002**, *33* (7), 743−759.

DOI: 10.1021/acs.energyfuels.5b01116
Energy Fuels 2015, 29, 4886−4892