Biological activity in the deep subsurface and the origin of heavy oil

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At temperatures up to about 80 °C, petroleum in subsurface reservoirs is often biologically degraded, over geological timescales, by microorganisms that destroy hydrocarbons and other components to produce altered, denser 'heavy oils'. This temperature threshold for hydrocarbon biodegradation might represent the maximum temperature boundary for life in the deep nutrient-depleted Earth. Most of the world's oil was biodegraded under anaerobic conditions, with methane, a valuable commodity, often being a major by-product, which suggests alternative approaches to recovering the world's vast heavy oil resource that otherwise will remain largely unproduced.

Biodegraded oils dominate the world petroleum inventory[1], with the largest oil reserves being found, not in the Middle East, but on the flanks of foreland basins in the Americas (Box 1 Fig. 1). Biodegraded oils also represent a significant fraction of the petroleum in conventional oil reserves and will be common among future oil discoveries likely to be made in deep-water areas of the world (for example, the Atlantic margin basins of Africa, South America, Canada and the Gulf of Mexico). In these areas, low thermal gradients and shallow exploration targets combine to produce many petroleum reservoirs cooler than 80 °C, which is an ideal environment for microbial degradation of crude oil and natural gas[2].

The presence and role of microorganisms in deep subsurface sediments have been the subject of much interest recently (Box 2), despite the fact that geologists have had evidence of active microbial communities in petroleum reservoirs since the 1930s[3,4]. Plate-tectonics, basin and petroleum-system formation are vital processes that affect deep subsurface biological systems. They control the formation and distribution of organic carbon, the delivery of nutrients and oxidants that can be used by microorganisms in the subsurface, and alter the physical environment that they inhabit. Plate tectonics, basin and petroleum-system formation are thus integral to the behaviour of the deep biosphere, and interactions between the biosphere and the geosphere in petroleum reservoirs provide a unique portal into the operation of life in Earth. Here, we summarize current knowledge of the composition of heavily biodegraded oils, factors that dictate their composition, and how the interplay of geological and biological phenomena influence the occurrence of biodegraded petroleum systems in the deep subsurface.

Effects of biodegradation on petroleum properties

Compositional changes, such as sequential and systematic removal of various hydrocarbons and other compounds, selective degradation of specific isomers within individual compound classes and the production of acidic compounds, seen in shallow petroleum reservoirs, are similar to those seen in surface oil seeps and laboratory biodegradation experiments. These changes are distinct from alterations caused by physical processes, such as water washing or phase fractionation, and suggest biological alteration of oils *in situ*[2,5–14].

The effects of biodegradation on the composition and physical properties of crude oil and natural gases are well known. Oxidation of oil (C_{6+} components) during biodegradation leads to a decrease in saturated hydrocarbon content (and to a smaller decrease in aromatic hydrocarbon content) and API gravity, a measure that correlates with economic value, whereas oil density, sulphur content, acidity, viscosity and metal content increase[2,8,9,11,13,15,16] (Fig. 1), which negatively affects oil production (by reducing well-flow rates) and refining operations, and reduces oil value.

Hydrocarbons are preferentially destroyed during biodegradation, but sulphur-, oxygen- and nitrogen-containing compounds can also be degraded[17–19]. New compounds such as acyclic and cyclic, saturated and aromatic carboxylic acids and phenols are produced from hydrocarbons[11,12,20], and a complex variety of acidic non-hydrocarbons is generated from the aromatic heterocycles found in oil[21]. The heterocyclic acids with multiple, different heteroatoms are the main cause of corrosion problems during processing of heavy, degraded oils[22].

Light hydrocarbons and gases (C2–C5 hydrocarbons) can also be biodegraded[23–25]; propane is degraded most rapidly, and *n*-butane is more susceptible to degradation than iso-butane (Fig. 1). Biodegradation of C2–C5 hydrocarbons increases the methane content, and this, with the reduction in oil alkane content during biodegradation, reduces the ability of the oil to dissolve gas, reducing the gas:oil ratio of any trapped oil leg (the oil-saturated part of the reservoir)[13] (Fig. 1). This may result in production of a gas cap enriched in methane following removal of wet gas components (C2–C5 alkanes) and probably also by direct production of methane during biodegradation. Large dry gas caps associated with residual biodegraded oil are common[24,26] (S. L. and Di Primio, unpublished data), and probably result directly from oil biodegradation. There is little direct evidence of methane oxidation during petroleum biodegradation *in situ,* but we recently recovered archaeal 16S rRNA gene sequences characteristic of anaerobic methane oxidizers from biodegraded oil reservoirs, suggesting that anaerobic methane oxidation occurs at some point during the subsurface degradation process.

All oils are mixtures

The composition of oil in reservoirs is the result of the processes occurring during petroleum generation, migration, trapping and subsequent alteration. Petroleum gener-

Box 1

The mothers of all petroleum systems

The world's oil reserves are dominated by biodegraded heavy and super-heavy oils in the super-giant tar sands common in shallow reservoirs on the flanks of foreland basins in North and South America and elsewhere.

Oils are classified for economic value according to API gravity, based on a surface measurement of the specific gravity of degassed oil[16]. Heavy oils have API gravities of 20 or less, super-heavy oils have API gravities of 10 or less, and a typical light marine non-biodegraded oil has an API gravity around 36–38 API. Tar sands are sandstones saturated with heavy or super-heavy oil: the oils in the Canadian and Venezuelan tar sands have API gravities of 6–12. The vast majority of heavy oils result from microbial alteration of oils in the reservoir[1,15,16,37,84,85], with over 50% of the Earth's oil inventory occurring as biodegraded oils in heavy oil and tar sand accumulations[1]. The largest single accumulations are the supergiant deposits of tar sands trapped on the flanks of the Alberta (Canada) and Eastern Venezuelan (Venezuela) foreland basins[1,84]. The Orinoco heavy oil belt in Venezuela has the largest known petroleum accumulation in the world (1,200 billion barrels — one barrel is 0.159 m³), with the Athabasca accumulation in Canada in second place (nearly 900 billion barrels). These accumulations dwarf the largest light oil fields such as Ghawar (Saudi Arabia) and Burghan (Kuwait), which contain a measly 190 billion barrels each[1].

