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The thermodynamic landscape of methanogenic PAH degradation

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Summary

Methanogenic degradation of polycyclic aromatic hydrocarbons (PAHs) has long been considered impossible, but evidence in contaminated near surface environments and biodegrading petroleum reservoirs suggests that this is not necessarily the case. To evaluate the thermodynamic constraints on methanogenic PAH degradation we have estimated the Gibbs free energy values for naphthalene, phenanthrene, anthracene, pyrene and chrysene in the aqueous phase, and used these values to evaluate several possible routes whereby PAHs may be converted to methane. Under standard conditions (25°C, solutes at 1 M concentrations, and gases at 1 atm), methanogenic degradation of these PAHs yields between 209 and 331 kJ mol⁻¹. Per mole of methane produced this is 27-35 kJ mol⁻¹, indicating that PAHbased methanogenesis is exergonic. We evaluated the energetics of three potential PAH degradation routes: oxidation to H₂/CO₂, complete conversion to acetate, or incomplete oxidation to H₂ plus acetate. Depending on the in situ conditions the energetically most favourable pathway for the PAH-degrading organisms is oxidation to H₂/CO₂ or conversion into acetate. These are not necessarily the pathways that prevail in the environment. This may be because the kinetic theory of optimal length of metabolic pathways suggests that PAH degraders may have evolved

towards incomplete oxidation to acetate plus H_2 as the optimal pathway.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) enter the near surface biosphere through human activities such as crude oil spillage, fossil fuel combustion and gasoline leakage as well as natural inputs like forest fire smoke and natural petroleum seepage. Here PAHs are regarded as pollutants by environmental and health agencies because of their toxic, mutagenic and carcinogenic effects on living organisms (Samanta et al., 2002). However, in petroleum reservoirs they are part of the natural mixture that makes up crude oil. Recent findings indicate that significant fractions of crude oil can be degraded in the deep subsurface under anaerobic conditions and it appears that this biodegradation is principally coupled to methanogenic terminal oxidation processes (Jones et al., 2008). Furthermore, it appears that this methanogenic oil degradation has been a major factor in the development of the world's vast heavy oil deposits and represents a significant and ongoing process in conventional deposits today (Jones et al., 2008). To assess the extent of this process, petroleum geochemists use systematic changes in oil composition to produce indices of degradation. The most widely used of these is the Peters and Moldowan (PM) scale which ranges from 0 to 10 with most mass removal and the greatest compositional changes occurring prior to PM level 5 (Head et al., 2003). The typical order of compound removal observed during biodegradation follows the sequence n-alkanes, alkylcyclohexanes, acyclic isoprenoid alkanes, bicyclic alkanes, steranes, hopanes. Interestingly naphthalenes are removed at PM levels 2-5 and phenanthrenes at PM 4-6 and as such these compounds are not classified as being particularly resistant to biodegradative processes. We recently evaluated thermodynamic constraints on methanogenic crude oil degradation (Dolfing et al., 2008). In that study we focused on linear alkanes in the range C_8 to C_{80} . Here we evaluate thermodynamic constraints on methanogenic PAH degradation.

In this paper we use naphthalene as an example of a typical PAH and evaluate the thermodynamics of several possible routes of methanogenic PAH degradation, namely:

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Compound	Substrates		Products	kJ/reaction	kJ mol⁻¹	kJ mol⁻¹ CH₄
Naphthalene	$4C_{10}H_8 + 32H_2O$	\rightarrow	24CH ₄ + 16CO ₂	-835.1	-208.8	-34.8
Phenanthrene	$4C_{14}H_{10} + 46H_2O$	\rightarrow	33CH ₄ + 23CO ₂	-1064.5	-266.1	-32.3
Anthracene	$4C_{14}H_{10} + 46H_2O$	\rightarrow	33CH ₄ + 23CO ₂	-1166.8	-291.7	-35.4
Pyrene Chrysene	$4C_{16}H_{10} + 54H_2O$	\rightarrow	$37CH_4 + 27CO_2$	-1001.2 -1325 7	-250.3 -331 4	-27.1 -31.6

Table 1. Change in Gibbs free energy (ΔG°) values for the methanogenic conversion of selected PAHs.^a

a. Data for standard conditions (25°C, solutes at 1 M concentrations, and gases at a partial pressure of 1 atm).

(i) complete oxidation of PAHs to H_2 and CO_2 , linked to methanogenesis from CO_2 reduction;

	$\begin{array}{l} C_{10}H_8 + 20H_2O\\ 24H_2 + 6CO_2 \end{array}$	$\stackrel{\rightarrow}{\rightarrow}$	$10CO_2 + 24H_2$ $6CH_4 + 12H_2O$	(reaction 1) (reaction 2)
sum	$C_{10}H_8 + 8H_2O$	\rightarrow	$4CO_2 + 6CH_4$	(reaction 3)

(ii) oxidation of PAHs to acetate and H₂, linked to acetoclastic methanogenesis and CO₂ reduction:

	$\begin{array}{l} C_{10}H_8 + 10H_2O \\ 5CH_3COO^- + 5H^+ \\ 4H_2 + CO_2 \end{array}$	\rightarrow \rightarrow \rightarrow	$\begin{array}{l} 5CH_3COO^-+5H^++4H_2\\ 5CO_2+5CH_4\\ CH_4+2H_2O \end{array}$	(reaction 4) (reaction 5) (reaction 6)
sum	$C_{10}H_8 + 8H_2O$	\rightarrow	4CO ₂ + 6CH ₄	

or (ii_a) oxidation of PAHs to acetate and H_2 , linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction;

