

Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs

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Biodegradation of crude oil in subsurface petroleum reservoirs is an important alteration process with major economic consequences¹. Aerobic degradation of petroleum hydrocarbons at the surface is well documented² and it has long been thought that the flow of oxygen- and nutrient-bearing meteoric waters into reservoirs was necessary for in-reservoir petroleum biodegradation³. The occurrence of biodegraded oils in reservoirs where aerobic conditions are unlikely⁴, together with the identification of several anaerobic microorganisms in oil fields⁵ and the discovery of anaerobic hydrocarbon biodegradation mechanisms^{6,7}, suggests that anaerobic degradation processes could also be responsible. The extent of anaerobic hydrocarbon degradation processes in the world's deep petroleum reservoirs, however, remains strongly contested. Moreover, no organism has yet been isolated that has been shown to degrade hydrocarbons under the conditions found in deep petroleum reservoirs⁸. Here we report the isolation of metabolites indicative of anaerobic hydrocarbon degradation from a large fraction of 77 degraded oil samples from both marine and lacustrine sources from around the world, including the volumetrically important Canadian tar sands. Our results therefore suggest that anaerobic hydrocarbon degradation is a common process in biodegraded subsurface oil reservoirs.

Aerobic biodegradation of hydrocarbons is often fast enough to be observable on a short human timescale and thus aerobic processes dominated petroleum geological thinking about subsurface petroleum biodegradation for many years. Although

aerobically degraded oilfields are described in the literature⁹, even conservative mass balances of the volumes of water needed to transport sufficient oxygen present overwhelming problems geologically in most reservoirs¹⁰. Even where meteoric water has flushed basins it does not follow that oxygen was carried to the deep reservoirs¹¹.

The first bacteria isolated from oilfield waters¹² were anaerobes and Russian workers have considered anaerobic hydrocarbon degradation in deep reservoirs to be the primary process for many years¹³. Subsurface waters are typically anaerobic and there is increasing evidence of the occurrence of viable anaerobic hydrocarbon degradation processes (for example, ref. 7 and references therein, and ref. 14), so we might reasonably expect subsurface hydrocarbon degradation to be anaerobic. However, until now, only one thermophilic anaerobic hydrocarbon degrader potentially capable of living in the deepest degraded reservoirs has been identified¹⁵ and none have been isolated which has been shown to degrade hydrocarbons under conditions found in deep petroleum reservoirs⁸. Magot and co-workers¹⁶ have shown that diverse anaerobic organisms commonly occur in oilfields. They concluded from laboratory and field studies that anaerobes were indeed responsible for most subsurface hydrocarbon degradation, and methanogenesis, an exclusively anaerobic process, seems common in oil-contaminated aquifers and reservoirs (compare with refs 8 and 17) and is a probable fate for much of the carbon dioxide produced during biodegradation⁴. These results, together with the availability of long periods of time in which degradation can occur, makes it likely that anaerobic hydrocarbon degradation is a common process in most petroleum reservoirs although direct evidence for this remains elusive¹¹.

It is only in the past twenty years that the use of hydrocarbons as substrates by anaerobic microorganisms has been investigated and the identification of metabolites and possible metabolic pathways reported (for reviews see ref. 6, and ref. 7 and references therein). The existence of indigenous thermophilic bacteria and hyperthermophilic *Archaea* in reservoirs was reported by L'Haridon and co-workers¹⁸ who also suggested that *in situ* bioconversion of crude oil fractions (probably aliphatic and aromatic hydrocarbons) was linked to sulphate reduction. A number of laboratory studies using aliphatic, aromatic, and polycyclic aromatic hydrocarbons as substrates for a variety of sulphate-reducing, de-nitrifying and