Foreland basins are extensive (up to thousands of kilometres long, hundreds of kilometres wide), elongated basins, which are formed as a result of isostatic down warping of the crust, owing to the tectonic load imposed during a mountain-building (orogenic) event. When source rocks are present in the older platform sequences or in the foreland basins sediments themselves, these basins develop enormous large elongated oil source kitchens, which produce vast volumes of oil. This oil migrates laterally up the sloping strata from the source rocks for up to several hundred kilometres to structural and stratigraphic traps at the margins of the basin[84–87]. Viable petroleum traps occur throughout foreland basins, and light oils may be trapped along the migration fairway, in reservoirs found deeper in the basin. At the flanks of the basins, reservoir sediments (typically sandstones) lay onto older basement rocks and produce reservoirs that are shallow, cool and may have local active meteoric water circulation[59], conditions ideal for biological activity. The formation of foreland basin petroleum systems feeds the deep biosphere at the basin flanks, where stratigraphic traps may accumulate vast volumes of biodegraded heavy oil trapped significantly by immobility owing to the high density and viscosity resulting from severe biodegradation (Box 1 Fig. 1).

In the case of the Western Canada Basin the Lower Cretaceous Mannville Group sandstones onlap onto subcropping Palaeozoic carbonates, which together contain in excess of 1,700 billion barrels of severely biodegraded heavy oils of 8–12 API in the Athabasca, Cold Lake, Wabasca and Peace River accumulations, representing over 97% of the Western Canada Basin oil reserves[85]. More than 36% of Canadian oil production is currently derived from this resource[59] either by mining the near-surface deposits or by heating the oil by steam injection to reduce the viscosity and produce the oil through boreholes[59,88].

The source of the oils is controversial, although it is agreed they represent biodegraded mature marine oils charged from pre-Cretaceous source rocks during the development of the Rocky Mountain Fold and Thrust Belt (Laramide orogeny) in Late Cretaceous–Early Tertiary time[85,86,89,90]. The Eastern Venezuelan Basin contains the 460 km by 40 km wide Orinoco Tar Belt, containing 1,200 billion barrels of severely degraded oil found predominantly in stratigraphic traps formed by onlap of Miocene sandstones onto the Guayana shield basement. Reservoirs were charged in the Neogene from Upper Cretaceous marine source rocks from up to 200 km away in the foreland basin depocentre created by formation of the Interior range[87]. These vast foreland basin petroleum generation, accumulation and alteration engines show graphically the dynamic interplay of tectonics, petroleum geology and and active biosphere.
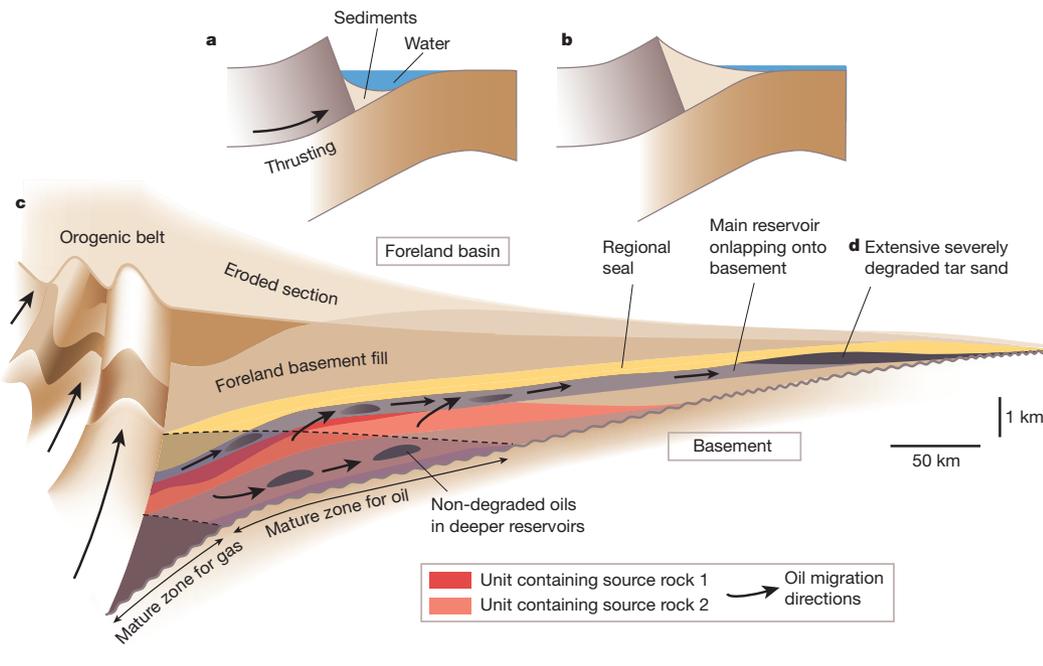
Box 1 Figure 1 Foreland basin petroleum systems are major heavy oil provinces. An idealized foreland basin petroleum system with source rocks in the platform sediments, a regionally extensive reservoir sandstone sequence and a regionally extensive seal. Mature oil source rocks in the basin depocentre charge the flank traps where the oils biodegrade and tar sands accumulate. **a**, **b**, The foreland basin forms during the period of active deformation and subsequently buries the source rocks present in the pre-foreland basin passive margin sequences and any source rocks formed in the foreland basin itself into the oil window (temperatures of ~100–150 °C). **c**, Oil migrates to the shallow cool basin flanks over distances of several hundred kilometres. Biodegradation of the oil occurs, increasing viscosities and helping to trap the degraded oil.

ation is the rate-determining step in charging reservoirs with new oil[27], and source rocks typically charge petroleum traps for timescales of the order of a few million to tens of millions of years. During this period, the petroleum expelled from the source(s) and subsequently trapped shows a progressive change in composition. This change results from alterations in the composition of the oil from the source rock(s) over time, temporal differences in migration-related phase fractionation effects and mixing of oils in the trap. Mixing of fresh and degraded oils dominates the composition and physical properties of biodegraded oils[24,28–30], rendering geochemical schemes that rank the level of biodegradation of oil unachievable. Nevertheless several utilitarian schemes have been proposed that have had wide and successful application in petroleum exploration[2,8,13,31–33], with the schemes of Volkman *et al.* (ref. 8) and Peters and Moldowan (ref. 32) being the standard descriptions of the extent of biodegradation of produced oil. These schemes are based heavily on alteration of biomarker compounds; however, much of the commercially significant changes in oil properties occur before significant alteration of cyclic biomarker hydrocarbons takes place. The more pragmatic scale of Wenger *et al.* (ref. 13) defines five broad levels (from slight to severe) of biodegradation and is used as the framework for Fig. 1.

