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ORIGINAL ARTICLE

Thermodynamic constraints on methanogenic crude oil biodegradation

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Methanogenic degradation of crude oil hydrocarbons is an important process in subsurface petroleum reservoirs and anoxic environments contaminated with petroleum. There are several possible routes whereby hydrocarbons may be converted to methane: (i) complete oxidation of alkanes to H_2 and CO_2, linked to methanogenesis from CO_2 reduction; (ii) oxidation of alkanes to acetate and H_2, linked to acetoclastic methanogenesis and CO_2 reduction; (iii) oxidation of alkanes to acetate and H_2, linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction; (iv) oxidation of alkanes to acetate alone, linked to acetoclastic methanogenesis and (v) oxidation of alkanes to acetate alone, linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction. We have developed the concept of a 'window of opportunity' to evaluate the range of conditions under which each route is thermodynamically feasible. On this basis the largest window of opportunity is presented by the oxidation of alkanes to acetate alone, linked to acetoclastic methanogenesis. This contradicts field-based evidence that indicates that in petroleum rich environments acetoclastic methanogenesis is inhibited and that methanogenic CO_2 reduction is the predominant methanogenic process. Our analysis demonstrates that under those biological constraints oxidation of alkanes to acetate and H_2, linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction offers a greater window of opportunity than complete oxidation of alkanes to H_2 and CO_2 linked to methanogenic CO_2 reduction, and hence is the process most likely to occur.
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Introduction

The largest deposits of petroleum on Earth are not, as conventionally assumed, in the Middle East. The vast Saudi Arabian and Kuwaiti oilfields of Ghawar (2.6×10^{11} barrels (bbl) in place) and Burgan (7.0×10^{10} bbl in place) are dwarfed by the trillion bbl deposits of western Canada (Athabasca tar sands; 1.7×10^{12} bbl) and Venezuela (Orinoco heavy oil belt; 1.2×10^{12} bbl). These so-called super-giant heavy oil fields are the result of biodegradation of the lighter, more readily produced and valuable oil fractions over geological time. Biodegraded oil fields are more difficult to produce and the oils more difficult to refine than oil from conventional fields and are thus less economically attractive. On the basis of known mechanisms of hydrocarbon degradation, conventional wisdom among petroleum geologists has for some time, been that biodegradation in oilfields was driven by oxygen delivered to petroleum reservoirs in meteoric water. This paradigm has been questioned in light of the discovery of a range of bacteria capable of coupling the oxidation of aliphatic or aromatic hydrocarbons to the reduction of nitrate, iron and sulphate (Widdel and Rabus, 2001) and microbial consortia capable of linking aliphatic hydrocarbon oxidation to methane generation (Zengler *et al.*, 1999; Anderson and Lovley, 2000; Townsend *et al.*, 2003).

Evidence is emerging to support the notion that in-reservoir petroleum biodegradation is caused by anaerobic hydrocarbon degrading bacteria. Reduced naphthoic acids, metabolites characteristic of anaerobic hydrocarbon degradation have been detected in biodegraded petroleum reservoirs, but not in non-degraded reservoirs (Aitken *et al.*, 2004). Geochemical and isotopic evidence also suggests that in many cases the end product of hydrocarbon degradation in petroleum reservoirs is methane (Scott *et al.*, 1994;

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Larter *et al.*, 1999; Sweeney and Taylor, 1999; Pallasser, 2000; Boreham *et al.*, 2001; Masterson *et al.*, 2001). Compositional gradients in oil columns towards the underlying water leg in biodegraded petroleum reservoirs suggests that the oil water transition zone is the primary site of biodegradation in petroleum reservoirs (Head *et al.*, 2003; Larter *et al.*, 2003).

On the basis of these and other data, a new conceptual model of in-reservoir petroleum biodegradation has been developed (Head *et al.*, 2003). In this model, anaerobic degradation of petroleum occurs most actively at the oil water transition zone. Electron donor, mainly hydrocarbons, is delivered to the oil water transition zone by diffusion from the oil column, with inorganic nutrients such as ammonium ions provided from the water leg (Head *et al.*, 2003; Manning and Hutcheon, 2004). This is consistent with reports from other deep subsurface environments that microbial activity is stimulated at geochemical interfaces (Parkes *et al.*, 2005). When the water leg contains low levels of sulphate, hydrocarbon degradation is driven by methanogenesis; indeed, many biodegraded petroleum reservoirs contain isotopically light methane indicative of a mixed secondary biogenic and thermogenic source (Scott *et al.*, 1994; Larter *et al.*, 1999; Sweeney and Taylor, 1999; Pallasser, 2000; Boreham *et al.*, 2001; Masterson *et al.*, 2001; Head *et al.*, 2003).

The significance of methanogenic crude oil degradation in petroleum reservoirs goes beyond its potential role in the biodegradation of petroleum reservoirs; it may ultimately be crucial for processes that can enhance the recovery of residual oil. Typically, over 60% of the oil in place in a petroleum reservoir remains unextractable following standard production procedures and the possibility that methanogenic degradation of this residual oil can re-pressurize a petroleum reservoir, has some potential for enhancing oil recovery. Furthermore, the volumetrics of gas recovery are far better than for oil (typically 70% of gas in place can be recovered) and methanogenic conversion of non-recoverable residual hydrocarbons to recoverable gas may be an economically viable way of extending the operational life of petroleum reservoirs (Parkes, 1999; Larter *et al.*, 1999; Head *et al.*, 2003). In addition, methanogenic hydrocarbon degradation may be a significant process in the attenuation of contaminated anoxic sediments and aquifers (Weiner and Lovley, 1998; Anderson and Lovley, 2000; Bekins *et al.*, 2005).

Because of the potential importance of methanogenic crude oil biodegradation and our limited knowledge of the organisms and mechanisms involved, it is important that we learn more about what governs the microbial conversion of oil to methane.