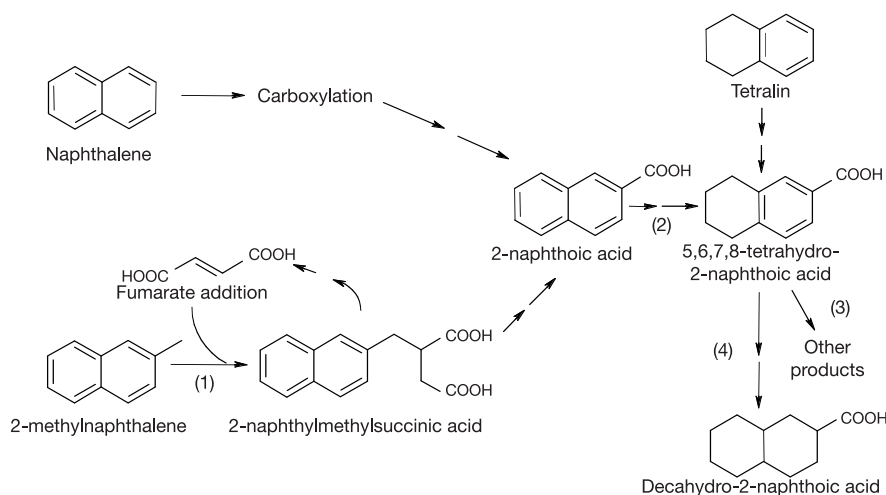


Figure 1 Part of a proposed reductive pathway for the anaerobic degradation of naphthalene, 2-methylnaphthalene and tetralin by sulphate-reducing bacteria²³. Initial carboxylation of naphthalene is followed by degradation to 2-naphthoic acid and, via a series of hydrogenation steps, to 5,6,7,8-tetrahydro-2-naphthoic acid (2), followed either by hydration and further degradation steps (3) or by further hydrogenation steps (4) to give

decahydro-2-naphthoic acid (a possible dead-end metabolite). Degradation of 2-methylnaphthalene is initiated by fumarate addition to form naphthyl-2-methylsuccinic acid (1), which is further degraded to give 2-naphthoic acid. The regeneration of fumarate shown here is not shown in the original proposed pathway.

letters to nature

methanogenic cultures have identified succinates—formed by the addition of fumarate either to a subterminal carbon of an alkane or to an alkyl substituent of an aromatic hydrocarbon—as the initial metabolites in the degradation process^{6,7,19,20}. Succinates have also been reported as metabolites from the biodegradation of both saturated and aromatic hydrocarbons in anoxic zones of petroleum-contaminated aquifers (for example, ref. 21). 2-Naphthoic acid and reduced 2-naphthoic acids have been reported as metabolites in a laboratory study of the anaerobic degradation of naphthalene under sulphate-reducing conditions²²; in a laboratory study of the anaerobic degradation of naphthalene, 2-methylnaphthalene and tetralin, using a sulphate-reducing enrichment culture isolated from a hydrocarbon-contaminated aquifer, 2-naphthoic acid and naphthyl-2-methylsuccinic acid were identified as initial intermediates in a metabolic pathway that also identified reduced 2-naphthoic acids as further metabolites²³ (Fig. 1). Studies of anoxic zones in petroleum-contaminated aquifers have also identified 2-naphthoic acid and reduced 2-naphthoic acids as evidence of anaerobic degradation^{21,24}. We report here the identification of these metabolites in a large suite of biodegraded oils from various petroleum systems in the world.

Biodegradation in oil reservoirs requires that water, nutrients and hydrocarbons are available to sustain microbial growth. This situation is most likely to occur near the oil–water contact in the reservoir⁴. Both the transitory nature of the metabolites in the metabolic process and the extremely small amounts likely to be present contribute to increasing the difficulty of isolating and detecting such metabolites in oil samples where, with their

increased water solubility compared to hydrocarbons, they would have partitioned into the oil from material in the water-saturated portions of the reservoir. The metabolite concentrations in reservoirs containing degraded oils will also be affected by whether biodegradation is at present occurring or is inactive.

In this study the methylated acid fractions from 77 biodegraded oils, which included reservoir core solvent extracts, oil seep and tar sand samples of both marine and lacustrine shale sources from well-studied, major petroleum basins around the world were analysed by gas chromatography-mass spectrometry (GC-MS) and their spectra compared to those of known metabolite standards. Seven comparable non-biodegraded oils were similarly characterized. The oils were categorized as non-biodegraded or biodegraded and classified according to their degree of biodegradation by analysis of their hydrocarbon distributions using the criteria proposed by ref. 25. Briefly, these criteria are based on the quasi-stepwise disappearance of certain hydrocarbon classes in oils, depending on their susceptibility to biodegradation. n-alkanes are the most susceptible, followed by simply branched alkanes, whereas polycyclic alkanes are generally the most resistant. Thus, on a scale of 0 to 10, level 0 is undegraded, level 1 represents some depletion of n-alkanes, level 4 represents complete removal of n-alkanes and depletion of acyclic isoprenoids, level 8 represents destruction of the regular steranes and alteration of the hopane polycyclic hydrocarbon distributions, and so on. However, mixtures of oils at different degradation levels are often encountered in reservoirs and the scale is thus indicative rather than absolute.