Quantitative evaluation of oil biodegradation

All biodegraded oils are mixtures; therefore, estimating the fraction of oil lost through degradation is not simple. The most obvious biodegradation-resistant tracers of original oil mass, such as oil vanadium or nickel content, vary in concentration by orders of magnitude as oils are generated through the oil-generation window (the temperature range over which oil is generated and expelled from source rocks). Thus variations in oil–metal content through biodegradation may not accurately indicate oil mass lost during degradation when maturity of the oil charge varies. In an attempt to link qualitative indicators of oil biodegradation (for example, the Peters and Moldowan scale; Fig. 1) with quantitative estimates of the mass of oil destroyed, data on bulk oil composition and variations in the concentration of degradation-resistant compounds in oil (where little variation in the maturity of the charged oil has occurred) have been analysed. These data, used with oil-charging biodegradation models[29], suggest that oils exhibiting heavy levels of biodegradation (Fig. 1) have typically lost up to 50% of their mass of C_{6+} components. Beyond this level of degradation, loss of oil mass from marine oils is less significant (only a further ~10–20% of original oil mass is lost), and it seems likely that structural rearrangements dominate subsequent changes in oil composition. The extent to which degradation is resistant to microbial necromass addition to oil during biodegradation is unclear, but it seems likely that addition of microbial necromass occurs at some level.

Controls on biodegradation

Most petroleum (oil and gas) is trapped in reservoirs in porous and permeable sediments such as sandstones and limestones. Typically, in the oil leg, oil, as the continuous fluid phase, accounts for ~80% of the pore space, with discontinuous water filling the rest. Below the oil leg in the water-saturated part of the reservoir (the water leg), 100% of the pore space is saturated with waters of variable salinity (Fig. 2). Free water is essential for biological activity, and biodegradation of oil could, in principle, occur throughout a reservoir. Biodegradation could also occur during early reservoir filling, when many oil–water contacts exist[27]. Significantly, however, degradation-related compositional gradients are seen in many oilfields (Fig. 2), with the most-degraded oil near oil–water contacts[29], or highly degraded residual oil[24]. This suggests that the site of most biodegradation is at the base of the oil column. The oil–water contact provides conditions that are the most conducive to microbial activity. Transport of hydrocarbons from the oil column will provide a plentiful supply of electron donors, whereas inorganic nutrients required for microbial growth

can, in principle, be transported by water flow or diffusion in the water column to the biosphere at the oil–water contact. This notion is supported by our observation that 16S rRNA genes from bacteria and archaea are more readily detected in sediment samples from the vicinity of the oil–water contact.

Large-scale tectonic processes

Biodegraded oils are found at depths of up to about ~4 km (ref. 2), with most biodegraded reservoirs at up to 2.5 km below the sediment surface. Deep subsurface petroleum reservoirs are characterized by high temperatures, with the temperature increasing ~2–3 °C per 100 m depth. The probability of observing oil biodegraded to a given extent has been noted empirically to increase with decreasing reservoir temperatures below about 80 °C (refs 2, 15, 34, 35), with the sensitivity of degradation to temperature seemingly independent of oil type[34]. Thus the probability of finding heavily degraded oils (level 5 on the Peters and Moldowan biodegradation scale; Fig. 1) is close to 0 for reservoirs near 80 °C, whereas for reservoirs near 50 °C, 70% of reservoirs might typically contain heavily degraded oil[34]. Thus significant biodegradation decreases with increasing reservoir temperature and essentially ceases around 80 °C. Biodegraded oils are only rarely found in currently subsiding reservoirs above 80 °C, which suggests that most biodegraded oils in reservoirs in these basins at lower temperatures have been charged and degraded recently, close to their present depths of burial[29].

Not all low-temperature reservoirs contain degraded petroleum. This may be because either they have been recently charged with fresh oil or they have been uplifted from deeper, hotter subsurface regions. Wilhelms *et al.* proposed a 'palaeopasteurization' model in which these petroleum reservoirs were pasteurized at 80–90 °C during deep burial[35], inactivating any hydrocarbon-degrading microorganisms present, before the main oil charge and subsequent uplift of the reservoir to cooler conditions (Fig. 3). This implies an important role for large-scale geological processes in shaping the extent of the deep subsurface biosphere. An important implication of this model is that following uplift, recharge of fluids and/or microorganisms from the surface and migration of microorganisms into pasteurized deep subsurface petroleum reservoirs are insignificant. This also implies that in biodegraded/biodegrading petroleum reservoirs in currently subsiding basins cooler than 80 °C, microorganisms were already present during burial, having survived and evolved since reservoir deposition. Even in coarse-grained sediments deposited under oxygenated surface conditions, oxygen is rapidly consumed as a result of aerobic organic matter mineralization. Most shallow subsurface sediments therefore become anoxic with depth, and anaerobic microbial communities dominate most shallow subsurface sediments. The consumption of labile organic matter leads to sediments that are dominated by residual recalcitrant organic carbon harbouring an anaerobic microbial community. During burial of such sediments and formation of potential reservoir rocks, acetate may be produced biogenically from more recalcitrant organic matter with increasing depth and temperature[36]. This may sustain an indigenous heterotrophic anaerobic microbial community until oil arrives in the reservoir. We conjecture that the base of oil biodegradation effectively marks the base of heterotrophic microbial activity in the Earth's crust.