Quantitatively, the most important component of crude oil is the saturated hydrocarbon fraction and little is known about the methanogenic degradation of long-chain aliphatic hydrocarbons; only three reports in the literature provide strong evidence of methanogenic degradation of aliphatic hydrocarbons or crude oil. Zengler *et al.* (1999) report degradation of pure hexadecane by an enrichment culture; Anderson and Lovley (2000) documented rapid mineralization of ^{14}C-labelled hexadecane in sediments from a crude oil-contaminated aquifer; and Townsend *et al.* (2003) observed methanogenic transformation of crude oil in sediments from a gas condensate contaminated aquifer.

In this paper, we evaluate the thermodynamics of five possible routes of methanogenic hydrocarbon degradation, viz (with hexadecane as example):

(i) complete oxidation of alkanes to H_2 and CO_2, linked to methanogenesis from CO_2 reduction:

$$4C_{16}H_{34} + 128H_2O \rightarrow 64CO_2 + 196H_2 \quad \text{(reaction 1)}$$

$$196H_2 + 49CO_2 \rightarrow 49CH_4 + 98H_2O \quad \text{(reaction 2)}$$

$$\text{sum } 4C_{16}H_{34} + 30H_2O \rightarrow 15CO_2 + 49CH_4 \quad \text{(reaction 3)}$$

(ii) oxidation of alkanes to acetate and H_2, linked to acetoclastic methanogenesis and CO_2 reduction:

$$4C_{16}H_{34} + 64H_2O \rightarrow 32CH_3COO^- + 32H^+ + 68H_2 \quad \text{(reaction 4)}$$

$$32CH_3COO^- + 32H^+ \rightarrow 32CO_2 + 32CH_4 \quad \text{(reaction 5)}$$

$$68H_2 + 17CO_2 \rightarrow 17CH_4 + 34H_2O \quad \text{(reaction 6)}$$

$$\text{sum } 4C_{16}H_{34} + 30H_2O \rightarrow 15CO_2 + 49CH_4$$

(iii) oxidation of alkanes to acetate and H_2, linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction:

$$4C_{16}H_{34} + 64H_2O \rightarrow 32CH_3COO^- + 32H^+ + 68H_2$$

$$32CH_3COO^- + 32H^+ + 64H_2O \rightarrow 64CO_2 + 128H_2 \quad \text{(reaction 7)}$$

$$196H_2 + 49CO_2 \rightarrow 49CH_4 + 98H_2O$$

$$\text{sum } 4C_{16}H_{34} + 30H_2O \rightarrow 15CO_2 + 49CH_4$$

(iv) oxidation of alkanes to acetate alone, linked to acetoclastic methanogenesis:

$$4C_{16}H_{34} + 30H_2O + 34CO_2 \rightarrow 49CH_3COO^- + 49H^+ \quad \text{(reaction 8)}$$

$$49CH_3COO^- + 49H^+ \rightarrow 49CO_2 + 49CH_4 \quad \text{(reaction 9)}$$

$$\text{sum } 4C_{16}H_{34} + 30H_2O \rightarrow 15CO_2 + 49CH_4$$

and (v) oxidation of alkanes to acetate alone, linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction:

$$4C_{16}H_{34} + 30H_2O + 34CO_2 \rightarrow 49CH_3COO^- + 49H^+ \quad \text{(reaction 10)}$$

$$49CH_3COO^- + 49H^+ + 98H_2O \rightarrow 98CO_2 + 196H_2 \quad \text{(reaction 11)}$$

$$196H_2 + 49CO_2 \rightarrow 49CH_4 + 98H_2O \quad \text{(reaction 12)}$$

$$\text{sum } 4C_{16}H_{34} + 30H_2O \rightarrow 15CO_2 + 49CH_4$$

The effects of temperature, pH, acetate and H_2 concentration on each of these processes is determined and conditions under which each process is likely to be most favourable are identified and related to conditions typical of petroleum reservoirs. We consider only the case of alkanes with even chain length. It is likely that similar patterns will be observed with odd chain alkanes, which will generate propionate in addition to acetate and H_2. This would require incorporation of syntrophic propionate oxidation as an intermediate reaction. Any effect of propionate will decrease with increasing alkane chain length.

Methods

Gibbs free energy calculations were made after Thauer *et al.* (1977) and Amend and Shock (2001).

Temperature corrections for ΔG^o were made with the Gibbs–Helmholtz equation according to:

$$\Delta G^o_{Tact} = \Delta G^o_{Tref}.(T_{act}/T_{ref}) + \Delta H^o_{Tref}.(T_{ref} - T_{act})/T_{ref}$$

with T in K; $T_{ref} = 298.15$ K.

Gibbs free energies and enthalpies of formation data for alkanes in the liquid state were taken from (Helgeson *et al.*, 1998). For all other compounds the data were taken from Hanselmann (Hanselmann, 1991), with acetate in the aqueous phase and methane, hydrogen and carbon dioxide in the gaseous phase at partial pressures of 1 atm. Calculations were made for neutrality rather than for pH = 7, with neutrality defined as the pH where activities of H^+ and OH^- are equal. The pH representing neutrality varies with temperature (Harned and Owen, 1943).

Sample calculations

Table 1 lists change in Gibbs free energy values for various reactions of importance to anaerobic hexadecane degradation. The values shown are $\Delta G^{o'}$. From these data the threshold concentrations of products and reactants, which result in $\Delta G' < 0$ were calculated as follows. The example given is for the threshold H_2 concentration for hydrogenotrophic methanogenesis.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O;$$

$$\Delta G^{o'} = -130.7 kJ/mol CH_4$$

Hence (for example Thauer *et al.*, 1977): $\Delta G' = -130.7 + RT \ln([CH_4]/[CO_2].[H_2]^4)$

(note that in biological systems $\ln[H_2O]$ is assumed to be 0)

Therefore, under otherwise standard conditions: $\Delta G' = -130.7 - 5.71 \log[H_2]^4$ (where 5.71 logx equals $R.T_{298.15}$ lnx).