Initial efforts were concentrated on the search for alkyl- and aryl-

Table 1 Metabolites indicative of anaerobic degradation found in clastic oil reservoir samples obtained from around the world

Sample	Degradation level*	Source, system, reservoir	2-naphthoic acid (p.p.m.)	5,6,7,8-tetra-hydro-2-naphthoic acid (p.p.m.)	decahydro-2-naphthoic acid (isomer 1) (p.p.m.)	decahydro-2-naphthoic acid (isomer 2) (p.p.m.)
1	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	n.d.	n.d.	0.07	0.09
2	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	n.d.	n.d.	0.18	0.16
3	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	n.d.	n.d.	n.d.	0.01
4	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	0.04	n.d.	n.d.	0.08
5	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	n.d.	n.d.	n.d.	0.11
6	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	n.d.	n.d.	n.d.	0.10
7	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	n.d.	n.d.	n.d.	0.06
8	1–4 (mixed)	Eocene lacustrine sourced oil, Eocene reservoir	n.d.	n.d.	n.d.	0.08
9	4	Cretaceous lacustrine sourced oil, Tertiary reservoir	0.33	0.30	0.06	0.28
10	4	Cretaceous lacustrine sourced oil, Tertiary reservoir	0.01	0.01	n.d.	n.d.
11	1–4 (mixed)	Miocene marine sourced oil, Miocene reservoir	0.02	n.d.	0.01	0.03
12	3	Cambrian marine sourced oil, Cretaceous reservoir	0.01	0.01	n.d.	n.d.
13	5	Eocene lacustrine sourced reservoir core, Eocene reservoir	0.30	0.05	n.d.	n.d.
14	6	Eocene lacustrine sourced reservoir core, Eocene reservoir	0.19	0.03	n.d.	n.d.
15	8	Eocene lacustrine sourced reservoir core, Eocene reservoir	0.22	0.03	n.d.	n.d.
16	2	Eocene lacustrine sourced reservoir core, Eocene reservoir	1.18	0.20	n.d.	n.d.
17	3	Eocene lacustrine sourced reservoir core, Eocene reservoir	0.67	0.06	n.d.	n.d.
18	5	Eocene lacustrine sourced reservoir core, Eocene reservoir	0.45	0.06	n.d.	n.d.
19	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	1.61	0.18	n.d.	n.d.
20	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	2.85	0.06	n.d.	n.d.
21	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	2.08	0.11	n.d.	n.d.
22	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	2.57	0.39	n.d.	0.24
23	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	1.09	0.12	n.d.	n.d.
24	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	9.40	0.45	n.d.	n.d.
25	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	5.23	0.34	n.d.	n.d.
26	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	8.15	0.31	n.d.	n.d.
27	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	3.18	0.40	0.12	0.22
28	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	6.70	0.46	0.13	0.27
29	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	3.87	0.44	0.17	0.28
30	1–4 (mixed)	Eocene lacustrine sourced reservoir core, Eocene reservoir	8.95	0.51	0.18	0.28
31	4	Cretaceous lacustrine sourced reservoir core, Tertiary reservoir	1.92	0.23	n.d.	n.d.
32	4	Cretaceous lacustrine sourced reservoir core, Tertiary reservoir	3.04	0.31	n.d.	n.d.
33	4	Cretaceous lacustrine sourced reservoir core, Tertiary reservoir	1.07	0.21	n.d.	n.d.
34	4/5	Marine-sourced Cretaceous reservoir tar sands	0.50	n.d.	n.d.	0.01
35	7	Marine-sourced Cretaceous reservoir tar sands	0.32	nd	0.19	0.10
36	7	Marine-sourced Cretaceous reservoir tar sands	3.35	nd	0.32	0.52
37	7	Marine sourced Cretaceous reservoir tar sands	3.59	nd	0.20	0.40
38	8	Jurassic marine sourced seeps	1.75	nd	nd	0.14
39	7	Jurassic marine sourced seeps	1.46	nd	nd	0.10
40	7/8	Jurassic marine sourced seeps	1.45	0.14	nd	nd

*Assigned according to ref. 25; n.d., none detected.