Sustaining petroleum degradation: oxidant sources

Aerobic biodegradation of hydrocarbons dominated thinking about the mechanisms of subsurface oil biodegradation for many years[15,16,37], despite conservative estimates of the water volumes needed to transport enough oxygen presenting overwhelming problems geologically in most petroleum reservoirs[38]. In our experience, deep biodegraded oilfields in marine basins often contain saline water, indicating minimal flushing of deep aquifers with fresh water, and in the North Sea, reservoir water salinities in the largest biodegraded petroleum accumulation in the basin, the Troll field, are equal to or
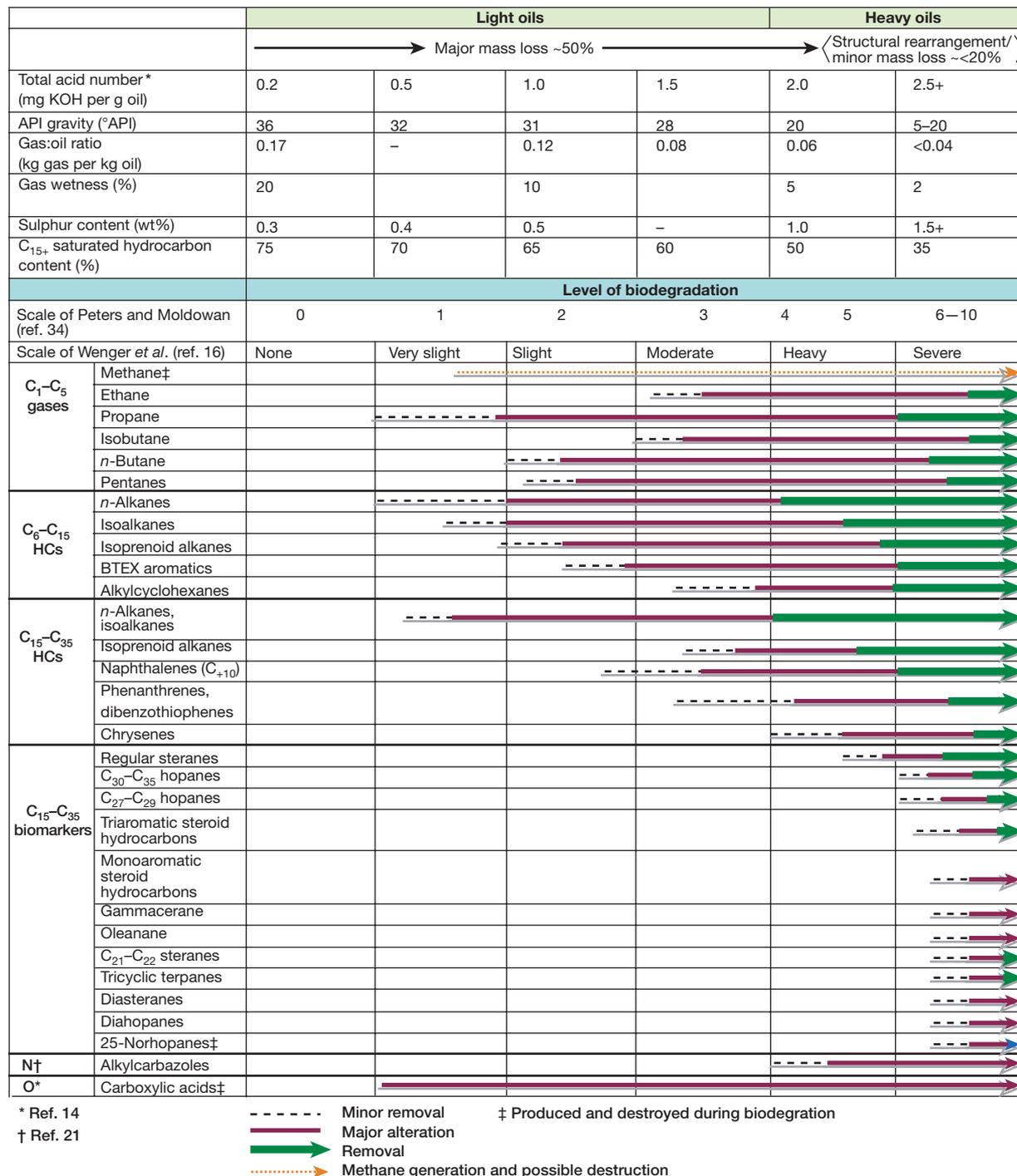
	Light oils				Heavy oils	
	⟶ Major mass loss ~50% ⟶				⟨Structural rearrangement/ minor mass loss ~<20%⟩	
Total acid number* (mg KOH per g oil)	0.2	0.5	1.0	1.5	2.0	2.5+
API gravity (°API)	36	32	31	28	20	5–20
Gas:oil ratio (kg gas per kg oil)	0.17	–	0.12	0.08	0.06	<0.04
Gas wetness (%)	20		10		5	2
Sulphur content (wt%)	0.3	0.4	0.5	–	1.0	1.5+
C_{15+} saturated hydrocarbon content (%)	75	70	65	60	50	35

	Level of biodegradation					
Scale of Peters and Moldowan (ref. 34)	0	1	2	3	4 5	6—10
Scale of Wenger et al. (ref. 16)	None	Very slight	Slight	Moderate	Heavy	Severe

C_1–C_5 gases: Methane‡, Ethane, Propane, Isobutane, n-Butane, Pentanes

C_6–C_{15} HCs: n-Alkanes, Isoalkanes, Isoprenoid alkanes, BTEX aromatics, Alkylcyclohexanes

C_{15}–C_{35} HCs: n-Alkanes, isoalkanes; Isoprenoid alkanes; Naphthalenes (C_{+10}); Phenanthrenes, dibenzothiophenes; Chrysenes

C_{15}–C_{35} biomarkers: Regular steranes; C_{30}–C_{35} hopanes; C_{27}–C_{29} hopanes; Triaromatic steroid hydrocarbons; Monoaromatic steroid hydrocarbons; Gammacerane; Oleanane; C_{21}–C_{22} steranes; Tricyclic terpanes; Diasteranes; Diahopanes; 25-Norhopanes‡

N†: Alkylcarbazoles
O*: Carboxylic acids‡

* Ref. 14
† Ref. 21

- – – – – Minor removal
- —— Major alteration
- ⟶ (green) Removal
- ······▸ Methane generation and possible destruction
- ‡ Produced and destroyed during biodegration