Since the threshold value is the value where $\Delta G' = 0$ it follows that

$$-130.7 - 5.71 \log[H_2]^4 = 0$$

$$\text{Thus } 4 \log[H_2] = 130.7/-5.71, \text{ and hence}$$

$$[H_2] = 10^{(-130.7/22.84)} = 10^{-5.72}$$

$$\text{Thus } [H_2]_{crit} = 1.89 \times 10^{-6} \text{ atm.}$$

Results

We have examined the effect of alkane chain length on the free energy yield of methanogenic alkane degradation. Hexadecane was chosen as an exemplar to determine the effect of temperature, H_2 concentration, acetate concentration and pH on the thermodynamics of alkane degradation via routes (i) to (v) outlined above. By determining threshold conditions at which the different component reactions for each route become exergonic we have defined 'windows of opportunity' under which different individual reactions can be linked, and thus what conditions are permissive for each of the five routes. The windows of opportunity determined, are discussed in the context of

Table 1 Stoichiometry and change in Gibbs free energy values for reactions potentially involved in methanogenic hexadecane degradation

Substrates	*Products*	$\Delta G^{o'}$	
		kJ/reaction	*kJ/mol*
Hexadecane degradation			
$4C_{16}H_{34}+128H_2O$	$64CO_2+196H_2$	4922.1	1230.5[a]
$4C_{16}H_{34}+64H_2O$	$32CH_3COO^-+32H^++68H_2$	1883.1	470.8[a]
$4C_{16}H_{34}+30H_2O+34CO_2$	$49CH_3COO^-+49H^+$	268.6	67.2[a]
$4C_{16}H_{34}+30H_2O$	$15CO_2+49CH_4$	−1487.1	−371.8[a]
Conversion of potential intermediates			
$CH_3COO^-+H^+ +2H_2O$	$2CO_2+4H_2$	94.9	94.9[b]
$CH_3COO^-+H^+$	CO_2+CH_4	−35.8	−35.8[c]
$4H_2+CO_2$	CH_4+2H_2O	−130.7	−130.7[c]

[a]Per mol hexadecane.
[b]Per mol acetate.
[c]Per mol methane.

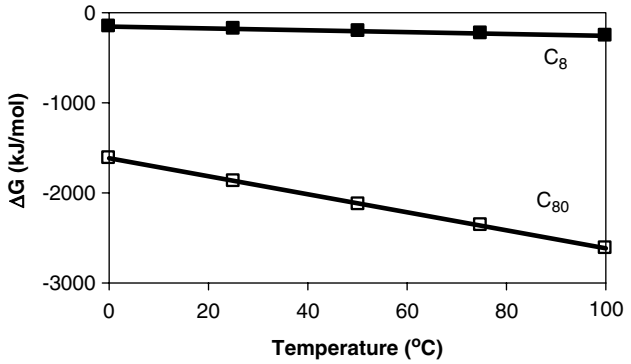

Figure 1 Effect of temperature on change in Gibbs free energy for methanogenesis from alkanes; closed symbols C_8, open symbols C_{80}.

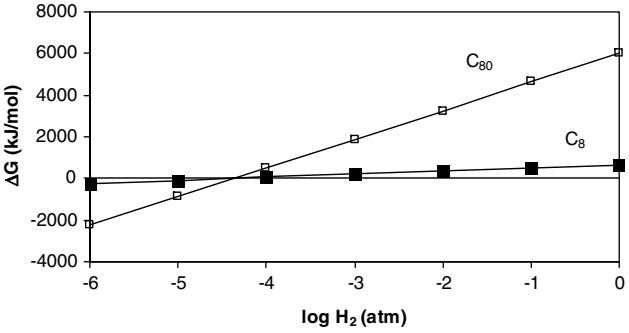

Figure 2 Effect of hydrogen partial pressure on the change in Gibbs free energy for oxidation of alkanes to H_2 plus CO_2; closed symbols C_8, open symbols C_{80}.

empirical data on crude oil degradation in petroleum reservoirs and used to assess the relative importance of thermodynamics relative to biological factors in determining the routes of methanogenic crude oil degradation, observed in nature.

Effect of chain length on the thermodynamics of anaerobic alkane degradation

The effect of chain length on the free energy yield of methanogenic degradation of alkanes was investigated to evaluate if inferences made using a single model alkane would be generally applicable across all alkanes.

Thermodynamic calculations for all alkanes in the range C_8–C_{80}, demonstrated that conversion to methane was exergonic. ΔG^o varied between -185 and -1870 kJ per mol for C_8 to C_{80} alkanes respectively (Figure 1). In all cases, the free energy yield increased with increasing temperature and the increase in energy yield per degree Kelvin was greater for longer chain hydrocarbons ($-0.93\,kJ\,mol^{-1}\,K^{-1}$ for C_8 and $-10.0\,kJ\,mol^{-1}\,K^{-1}$ for C_{80}). Similar trends were observed for complete oxidation of alkanes to H_2 and CO_2 and incomplete oxidation of alkanes to H_2 and acetate (reactions 1 and 4). In contrast, oxidation of alkanes to acetate alone (reaction 8) became less energetically favourable with increasing temperature, especially for longer chain alkanes ($+0.73\,kJ\,mol^{-1}\,K^{-1}$ for C_8 and $+6.0\,kJ\,mol^{-1}\,K^{-1}$ for C_{80}: see Supplementary Information for data). When normalized for the number of carbon atoms, there is a slightly greater energy yield per carbon from longer chain length alkanes (for C_8 $\Delta G^o = -23.1$ kJ per mol C, for C_{80} $\Delta G^o = -23.4$ kJ per mol C).