Seven non-degraded oils from around the world (North Africa, Canada, China, the Middle East, the North Sea, and the Mediterranean) were also analysed and none were found to contain reduced naphthoic acids.

succinate metabolites, because these have been widely reported as anaerobic markers for both saturated and aromatic hydrocarbon degradation, both in laboratory studies and from petroleum-contaminated aquifers^{7,19–21}, and we have indeed identified such metabolites in laboratory anaerobic hydrocarbon degradation experiments²⁶. Nevertheless, attempts to confirm their presence in the oils we have studied have been inconclusive. The transient nature of these compounds, which appear to be formed in the initial step of metabolic pathways, where fumarate is regenerated as part of a cyclic process^{6,7}, suggests that they are unlikely to be detected in reservoir systems that are no longer actively degrading and, in actively degrading systems, will only be detected if sampling occurs at a currently biologically active zone. Although succinates were not reproducibly detectable, 52 of the 77 degraded oils studied were found to contain 2-naphthoic acid in concentrations ranging from 0.01 to ~10 p.p.m. and, more significantly, trace amounts of reduced 2-naphthoic acids, 5,6,7,8-tetrahydro-2-naphthoic acid and two isomers of decahydro-2-naphthoic acid were identified in 40 samples in concentrations ranging from 0.01 to ~0.5 p.p.m. (Table 1). Identification of metabolites in samples was made by comparison of mass spectral data for these samples (Fig. 2) with those of the methyl esters of standard compounds confirmed by co-injection studies with standards. To investigate the possibility that metabolites such as succinates and reduced naphthoic acids, if present, may be in the form of 'bound' biomarkers, for example, in the asphaltene fraction of degraded oils and extracts, alkaline saponification was performed on several reservoir samples before extraction and analysis of the acid fraction. Although succinates were not detected in the acid fractions of any of the saponified samples, the presence of 2-naphthoic acid and/or reduced 2-naphthoic acids was confirmed in samples in which these metabolites had previously been detected.

2-Naphthoic acid and reduced 2-naphthoic acids are formed in a reductive pathway for the anaerobic degradation of both 2-methylnaphthalene and naphthalene (Fig. 1) and as such, with abundant ubiquitous precursors in crude oils, may ultimately accumulate in sufficient quantities to be detectable in reservoir samples. The absence of reduced 2-naphthoic acids in the acid fractions of seven non-degraded oils from a variety of sources worldwide that we also analysed, and the absence of reduced 2-naphthoic acids from the acid fractions of oils that have been aerobically biodegraded in the laboratory²⁷, suggests that such compounds are indeed exclusively the products of anaerobic hydrocarbon biodegradation. Why these anaerobic metabolites were not found in all the degraded oils analysed is not known but perhaps they are only detectable in actively degrading or recently degraded reservoirs.

Although 2-naphthoic acid has been reported as both an aerobic²⁸ and an anaerobic metabolite of 2-methylnaphthalene biodegradation, 5,6,7,8-tetrahydro-2-naphthoic acid and two isomers of decahydro-2-naphthoic acid have been reported only as exclusively anaerobic metabolites^{23,24} of naphthalene and 2-methylnaphthalene biodegradation. The production of high-molecular-mass carboxylic acids by abiotic oxidation of hydrocarbons in petroleum reservoirs, such as occurs during thermochemical sulphate reduction, is in principle possible, but it only takes place at relatively high temperatures (usually $\gg 120^\circ\text{C}$)²⁹, whereas the maximum temperatures of the degraded oil reservoirs sampled in this study are all well below 85°C and high concentrations of sulphate are absent from the reservoirs. This, and the specificity of the acids seen, indicates that these are biological oxidation products.