Figure 1 Schematic diagram of physical and chemical changes occurring during crude oil and natural gas biodegradation. The general sequence of removal of saturated hydrocarbon types during biodegradation is n-alkanes, alkylcyclohexanes, acyclic isoprenoid alkanes, bicyclic alkanes–steranes–hopanes[7], with some production of new hydrocarbons, such as 17α,25-norhopanes from demethylation of hopanes at advanced levels of degradation[32,33]. Similarly, for aromatic hydrocarbons, alkylbenzenes are removed before diaromatic and triaromatic hydrocarbons[8], with aromatic steroid hydrocarbons being resistant until very severe levels of biodegradation are achieved[32]. With both alkanes and aromatic hydrocarbons, stereochemistry and the position of alkyl substituents confound such simplistic scenarios, and there is frequently much overlap and synchronous removal of the various compound types. Removal of gas chromatography (GC)-resolvable components during degradation contributes to the production of the 'humps' of GC-unresolvable hydrocarbon mixtures that are typically found in degraded oils[91]. However, even when biodegradation levels are slight, mass balance calculations suggest that non-GC-resolvable components are also degraded. The indicative compositional and physical property changes with biodegradation assume that it starts with a light, low-sulphur undegraded oil. The lower diagram of compositional change is modified from Wenger et al. (ref. 13). Pervasive oil mixing and synchronous removal of several compound types result in the degradation scales being indicative rather than being a precise consistent form.
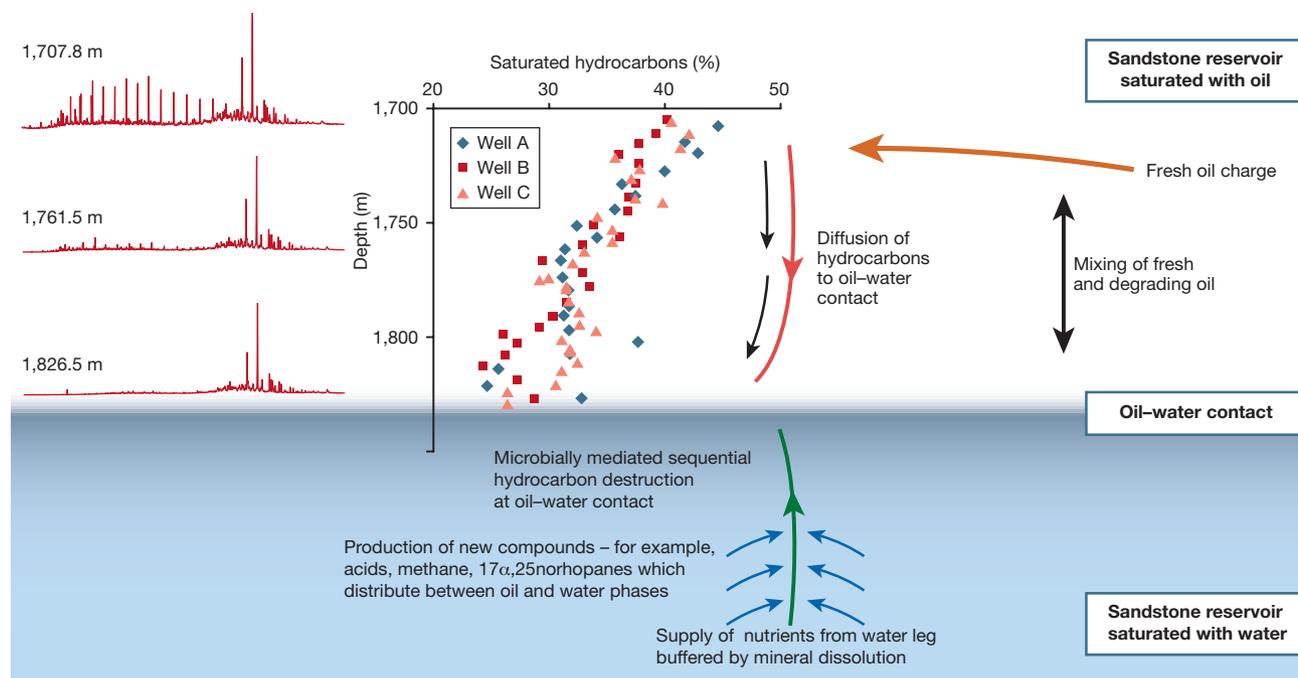
Figure 2 Saturated hydrocarbon contents and gas chromatograms of petroleum extracted from reservoir cores show a progressive increase in biodegradation in three wells from a Chinese oilfield[19]. Hydrocarbons diffuse towards the oil–water contact, where they are degraded by microorganisms living near the oil–water contact using nutrients derived from the water-saturated zone below the oil column. Nutrients such as phosphorus are probably buffered by mineral dissolution reactions[72]. Fresh oil is charged to the reservoir at the same time that degradation occurs. Compositional gradients reflect this complex charge and degradation scenario.

greater than sea water[39]. Recent microbiological evidence from our group and others suggests, even when reservoirs are shallow (< 500 m) and contain fresh water, that the microbial flora is anaerobic. There is strong evidence for the widespread occurrence of obligate anaerobes in subsurface petroleum systems[40–42] (Box 3).

Flushing of meteoric water in itself does not indicate that highly reactive oxygen survives transport to deep reservoirs, past reactive organic matter and minerals, because even small concentrations of organic compounds can swiftly remove oxygen from an aquifer. It is therefore almost certain that oil biodegradation in deep subsurface petroleum reservoirs proceeds through anaerobic microbial metabolism rather than through aerobic mechanisms. This was proposed many years ago[43,44], but was not widely accepted in the face of overwhelming evidence that hydrocarbon degradation only occurred rapidly under oxic conditions. The first bacteria isolated from oilfield waters were anaerobes[3], and the widespread occurrence of anaerobic bacteria and archaea in subsurface petroleum systems is supported by many more recent studies[41,42,44–46] (Box 3). In addition, viable anaerobic hydrocarbon degradation processes have recently been established for both saturated and aromatic hydrocarbons[47–49] (Fig. 4), and metabolites characteristic of anaerobic hydrocarbon degradation such as reduced 2-naphthoic acids[50] have been identified in most biodegraded oil reservoirs that we have examined, including the Canadian tar sands[51]. Although anaerobic bacteria have been widely reported in petroleum reservoirs, none of these are anaerobic hydrocarbon degraders, and only one thermophilic anaerobic hydrocarbon degrader potentially capable of living in the deepest biodegraded reservoirs has been identified[52]. It does seems certain, however, that hydrocarbon degraders inhabit deep reservoirs. The subsurface petroleum reservoir biosphere awaits the explorers of the twenty-first century.

Slow anaerobic processes dominate the deep subsurface, with subsurface hydrocarbon degradation probably linked to iron reduction and methanogenesis[10,47,49,53] or, in petroleum reservoirs where free sulphate is abundant, to microbial sulphate reduction[54]. The high hydrogen sulphide concentrations produced may ultimately stop biodegradation[14]. Methanogenesis, an exclusively anaerobic process, appears to be commonly associated with biodegraded petroleum reservoirs that are free of abundant sulphate, and isotopically lighter methane is frequently found mixed with thermogenic methane[25,26,30,55–57]. Methanogenesis is thought to have contributed to the gas associated with the Canadian tar sand deposits[58,59], and similar processes have been proposed for biogenic methane generation in coals[55,57].