These results relate to standard conditions, where the initial oxidation reactions (reactions 1, 4 and 8) are all endergonic. When the effect of H_2 on the complete oxidation pathway is taken into account, it is clear that H_2 concentration has a more marked effect on long chain alkanes than short chain alkanes (Figure 2). However, it is interesting to note that the threshold H_2 concentration at which alkane oxidation to H_2 and CO_2 becomes energetically favourable under otherwise standard conditions, increases merely from 3.7×10^{-5} atm for *n*-octane (C_8) to 4.3×10^{-5} atm for *n*-octacontane (C_{80}), that is, is essentially independent of chain length (Figure 2).

Overall the same trends are observed, independent of alkane chain length and for simplicity all subsequent discussion is restricted to analysis of hexadecane oxidation, which we have used as an example to illustrate the effect of hydrogen concentration, acetate concentration, temperature and pH on different pathways of methanogenic alkane degradation.

Complete oxidation of hexadecane to H_2 and CO_2 linked to methanogenic CO_2 reduction

While hexadecane oxidation to H_2 and CO_2 becomes more thermodynamically favourable with increasing temperature, methanogenesis by CO_2 reduction becomes less thermodynamically favourable with increasing temperature (Figure 3). However, because the energy yield from hexadecane oxidation increases more steeply than the reduction in energy yield for the methanogenic reaction, the net result is an increase in the overall energy yield from methanogenic hexadecane degradation with increasing temperature (Figure 3).

When the H_2 concentration is taken into account, the energy yield from complete oxidation of hexadecane decreases with increasing H_2 concentration (Figure 4) whereas methanogenic CO_2 reduction becomes more exergonic with increasing H_2 concentration. Provided the overall reaction is exergonic, there is a 'window of opportunity' defined by the H_2 concentrations where both processes are exergonic (Figure 4). It is particularly interesting to note that this window of opportunity increases with increasing temperature and thus this pathway of methanogenic hexadecane degradation becomes less constrained with increasing temperature (that is the window of opportunity becomes larger. Note that the *y* axis is on a log scale (Figure 5)).

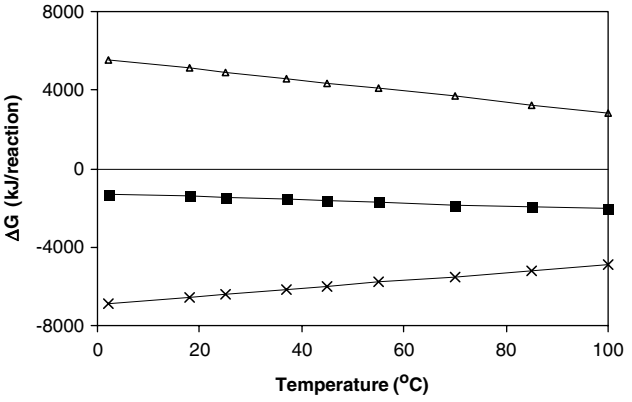

Figure 3 Effect of temperature on the change in Gibbs free energy for complete oxidation of hexadecane to H_2 and CO_2 (open symbols), for stoichiometric methanogenesis of the hydrogen produced (+) and for the sum of the aforementioned reactions, that is, methanogenic degradation of hexadecane (closed symbols). Reactions considered: $4C_{16}H_{34} + 128H_2O \rightarrow 196H_2 + 64CO_2$; $196H_2 + 49CO_2 \rightarrow 49CH_4 + 98H_2O$; $4C_{16}H_{34} + 30H_2O \rightarrow 15CO_2 + 49CH_4$.

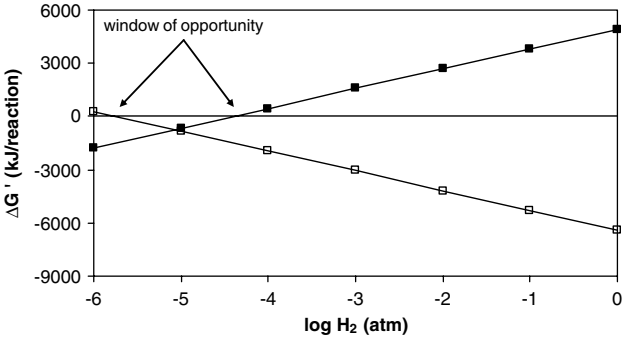

Figure 4 Effect of hydrogen partial pressure on the change in Gibbs free energy for oxidation of hexadecane to H_2 and CO_2 (open symbols) and for stoichiometric methanogenesis of the hydrogen produced. The arrows delineate the 'window of opportunity' where both reactions are exergonic. Reactions considered: $4C_{16}H_{34} + 128H_2O \rightarrow 196H_2 + 64CO_2$; $196H_2 + 49CO_2 \rightarrow 49CH_4 + 98H_2O$.

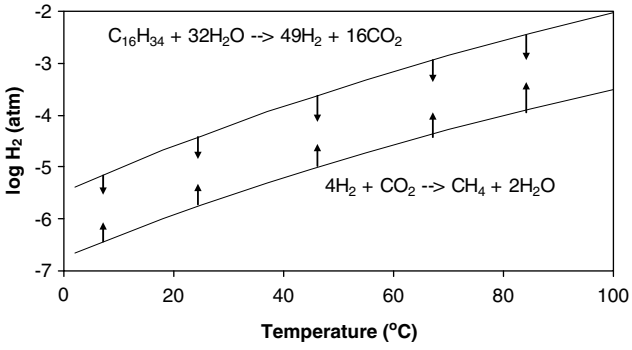

Figure 5 Effect of temperature on the range of H_2 partial pressures where both hexadecane oxidation and methanogenesis from H_2/CO_2 are exergonic. The lines represent the threshold at which the free energy change for each process is equal to zero and the arrows indicate conditions under which the processes become increasingly exergonic.