Our results, obtained for a number of oils from a variety of reservoir sources including from the vast Canadian tar sand belt, therefore confirm that oil biodegradation in most reservoirs must

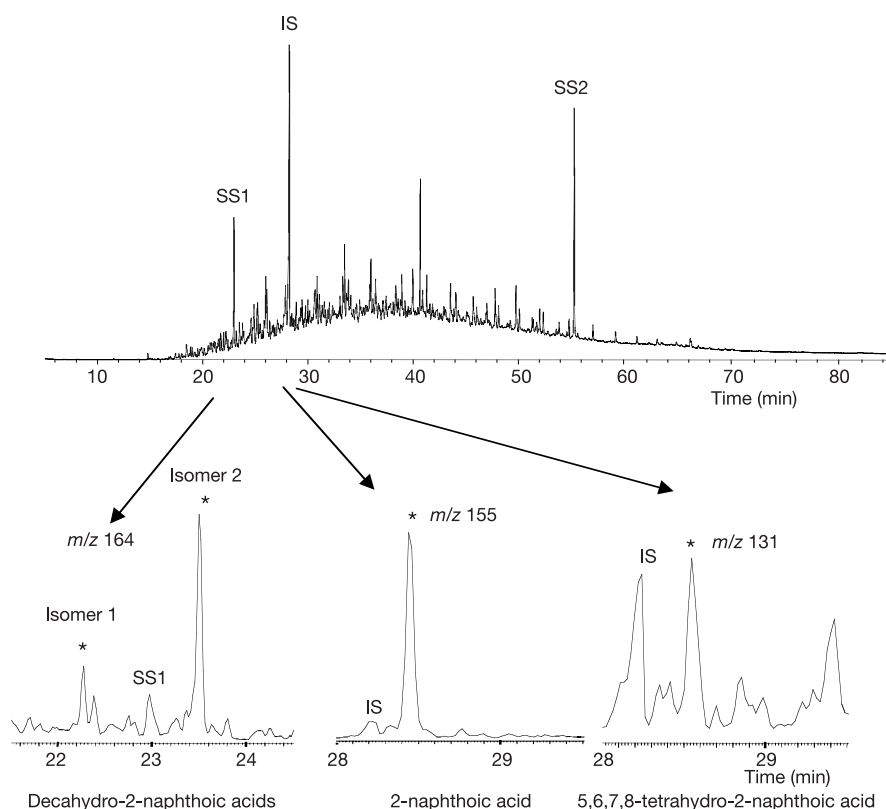


Figure 2 GC-MS data for the methylated acid fraction of a degraded oil. This shows the total ion chromatogram with recovery standard (SS) and internal standard (IS) peaks, and partial ion chromatograms for the metabolites 2-naphthoic acid (mass to charge ratio, m/z

$z = 155$); 5,6,7,8-tetrahydro-2-naphthoic acid ($m/z = 131$) and two isomers of decahydro-2-naphthoic acid ($m/z = 164$).

indeed have been anaerobic in nature at some point and indeed it seems probable that anaerobic hydrocarbon degradation is the prevailing mechanism for deep subsurface petroleum biodegradation, marking an important step in the understanding of reservoir degradation conditions. □

Methods

Total hydrocarbon fractions from crude oils were obtained by silica gel chromatography using hexane as eluent and were analysed by gas chromatography and gas chromatography-mass spectrometry (GC-MS) to provide hydrocarbon distribution patterns that provided information on the extent of biodegradation that had been suffered by the oil, as described by the scale of 0–10 proposed by ref. 25.

Acid fractions of crude oil samples were obtained using the method described in ref. 30. Briefly, ~150 mg of oil was loaded onto a strong anion exchange (SAX) quaternary amine solid phase extraction (SPE) column, and after removal of non-acid compounds, the crude carboxylic acid isolate was eluted with diethyl ether containing 2% (v/v) formic acid. This fraction was methylated with an ethereal solution of diazomethane, and cleaned up by eluting through a silica SPE column with a mixture of *n*-hexane and dichloromethane. 1-Adamantane carboxylic acid (Fluka) and 5 β cholanic acid (Sigma) were used as extraction efficiency standards and the methyl ester of 1-phenyl-1-cyclohexane carboxylic acid was used as an internal standard. Reference standards were 2-naphthoic acid (Lancaster Synthesis), 5,6,7,8-tetrahydro-2-naphthoic acid and decahydro-2-naphthoic acid. 5,6,7,8-Tetrahydro-2-naphthoic acid and decahydro-2-naphthoic acid were not commercially available and were therefore synthesized by the catalytic hydrogenation of 2-naphthoic acid using molecular hydrogen and a palladium/graphite catalyst; the identities of these compounds were confirmed by nuclear magnetic resonance spectroscopy (NMR) and by comparison of the standards' mass spectra with those in the literature.