The carbon isotopic composition $\delta^{13}C$ of methane associated with subsurface oil biodegradation conditions frequently seems to be in the range of −45‰ to −55‰, but the carbon dioxide associated with biodegraded oils shows a wide range and is, in contrast, often isotopically heavy ($\delta^{13}C$ up to +15‰), which is indicative of almost complete closed-system reduction of carbon dioxide to methane[25,26,30] (Fig 4).

Methanogens are probably indigenous members of petroleum reservoir microflora[45,60,61]. The methanogens described so far are those that reduce carbon dioxide to methane, although there are a few reports of acetoclastic methanogens from petroleum reservoirs[62]. Radiotracer experiments indicate that carbon dioxide reduction to methane is more prevalent than acetoclastic methanogenesis[63], and the high pressures in petroleum reservoirs would be expected to favour net-volume-reducing reactions such as methanogenesis from carbon dioxide reduction rather than from acetate. Methanogenesis is a likely fate for most carbon dioxide produced during oil biodegradation in the absence of abundant sulphate, as little carbonate cementation is seen in many biodegraded oilfields despite large fractions of the petroleum carbon being oxidized. Carbon dioxide contents of biodegraded petroleum reservoirs are generally well below 10% by volume of the produced gas phase and are often not much higher than nearby non-degraded oilfields, which typically contain only a few mol % of carbon dioxide. Although hydrogen for initial

methanogenesis is probably derived from water[47], secondary reduction of the remaining carbon dioxide requires an additional source of hydrogen. This may be supplied from depth by mineral hydrolysis[64], maturation of organic matter or even from the aromatization of alicyclic or naphthenoaromatic compounds present in the oil. The petroleum biosphere might have many of the characteristics of other hydrogenotrophic subsurface biota[65]. We speculate that methanogenesis through carbon dioxide reduction may be a dominant terminal process in petroleum biodegradation in the subsurface[42] (Fig. 4) and is probably active today in the giant Troll field in the North Sea[26].

Water itself is a likely reactant for the production of methane, carbon dioxide and free energy during hydrocarbon degradation in deep anoxic settings. Thermodynamic and microbiological evidence support this[47,53,66], and water is of course geologically the most plausible universal reactant in petroleum reservoirs. Oil plus water producing life — and a little gas (Fig. 4).

Sustaining petroleum degradation: inorganic nutrients

Although transport of oxygen to fuel hydrocarbon degradation in petroleum reservoirs seems unlikely, where water movement occurs it might enhance mineral dissolution and release nutrients such as phosphate, promoting microbial activity. Hydrocarbon degradation in surface environments is often limited by nitrogen or phosphorus, but nitrogen is unlikely to be limiting in petroleum reservoirs, because nitrogen in petroleum systems is as abundant as ammonium ions in water, and as dinitrogen gas and heterocyclic aromatic nitrogen compounds in petroleum[19,42,67,68]. Aromatic petroleum nitrogen compounds are degraded in heavily degraded oils[18,19], but it is likely that ammonium ions buffered by reservoir minerals[67] are the primary nitrogen source for the hydrocarbon-degrading biosphere in petroleum systems[68], with phosphorus much more likely to limit oil biodegradation in petroleum reservoirs. In pivotal studies, Bennett and co-workers[69–72] have shown a close relationship between the geomicrobiology of a petroleum-contaminated aquifer, mineral alteration and groundwater chemistry. On a large scale, biological activity perturbs general groundwater chemistry, and therefore mineral water equilibria, and on a small scale, attached organisms locally perturb mineral water equilibria, releasing limiting nutrients. In an oil-contaminated aquifer, feldspars weather exclusively near attached microorganisms in the anoxic region of the contaminant plume; indigenous bacteria colonize feldspars that contain potassium or trace phosphorus as apatite inclusions. Most of the phosphorus in sediments from clastic petroleum reservoirs is in feldspars[70], and feldspar dissolution in some oil reservoirs (for example, the Gullfaks field in the North Sea) may be related to biodegradation of the associated oils[73]. Indeed we think that supply of limiting nutrients (probably phosphorus) from mineral dissolution in reservoirs or encasing shales in many instances may be the rate-limiting step in subsurface petroleum biodegradation.

Rates of oil biodegradation in petroleum reservoirs

It has been suggested that significant oil biodegradation occurs naturally in a petroleum reservoir on a human timescale[74], and when sea water is injected as part of a production strategy, sulphate reduction may be stimulated[46]. Under subsurface conditions, however, biodegradation is usually a slow process. We have assessed biodegradation rates using oilfield charge models and observed oil compositions, diffusion-controlled oil column compositional gradients and by examining mixed degraded/non-degraded oils in oilfields and determining the net degradation rate in terms of the oil charge rate[29]. These three quite different approaches all estimate first-order biodegradation rate constants of between 10^{-6} to 10^{-7} yr^{-1} for hydrocarbons in reservoirs near 60–70 °C, if it is assumed that biodegradation takes place in the lowest 2% of the oil column. Considering global levels of biodegradation in oil reservoirs, we find that hydrocarbon destruction fluxes at the base of oil columns are of the order of 10^{-4} kg hydrocarbons m^{-2} yr^{-1} for reservoirs at 40–70 °C.

Our models suggest that biodegradation takes around 1–2 Myr to compositionally perturb an entire oil column of 100 m for lightly degraded oil reservoirs, and where we see continuous compositional gradients in oil columns related to biodegradation, degradation must have been occurring episodically for many millions of years (Fig. 2). It seems that to remove the n-alkanes from oil, around 5–15 Myr is needed, depending on the oil properties, and we estimate the Canadian tar sands could have been degraded to their current state in as little as ~35 Myr. The timescales of oilfield degradation and filling are thus very similar, and the degree of biodegradation and the physical properties of oil will be substantially controlled by oilfield charge history and mixing[29,75].