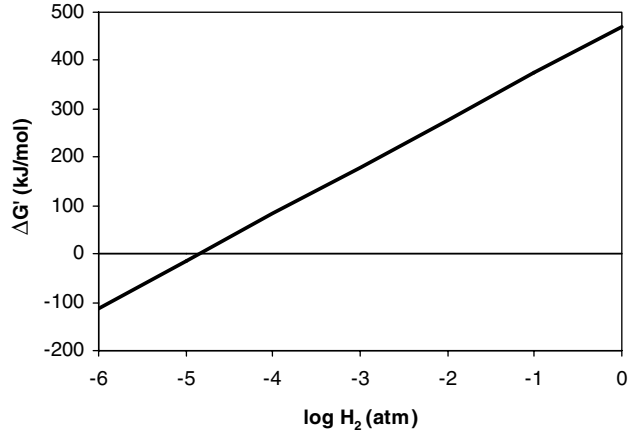

Figure 6 Effect of hydrogen partial pressure on the change in Gibbs free energy for incomplete oxidation of hexadecane to acetate and H_2, under otherwise standard conditions, that is acetate 1 M, hexadecane as liquid and pH = 7.

Incomplete oxidation of hexadecane to acetate and H_2 linked to acetoclastic methanogenesis and methanogenic CO_2 reduction

As with complete oxidation of hexadecane, the free energy yield of incomplete oxidation of hexadecane to acetate and H_2 is highly dependent on H_2 concentration (Figure 6). The range of H_2 concentrations where oxidation of hexadecane to acetate and H_2 is exergonic is also dependent on the acetate concentration (Figure 7). As acetate concentration decreases, the range of H_2 concentrations that are permissive for incomplete oxidation of hexadecane linked to methanogenic CO_2 reduction increases. Furthermore, the higher the temperature the more permissive incomplete hexadecane oxidation becomes with respect to H_2 concentration (Figure 7). The effect of acetate concentration on incomplete oxidation of hexadecane is therefore considerable, but is dependent upon H_2 concentration.

Oxidation of hexadecane to acetate and H_2 linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction

A variant of incomplete hexadecane oxidation linked to acetoclastic methanogenesis and methanogenic CO_2 reduction could involve syntrophic acetate oxidation linked exclusively to methanogenic CO_2 reduction, with no involvement of acetoclastic methanogenesis. Crude oil has been shown to have an adverse effect on acetoclastic methanogenesis (Warren *et al.*, 2004); under such circumstances it is possible that syntrophic acetate oxidation might permit complete conversion of hexadecane to methane and CO_2 via methanogenic CO_2 reduction. Interestingly, the range of H_2 concentrations that permit hexadecane oxidation linked to methanogenic CO_2 reduction is least for incomplete oxidation and greatest for syntrophic acetate oxidation, which is exergonic at H_2 partial pressures

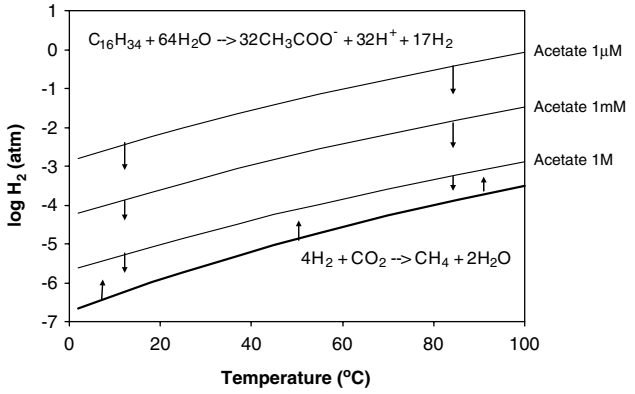

Figure 7 Effect of temperature on the range of H_2 partial pressures where both incomplete oxidation of hexadecane to acetate and H_2 and methanogenic CO_2 reduction are exergonic. The thin lines demarcate the hydrogen thresholds where the free energy yield equals zero at acetate concentrations of 1 μM, 1 mM and 1 M respectively. The thick line gives the threshold for methanogenic CO_2 reduction. The arrows indicate conditions under which the processes become increasingly exergonic.

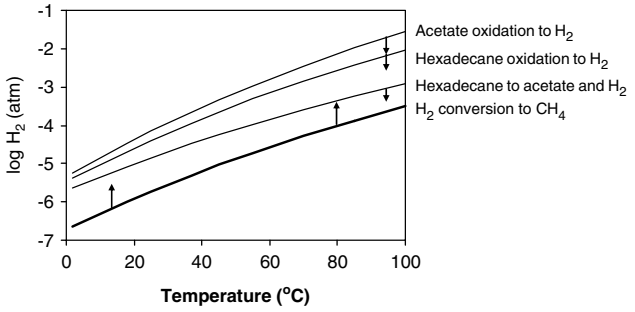

Figure 8 Effect of temperature on the range of H_2 partial pressures where complete oxidation of acetate to H_2, complete oxidation of hexadecane to H_2 and CO_2 and incomplete oxidation of hexadecane to acetate and H_2 are exergonic and can be coupled to methanogenic CO_2 reduction. The thin lines demarcate the hydrogen thresholds for acetate oxidation and for hexadecane oxidation; the thick line gives the threshold for methanogenic CO_2 reduction. Standard states are: acetate 1 M, hexadecane as liquid and CO_2 and CH_4 at 1 atm.

slightly greater than required to give a negative ΔG for complete oxidation of hexadecane to H_2 and CO_2 (Figure 8). However, as acetate concentrations decrease (less than 0.1 M acetate), syntrophic acetate oxidizers require lower H_2 concentrations than either complete or incomplete hexadecane oxidizers (Figure 9; sector II and III compared to sector IV) and the feasibility of this route is therefore dictated by the sensitivity of syntrophic acetate oxidation to H_2 concentration.