GS-MS analysis of carboxylic acid methyl esters was performed in full scan mode on a Hewlett Packard 6890 gas chromatograph linked to a HP 5973 MSD with some confirmatory analyses carried out using a Varian 1200 GC-MS-MS system. 2-Naphthoic acid, 5,6,7,8-tetrahydro-2-naphthoic acid and decahydro-2-naphthoic acids were quantified by comparison of their peak areas in the respective *m/z* (mass-to-charge ratio) 155, 131 and 164 mass chromatograms with the peak area of the internal standard in the *m/z* = 159 mass chromatogram. Response factors of the analyte compounds were assumed to be unity, while the response factors of the surrogate standards were calculated and corrected for. Detection limits for naphthoic and reduced naphthoic acids were 0.01 p.p.m.

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1. Connan, J. in *Advances in Petroleum Geochemistry* (eds Brooks, J. & Welte, D. H.) Vol. 1, 299–335 (Academic, London, 1984).
2. Atlas, R. M. & Bartha, R. Hydrocarbon biodegradation and oil-spill remediation. *Adv. Microb. Ecol.* **12**, 287–338 (1992).
3. Palmer, S. E. in *Organic Geochemistry* (eds Macko, S. A. & Engel, M. H.) 511–534 (Plenum, New York, 1993).
4. Head, I. M., Jones, D. M. & Larter, S. R. Biological activity in the deep subsurface and the origin of heavy oil. *Nature* **426**, 344–352 (2003).
5. Magot, M., Ollivier, B. & Patel, B. K. C. Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **77**, 103–116 (2000).
6. Boll, M., Fuchs, G. & Heider, J. Anaerobic oxidation of aromatic compounds and hydrocarbons. *Curr. Opin. Chem. Biol.* **6**, 604–611 (2002).
7. Widdel, F. & Rabus, R. Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Curr. Opin. Biotechnol.* **12**, 259–276 (2001).
8. Röling, W. F. M., Head, I. M. & Larter, S. R. The microbiology of hydrocarbon degradation in subsurface petroleum reservoirs: perspectives and prospects. *Res. Microbiol.* **154**, 321–328 (2003).
9. Evans, C. R., Rogers, M. A. & Bailey, N. J. L. Evolution and alteration of petroleum in Western Canada. *Chem. Geol.* **8**, 147–170 (1971).
10. Horstad, I., Larter, S. R. & Mills, N. A quantitative model of biological petroleum degradation within the Brent Group reservoir in the Gullfaks field, Norwegian North Sea. *Org. Geochem.* **19**, 107–117 (1992).
11. Larter, S. R. *et al.* The controls on the composition of biodegraded oils in the deep subsurface. Part 1: biodegradation rates in petroleum reservoirs. *Org. Geochem.* **34**, 601–613 (2003).
12. Bastin, E. S. The presence of sulphate reducing bacteria in oil field waters. *Science* **63**, 21–24 (1926).
13. Kartsev, A. A., Tabasarsanskii, Z. A., Subbota, M. I. & Mogilevskii, G. A. *Geochemical Methods of Prospecting and Exploration for Petroleum and Natural Gas* (English translation edited by Witherspoon, P. A. & Romey, W. D.) (Univ. California Press, Berkeley, California, 1959).
14. Zengler, K., Richnow, H. H., Rosselló-Mora, R., Michaelis, W. & Widdel, F. Methane formation from long-chain alkanes by anaerobic microorganisms. *Nature* **401**, 266–269 (1999).
15. Rueter, P. *et al.* Anaerobic oxidation of hydrocarbons in crude oil by new types of sulfate-reducing bacteria. *Nature* **372**, 455–458 (1994).
16. Magot, M., Connan, J. & Crolet, J.-L. Les bactéries des gisements pétroliers. *La Recherche* **25** (268), 936–937 (1994).
17. Bennett, P. C., Siegel, D. E., Baedecker, M. J. & Hult, M. F. Crude oil in a shallow sand and gravel aquifer. I. Hydrogeology and inorganic geochemistry. *Appl. Geochem.* **8**, 529–549 (1993).
18. L'Haridon, S., Reysenbach, A.-L., Glénat, P., Prieur, D. & Jeanthon, C. Hot subtterranean biosphere in a continental oil reservoir. *Nature* **377**, 223–224 (1995).
19. Elshahed, M. J., Gieg, L. M., McClurey, M. J. & Suflija, J. M. Signature metabolites attesting to the *in situ* attenuation of alkylbenzenes in anaerobic environments. *Environ. Sci. Technol.* **35**, 682–689 (2001).
20. Rios-Hernandez, L. A., Gieg, L. M. & Suflija, J. M. Biodegradation of an alicyclic hydrocarbon by a sulfate-reducing enrichment from a gas condensate-contaminated aquifer. *Appl. Environ. Microbiol.* **69**, 434–443 (2003).
21. Gieg, L. M. & Suflija, J. M. Detection of anaerobic metabolites of saturated and aromatic