The oil biodegradation rate is not limited by electron-donor supply (that is, hydrocarbons) but by supply of nutrients or oxidants. This suggests that supply of nutrients or electron acceptors to the site
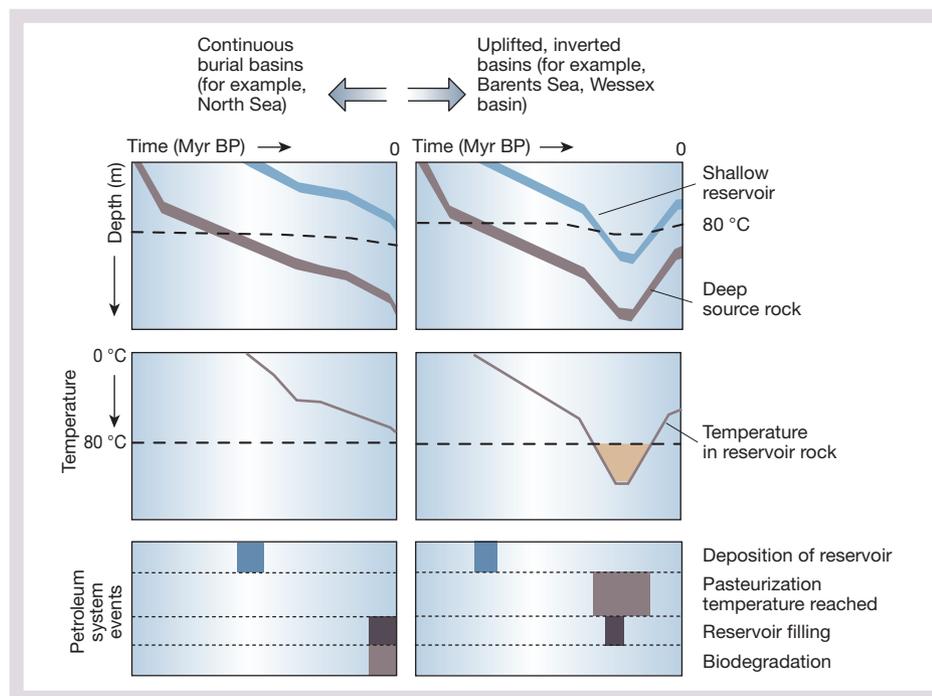

Figure 3 The palaeopasteurization model[35] of Wilhelms *et al.* compares continuously subsiding (for example, Viking Graben, North Sea) and uplifted sedimentary basins (for example, Barents Sea or Wessex Basin) and shows schematic burial history (top), reservoir temperature history (middle) and petroleum system (lower) events. This illustrates key differences with respect to biodegradation in petroleum reservoirs. Biodegradation of petroleum occurs only in sedimentary units that have not been exposed to temperatures exceeding 80 °C (palaeopasteurization) before oil charging. We speculate that this 'pasteurization temperature' may represent the effective maximum temperature boundary for most life in Earth struggling to survive over geological timescales in hot oligotrophic sediments.

Figure labels: Continuous burial basins (for example, North Sea); Uplifted, inverted basins (for example, Barents Sea, Wessex basin); Time (Myr BP); Depth (m); Shallow reservoir; 80 °C; Deep source rock; Temperature; 0 °C; 80 °C; Temperature in reservoir rock; Petroleum system events; Deposition of reservoir; Pasteurization temperature reached; Reservoir filling; Biodegradation

Box 2
The deep biosphere

Although bacteria putatively associated with deep subsurface environments were characterized in the first part of the twentieth century, it is only recently that the existence of a deep subsurface biosphere has been widely accepted in the scientific community. Scepticism about the deep subsurface biosphere stemmed largely from concerns about the provenance of organisms detected in samples taken from the subsurface, but with improved sampling techniques and methods to corroborate the authenticity of organisms recovered from deep within the Earth's crust many of these concerns have been dispelled.

Soils and surface sediments can harbour billions of prokaryotic cells per cubic centimetre, but their abundance drops off exponentially with increasing depth[82]. Nevertheless, some deep subsurface sediments still hold around 10^5 to 10^6 cells cm^{-3}, and where carbon and energy sources are abundant, numbers may increase at depth. Evidence suggests that a deep crustal biosphere beneath both land and sea has reached approximately 3 km below the Earth's surface, with oil biodegradation suggesting that this can be extended to at least 4 km. Although the number of cells per unit volume of deep subsurface sediments is relatively small, the vast extent of the sediments (estimated at 5×10^{25} cm^3) means that total

prokaryote cell numbers in all subsurface environments are of the order of 10^{30} (ref. 83). Although there are uncertainties in the estimates, and they are based on extrapolation from a small number of samples, deep subsurface microbial biomass may account for greater than 90% of global prokaryotic biomass, exceeding the values for all of the world's oceans and terrestrial environments by some margin.

Subsurface prokaryotic biomass may contain $3-5 \times 10^{17}$ g of carbon, representing 60–100% of the carbon present in global plant biomass. Prokaryotic cells typically contain a higher proportion of nitrogen and phosphorus than plant biomass (about 10 times more), and thus the contribution of deep crustal microorganisms to global nitrogen and phosphorus pools is extremely significant[83]. The deep biosphere is clearly of global significance simply in terms of key elemental budgets. However, the fact that many of the organisms present may be consuming and producing inorganic and organic compounds to generate energy and biomass extends their significance to the realms of biogeochemical processes. One such crucial process in the deep subsurface is the transformation of petroleum hydrocarbons to produce heavy oils by biodegradation.

of degradation is a major factor controlling the rate and extent of subsurface oil biodegradation, with diffusion of nutrients in the water leg probably being adequate. Biodegradation, like inorganic diagenesis in many petroleum reservoirs, may be isochemical[76], involving only mass transport of hydrocarbons, nutrients and oxidants largely within the reservoir[29]. The system may operate even if closed at the reservoir scale, and water flow may not be needed, although undoubtedly it would help mineral dissolution and nutrient supply. Consequently, reservoir topology will be an an important determinant of the rate and location of degradation. For example, in reservoirs filled by oil down to an under-seal (a shale for example) with limited water legs, the oil is frequently not extensively degraded, even when nearby oil columns with substantial water legs are heavily biodegraded. This may explain why degradation frequently stops before all reactive components are removed.

Hydrocarbon removal rates in biodegrading petroleum reservoirs

are of the order of 10^{-6} mmol oil l^{-1} d^{-1} in the degradation zone, which is comparable with many reported aquifer respiration rates[29,77]. We think that these slow rates of degradation in nutrient-limited reservoirs are inadequate to support rapid resynthesis of the biochemicals necessary to support the activity of microorganisms at temperatures above 80 °C. This falls short of 113 °C, the current highest authenticated temperature at which life can be sustained[78], and is considerably lower than 121 °C, which has recently been reported as the new upper temperature for life[79]. However, the majority of bacteria and archaea that are capable of growth at such high temperatures are isolated from environments rich in reduced electron donors, nutrients and electron acceptors, such as hydrothermal vent systems. Under such conditions, high metabolic rates required for the rapid regeneration of labile biomolecules can be sustained. This contrasts starkly with deep subsurface petroleum reservoirs, which are likely to be severely nutrient limited, and where low levels of metabolic activity appear to be the norm.