Oxidation of hexadecane to acetate alone, linked to acetoclastic methanogenesis

A further route for the primary oxidation of alkanes is their conversion to acetate alone, which may then be linked to acetoclastic methanogenesis. This pathway becomes thermodynamically less favourable with increasing temperature (Figure 10). Like incomplete hexadecane oxidation, this mechanism of primary oxidation of hexadecane, is dependent on the acetate concentration. Under otherwise standard conditions, this route of hexadecane oxidation becomes exergonic at concentrations less than 0.1 M acetate (Figure 9). This contrasts with incomplete hexadecane oxidation which is exergonic at higher acetate concentrations provided that the H_2 partial pressure is maintained at less than 1.4×10^{-5} atm (Figure 9, sector V; see also section 3.4).

Oxidation of hexadecane to acetate alone linked to syntrophic acetate oxidation and methanogenic CO_2 reduction

A variation on complete acetate oxidation linked to methanogenesis involves syntrophic acetate oxidation and methanogenic CO_2 reduction as the terminal reaction. This metabolic route has the effect of reducing the domain under which conversion of hexadecane to methane is feasible (Sector I through VI in Figure 9, for hexadecane conversion to acetate linked to acetoclastic methanogenesis, compared to Sector I through IV in Figure 9 if syntrophic acetate oxidation was involved). This is because acetoclastic methanogenesis is independent of H_2 concentration, but methanogenic CO_2 reduction is prevented at low H_2 concentrations, and in addition oxidation of hexadecane to acetate alone is prevented at acetate concentrations greater than 0.1 M (Figure 9).

Effect of pH on incomplete oxidation and acetogenic oxidation of hexadecane

Both incomplete oxidation of hexadecane and conversion of hexadecane to acetate are pH dependent. Both processes become increasingly energetically favourable with increasing pH. In line with the stoichiometry of acetate production, the effect is more pronounced for complete conversion of hexadecane to acetate (Figure 11). Higher pH values also increase the range of acetate concentrations where the reaction remains exergonic (Figure 12).

Discussion

The methanogenic alkane degradation landscape

Microbial degradation of hydrocarbons under anoxic conditions is of global significance. It is responsible for the in-reservoir degradation of oil that has lead to vast heavy oil and tar sand deposits that constitute the bulk of the world's petroleum inventory (Roadifer, 1987; Head *et al.*, 2003; Aitken *et al.*, 2004). Despite the importance of the process, relatively little is known about the

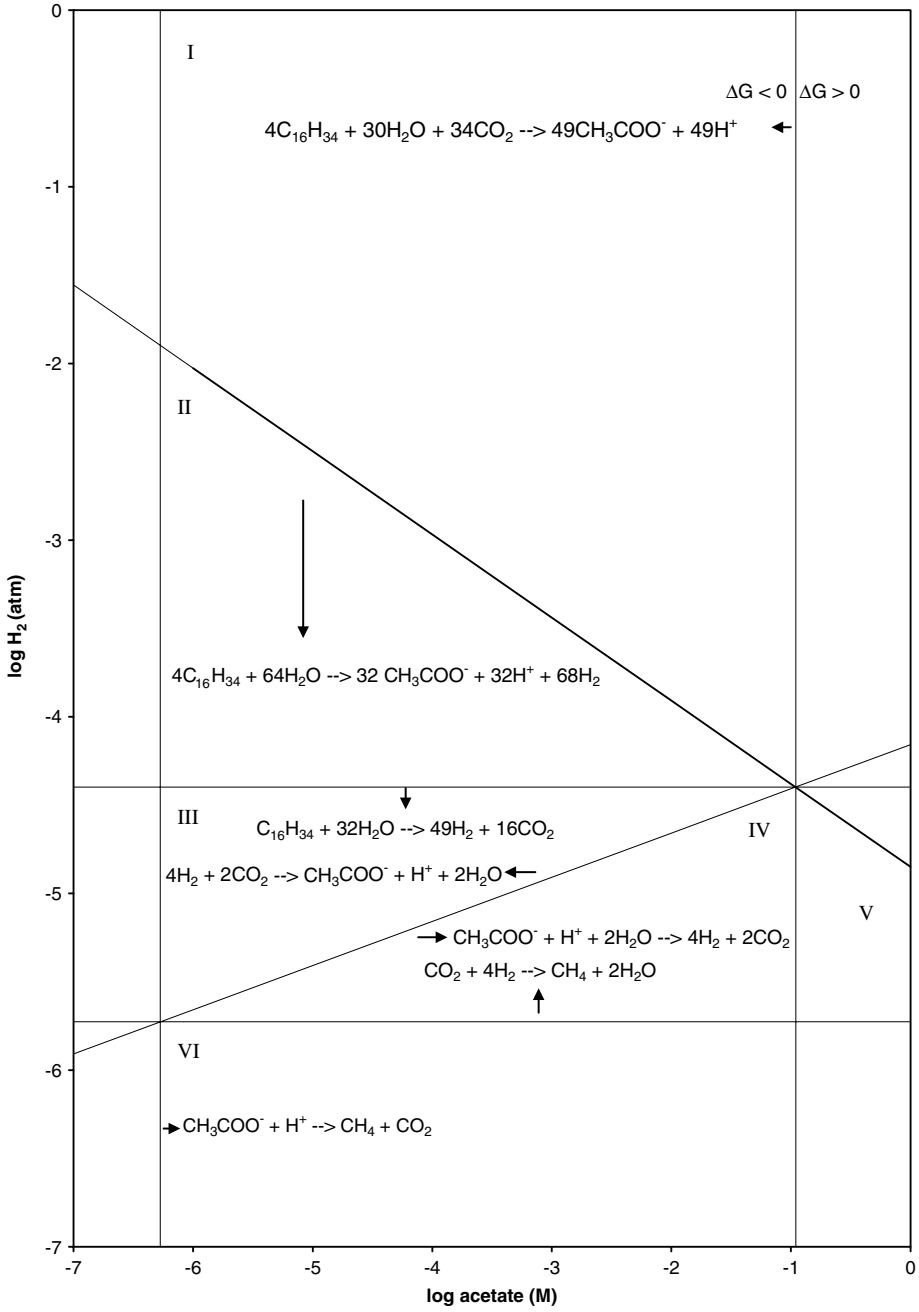

Figure 9 Hydrogen and acetate as thermodynamic constraints on methanogenic hexadecane degradation.