- hydrocarbons in petroleum-contaminated aquifers. *Environ. Sci. Technol.* **36**, 3755–3762 (2002).
22. Zhang, X., Sullivan, E. R. & Young, L. Y. Evidence for aromatic ring reduction in the biodegradation pathway of carboxylated naphthalene by a sulfate reducing consortium. *Biodegradation* **11**, 117–124 (2000).
23. Annweiler, E., Michaelis, W. & Meckenstock, R. U. Identical ring cleavage products during anaerobic degradation of naphthalene, 2-methylnaphthalene, and tetralin indicate a new metabolic pathway. *Appl. Environ. Microbiol.* **68**, 852–858 (2002).
24. Phelps, C. D., Battistelli, J. & Young, L. Y. Metabolic biomarkers for monitoring anaerobic naphthalene biodegradation *in situ*. *Environ. Microbiol.* **4**, 532–537 (2002).
25. Peters, K. E. & Moldowan, J. M. *The Biomarker Guide* (Prentice Hall, New York, 1993).
26. Aitken, C. M. *Identification of Non-Hydrocarbon Metabolites of Deep Subsurface Anaerobic Petroleum Hydrocarbon Biodegradation* PhD thesis, Univ. Newcastle-upon-Tyne, UK (2004).
27. Watson, J. S. *Hydrocarbon and Carboxylic Acid Compositions of Crude Oil Biodegraded in Marine Systems* PhD thesis, Univ. Newcastle-upon-Tyne, UK (1999).
28. Van der Linden, A. C. & Thijssse, G. J. E. The mechanisms of microbial oxidations of petroleum hydrocarbons. *Adv. Enzymol.* **27**, 469–546 (1965).
29. Seewald, J. S. Organic-inorganic interactions in petroleum-producing sedimentary basins. *Nature* **426**, 327–333 (2003).
30. Jones, D. M., Watson, J. S., Meredith, W., Chen, M. & Bennett, B. Determination of naphthenic acids in crude oils using non-aqueous ion exchange solid-phase extraction. *Anal. Chem.* **73**, 703–707 (2001).

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Complex organic chemical balms of Pharaonic animal mummies

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Millions of votive mummies of mammals, birds and reptiles were produced throughout ancient Egypt, with their popularity increasing during the reign of Amenhotep III (1400 BC) and thereafter. The scale of production has been taken to indicate that relatively little care and expense was involved in their preparation compared with human mummies^{1–3}. The accepted view is that animals were merely wrapped in coarse linen bandages and/or dipped in 'resin' before death^{2–4}. However, as with human mummification there was a range of qualities of treatments, and visual inspection of animal mummies suggests that the procedures used were often as complex as those used in humans (for example, evisceration and elaborate bandaging). Moreover, the ancient Egyptians treated animals with great respect, regarding them both as domestic pets and representatives of the gods; for example, the cat symbolized the goddess Bastet; the hawk, Horus; the ibis, Thoth, and so on. We report here the results of chemical investigations of tissues and wrappings from Pharaonic cat, hawk and ibis mummies using gas chromatography, gas chromatography–mass spectrometry, thermal desorption–gas chromatography–mass spectrometry and pyrolysis–gas chromatography–mass spectrometry^{5,6}. The analyses reveal the presence of highly complex mixtures of