Box 3
Microorganisms in petroleum reservoirs

One of the first microbiological studies of a deep subsurface environment resulted in the isolation of sulphate-reducing bacteria from produced waters from an oil well[3]. Since that time a wide range of bacteria have been isolated from petroleum systems. These have exclusively come from samples of produced waters. Produced waters from reservoirs undergoing water injection to enhance oil recovery are prone to contamination from the injected waters, thus samples from non-water flooded reservoirs are more likely to yield organisms native to the petroleum reservoir, but even then non-indigenous organisms may be introduced during drilling and grow in pipework in the oil well. Consequently many aerobic heterotrophic bacteria, many of which are capable of hydrocarbon degradation only in the presence of oxygen, have been isolated from well head water samples. These organisms, however, are unlikely to be native populations from the petroleum reservoir. A wide range of anaerobic bacteria and archaea with physiological properties consistent with a deep subsurface existence have also been isolated (for an excellent review, see ref. 40). These include

fermentative thermophilic heterotrophic bacteria including *Thermotoga*, *Thermoanaerobacter* and *Fervidobacterium*. Hyperthermophilic archaea such as *Thermococcus* and *Archaeoglobus* have also been isolated from non-flooded reservoirs. In addition, sulphate-reducing bacteria, iron-reducing bacteria and methanogenic archaea have been identified in wellhead waters[42]. No bacteria capable of degrading hydrocarbons under *in situ* conditions have yet been isolated from petroleum reservoirs. Sediment samples have now been recovered from many deep subsurface environments. These have yielded isolates with characteristics that suggest that they are true representatives of the subsurface biosphere, and it has been possible to measure important biogeochemical processes in these sediments. Interestingly, there are no reports in the literature of successful isolation or characterization of the microbial communities in sediments recovered from a petroleum reservoir. The isolation and characterization of the deep slow biosphere in petroleum reservoirs thus remains a major challenge.
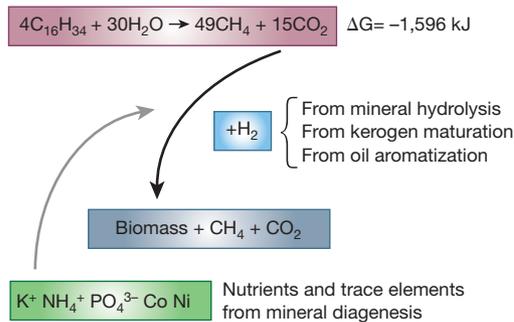
Figure 4 The putative chemistry of hydrocarbon degradation in most petroleum reservoirs with an absence of abundant sulphate. The overall conversion of hydrocarbons to biomass, methane and carbon dioxide may well involve water–hydrocarbon reactions[47,49], with the carbon dioxide produced being further reduced to methane using hydrogen produced either externally to or within the reservoir. Nutrient supply from mineral diagenesis may ultimately control the extent of hydrocarbon destruction and methane production[69–72]. The detailed mechanisms of anaerobic hydrocarbon degradation are now being elucidated and many mechanisms involve addition of fumarate[48] or carboxylation of hydrocarbons to produce functionalized intermediates, which can either be metabolized to carbon dioxide or accumulate as dead-end metabolites. For example, a reductive pathway for the anaerobic degradation of naphthalene and 2-methylnaphthalene has been proposed by Annweiler and co-workers[50]. Initial carboxylation of naphthalene is followed by degradation of 2-naphthoic acid and, then, via a series of hydrogenation steps, to 5, 6, 7, 8-tetrahydro-2-naphthoic acid. Further hydrogenation to octahydro-2-naphthoic acid may then be followed by further degradation steps to produce energy and carbon dioxide or by further hydrogenation to give decahydro-2-naphthoic acid (a possible dead-end metabolite). We have found these reduced naphthoic acid derivatives indicative of anaerobic hydrocarbon degradation in oils from all the biodegraded oil provinces that we have examined[51].

Regeneration of heat-labile cell components might be an important mechanism permitting the growth of hyperthermophilic organisms at extremely high temperatures[80], and starvation survival of hyperthermophilic archaea is reduced at high temperatures[81]. Petroleum reservoir isolates are more resistant to starvation than similar isolates from hydrothermal vent systems. But when the geological timescales involved in the biodegradation of oil in high-temperature petroleum reservoirs are considered in conjunction with the extreme conditions, it may not be so surprising that bacteria and archaea in these environments are unable to survive at the upper limit for biological activity observed in other environments. We therefore suggest that low metabolic rate rather than biomacromolecule stability is the primary controller of the upper temperature limit of the petroleum-biodegrading deep subsurface biosphere. One can also speculate that that this will be true of all life in oligotrophic deep crustal environments. The temperature base of the hydrocarbon-degrading biosphere may well be the base of life in Earth.

Perspective

Reservoir temperature and oil-charge histories are the primary controllers of the degree of oil biodegradation, with reservoir topology (relationships between oil and water volumes and interface areas) also exerting a control. Nutrient supply from temperature-dependent mineral diagenesis is a key factor, and methane is a major terminal product of oil biodegradation produced predominantly by carbon dioxide reduction with hydrogen. This has major implications both for conventional petroleum exploration and also for future strategies for recovery of heavy oil as methane through microbially engineered interventions.

The deep biosphere's origin and survival is intimately linked to the large scale tectonic processes that form, deform and destroy sedimentary basins. Observations of petroleum systems shed light on several key processes in deep biosphere evolution. Plate tectonics form and destroy sedimentary basins, accumulating organic carbon and converting it into mobile petroleum, which may migrate hundreds of kilometres before being consumed by microorganisms in transit or following accumulation in reservoirs. Mineral diagenesis and water flow in deep sediments control key nutrient supply to the petroleum biosphere, with low nutrient levels in petroleum reservoirs resulting in low metabolic rates and lower maximum temperatures for the survival of life. It appears from the distribution of biodegraded oils that temperatures near 80 °C represent the base of heterotrophic life in the crust, and this temperature may well be the base, in oligotrophic sediments, of all life in Earth. Uplifted reservoirs heated above 80–90 °C generally do not have biodegraded oils, indicating that they are effectively isolated from the surface over geological timescales, and thus the deep biosphere must be populated through burial processes, its organisms being inherited from the surface and sustained over geological timescales[35]. ☐

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