factors which dictate the occurrence of in-reservoir biodegradation. The geothermal history of petroleum basins has been shown to exert a broad control on the occurrence of biodegraded petroleum reservoirs and has led to the concept of palaeopasteurization or palaeosterilization, which prevents the biodegradation of crude oil in subterranean formations (Wilhelms *et al*., 2001). The limited availability of nutrients in aquifers associated with petroleum reservoirs and geological constraints on the physical interaction of water and oil legs have also been proposed as factors which may limit in-reservoir oil biodegradation (Head *et al*., 2003; Larter *et al*., 2006). As we have seen, there are several possible routes that lead to the conversion of crude oil hydrocarbons to methane, and the interplay between physical and chemical conditions in the reservoir and reservoir microbiology likely have a role in controlling in-reservoir oil biodegradation. In addition to these broad scale geological controls, thermodynamic factors may also have a bearing on crude oil biodegradation in petroleum reservoirs.

Thermodynamic calculations demonstrate that methanogenic alkane degradation becomes more energetically favourable at higher temperatures and

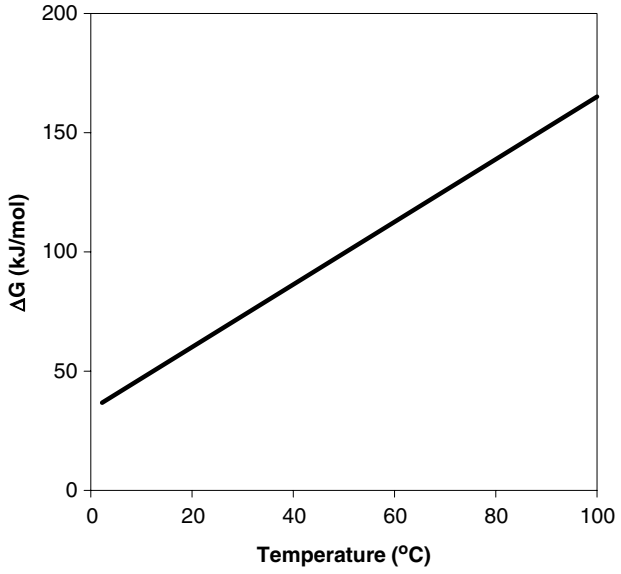

Figure 10 Effect of temperature on change in Gibbs free energy for oxidation of hexadecane to acetate alone.

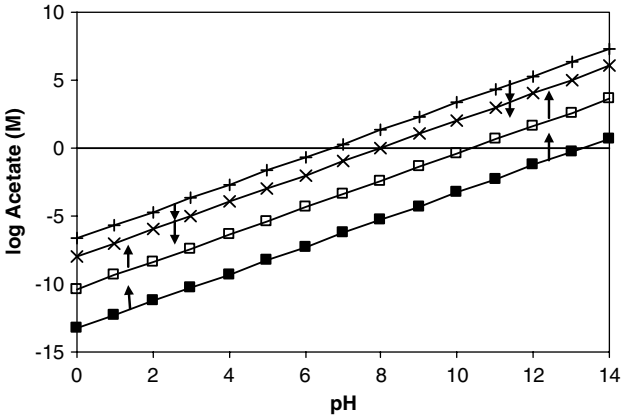

Figure 12 Effect of pH on the range of acetate concentrations where hexadecane conversion to acetate or acetate plus hydrogen are exergonic and can be coupled to methanogenesis from acetate at a hydrogen partial pressure of 10^{-5} atm; symbols: +, incomplete oxidation of hexadecane to acetate and H_2; x, oxidation of hexadecane to acetate; open squares, oxidation of acetate to H_2 and CO_2; closed squares, conversion of acetate to CH_4. The arrows indicate conditions under which the processes become increasingly exergonic.

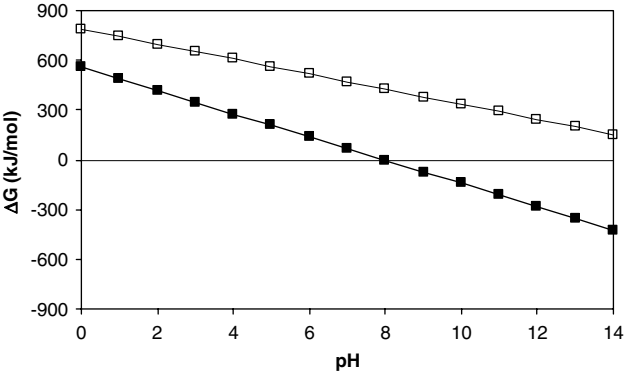

Figure 11 Effect of pH on change in Gibbs free energy for complete oxidation of hexadecane to acetate (closed symbols) and for incomplete oxidation to H_2 and acetate (open symbols).

although the three potential initial oxidation reactions examined here are all endergonic under standard conditions, at low concentrations of H_2 and/or acetate they are all thermodynamically feasible. Acetate concentrations ranging from less than 10 μM to over 17 mM have been measured in oilfield formation waters (Barth and Riis, 1992) with the majority (85 of 121 samples analyzed) having acetate concentrations below 5 mM. Such concentrations are well within the permissive range for the hydrocarbon oxidation reactions investigated here (Figure 9). There are very few data in the literature on hydrogen gas concentrations in petroleum reservoirs. Hydrogen however, seems to be present below detection limits in most oilfield waters examined and is unlikely to represent more than 1 mol% (equivalent to 10^{-2} atm) in associated gases (Hill *et al.*, 2007). This again is compatible with the hydrocarbon oxidation pathways examined here (Figure 9). However, it should be borne in mind that the gas data were obtained from well-head samples and may not reflect the true concentrations present in deep petroleum reservoirs.

To evaluate how the different initial oxidation processes can be linked to conventional methanogenic pathways we have introduced the concept of a 'window of opportunity', which defines the conditions under which both the initial oxidation process and the terminal methanogenic pathways are energetically favourable. This demonstrates that as temperature increases, so does the window of opportunity with respect to H_2 and acetate. Clearly this is only part of the story as it is well known that at temperatures in excess of 80–90 °C, in-reservoir petroleum biodegradation apparently ceases (Connan, 1984; Head *et al.*, 2003), illustrating that above these temperatures biological factors are more important than thermodynamic factors in controlling methanogenic hydrocarbon degradation. The windows of opportunity with respect to acetate and H_2, the central intermediates in methanogenic alkane degradation, have been summarized for the range of processes that are feasibly involved in methanogenic alkane degradation. Figure 9 represents the situation at 25 °C and pH 7; however, the activity domains identified from this analysis remain similar for other temperatures and pH values, with the proviso that they are shifted towards higher H_2 and lower acetate concentrations at higher temperatures (see Supplementary Information). The value of this analysis is that it identifies clear zones where different methanogenic alkane degradation pathways can occur. For example the window of opportunity for linking complete

conversion of hexadecane to acetate with acetoclastic methanogenesis (domain I to IV and VI in Figure 9) is much larger than the equivalent window for linking incomplete oxidation of hexadecane to both acetoclastic methanogenesis and methanogenic CO_2 reduction (domain II, III, IV and VI in Figure 9), or complete oxidation of hexadecane to methanogenic CO_2 reduction (domain III, IV and V in Figure 9). This might suggest that acetoclastic methanogenesis might predominate in methanogenic oil-degrading systems. However, several lines of evidence suggest that methanogenic CO_2 reduction, may be more prevalent in petroleum systems. The majority of methanogens identified from oil field waters are CO_2-reducing methanogens, and acetoclastic methanogens are apparently rare (Magot *et al.*, 2000; Orphan *et al.*, 2000, 2003; Grabowski *et al.*, 2005). Furthermore, experimental measurements of methanogenic pathways in oil field waters also suggest that CO_2 reduction to methane may be more important than acetoclastic methanogenesis in petroleum reservoirs. A synthesis of data from 138 measurements made across 6 different oilfields (Nazina *et al.*, 1995a, b; Rozanova *et al.*, 1995, 2001; Bonch-Osmolovskaya *et al.*, 2003) indicates that in 67% of the cases, the predominant methanogenic pathway was CO_2 reduction, whereas in 33% acetoclastic methanogenesis predominated. This is supported by field data which demonstrate that acetate is present at high concentrations in some oil field waters (Barth and Riis, 1992), and by modelling of gas isotope composition during in- reservoir biodegradation and data from methanogenic oil-degrading microcosms (Jones *et al.*, 2008 accepted for publication). Studies of methanogenesis in a crude oil contaminated aquifer also suggest that crude oil suppresses acetoclastic methanogenesis (Warren *et al.*, 2004). Interestingly predominance of acetoclastic methanogens has been observed in hydrocarbon contaminated aquifers (Dojka *et al.*, 1998; Struchtemeyer *et al.*, 2005). However, in these instances, refined petroleum products (jet fuel) and a gas condensate were the contaminants, not crude oil. Taken together these suggest that there are biological controls that override thermodynamic considerations and lead to a subordinate role for processes of crude oil alkane degradation that require a major role for acetoclastic methanogenesis. The thermodynamic analysis; however, demonstrates that there is a wide range of conditions under which alkane degradation can be supported by methanogenic CO_2 reduction linked either to the complete oxidation of alkanes (domain III to V), or incomplete oxidation of alkanes where the acetate generated can be oxidized to H_2 and CO_2 by syntrophic acetate oxidizing organisms (domain II to V in Figure 9). The window of opportunity, which is permissive for the pathway involving incomplete alkane oxidation coupled to syntrophic acetate oxidation and methanogenic CO_2 reduction is larger than the corresponding window for complete alkane oxidation coupled to methanogenic CO_2 reduction. Furthermore, if one considers the line relating ΔG to hydrogen concentration for complete and incomplete alkane oxidation, it is apparent that incomplete oxidation is more favourable at higher hydrogen concentrations and the slope is much steeper for complete oxidation (see Supplementary Figure S6). The second of these observations indicates that complete oxidation of alkanes is likely be more sensitive to small fluctuations in hydrogen concentrations around the point that the process shifts from being exergonic to endergonic. Thus under conditions where acetoclastic methanogenesis is inhibited one would expect a pathway via incomplete oxidation of alkanes linked to syntrophic acetate oxidation and methanogenic CO_2 reduction, rather than a pathway via complete oxidation to H_2/CO_2 linked to methanogenic CO_2 reduction. Interestingly, putative thermophilic acetate-oxidizing bacteria have been detected in a petroleum reservoir (Nazina *et al.*, 2006). Coupled with the inability to detect acetoclastic methanogens in formation waters or enrichment cultures, even when high rates of methanogenesis from acetate were measured, this has led to the suggestion that syntrophic acetate oxidation might be important for driving methanogenesis in a high temperature oil reservoir (Nazina *et al.*, 2006). Therefore it is feasible that the principal pathways leading to methanogenic oil degradation in petroleum reservoirs involve either complete oxidation of hydrocarbons to H_2 and CO_2 followed by methanogenic CO_2 reduction or incomplete hydrocarbon oxidation coupled with syntrophic acetate oxidation and methanogenic CO_2 reduction with the latter of these likely to occur more widely.

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