	$C_{10}H_8 + 10H_2O$ 5CH ₃ COO ⁻ + 5H ⁺ + 10H ₂ O	$\stackrel{\rightarrow}{\rightarrow}$	$\begin{array}{l} 5CH_{3}COO^{-}+5H^{+}+4H_{2}\\ 10CO_{2}+20H_{2} \end{array}$	(reaction 7)
	$24H_2 + 6CO_2$	\rightarrow	$6CH_4 + 12H_2O$	
sum	C10H8 + 8H2O	\rightarrow	4CO ₂ + 6CH ₄	

(iii) oxidation of PAHs to acetate alone, linked to acetoclastic methanogenesis;

	$\begin{array}{c} C_{10}H_8 + 8H_2O + 2CO_2 \\ 6CH_3COO^- + 6H^+ \end{array}$	$\stackrel{\rightarrow}{\rightarrow}$	$\begin{array}{l} 6CH_3COO^-+6H^+\\ 6CO_2+6CH_4 \end{array}$	(reaction 8) (reaction 9)
sum	$C_{10}H_8 + 8H_2O$	\rightarrow	$4CO_2 + 6CH_4$	

and (iii_a) oxidation of PAHs to acetate alone, linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction.

	$C_{10}H_8 + 8H_2O + 2CO_2$ $6CH_3COO^- + 6H^+$ $+ 12H_2O$	$\stackrel{\rightarrow}{\rightarrow}$	$\begin{array}{l} 6CH_{3}COO^{-}+6H^{+}\\ 12CO_{2}+24H_{2} \end{array}$	(reaction 10)
	$24H_2 + 6CO_2$	\rightarrow	$6CH_4 + 12H_2O$	(reaction 11)
sum	$C_{10}H_8 + 8H_2O$	\rightarrow	4CO ₂ + 6CH ₄	

Our analysis shows that methanogenic PAH degradation is exergonic and that PAH degradation would not necessarily be a syntrophic process in the traditional sense: a pathway via acetate only would allow stable PAH degradation with only a minor role for interspecies acetate transfer.

Results

Energetics of methanogenic PAH degradation

Thermodynamic calculations for the methanogenic degradation of five different PAHs (naphthalene, phenanthrene, anthracene, pyrene and chrysene) yielded ΔG° values in the range of –208.8 to –331.4 kJ mol⁻¹ (Table 1). Calculated on a per mole CH₄ produced basis this range collapsed to –27.1 to –34.8 kJ mol⁻¹. The change in Gibbs free energy values per mole of CH₄ produced increased with increasing C/H ratios: the less hydrogen substituents present on the aromatic ring, the more energy available from the methanogenic degradation of these compounds (Fig. 1).

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

Complete oxidation of PAHs to H₂ and CO₂ is an endergonic reaction under standard conditions, with ΔG^{or} values ranging between 575.7 and 1041.4 kJ mol⁻¹ PAH



Fig. 1. Gibbs free energy change for methanogenic degradation of PAHs as function of the C/H ratio.

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Table 2. Change in Gibbs free energy ($\Delta G^{\circ'}$) values for the complete oxidation of selected PAHs to H₂ and CO₂ and the hydrogen partial pressure below which the reaction becomes exergonic.^a

Compound	Substrates		Products	kJ/reaction	kJ mol ⁻¹ H ₂	H_2 threshold ^b	H₂ threshold ^c
Naphthalene	C ₁₀ H ₈ + 20H ₂ O	\rightarrow	10CO ₂ + 24H ₂	575.7	24.0	-4.20	-4.35
Phenanthrene	$C_{14}H_{10} + 28H_2O$	\rightarrow	$14CO_2 + 33H_2$	812.5	24.6	-4.31	-4.47
Anthracene	$C_{14}H_{10} + 28H_2O$	\rightarrow	14CO ₂ + 33H ₂	787.0	23.8	-4.18	-4.37
Pyrene	$C_{16}H_{10} + 32H_2O$	\rightarrow	16CO ₂ + 37H ₂	959.1	25.9	-4.54	-4.71
Chrysene	$C_{18}H_{12} + 36H_2O$	\rightarrow	18CO ₂ + 42H ₂	1041.4	24.8	-4.34	-4.53

a. Data for standard conditions (25°C, solutes at 1 M concentrations, and gases at a partial pressure of 1 atm, pH = 7).

b. log H_2 (atm).

c. H₂ threshold (log H₂ in atm) when the PAH is present at its aqueous solubility.

Table 3. Change in Gibbs free energy ($\Delta G^{o'}$) values for the incomplete oxidation of selected PAHs to acetate and H₂.^a

Compound	Substrates		Products	kJ/reaction	kJ mol⁻¹ acetate	kJ mol⁻¹ H₂
Naphthalene	$C_{10}H_8 + 10H_2O$	\rightarrow	5CH ₃ COO ⁻ + 5H ⁺ + 4H ₂	101.1	20.2	25.3
Phenanthrene	$C_{14}H_{10} + 14H_2O$	\rightarrow	7CH ₃ COO ⁻ + 7H ⁺ + 5H ₂	148.1	21.2	29.6
Anthracene	$C_{14}H_{10} + 14H_2O$	\rightarrow	7CH ₃ COO ⁻ + 7H ⁺ + 5H ₂	122.5	17.5	24.5
Pyrene	$C_{16}H_{10} + 16H_2O$	\rightarrow	8CH ₃ COO ⁻ + 8H ⁺ + 5H ₂	199.8	25.0	40.0
Chrysene	$C_{18}H_{12} + 18H_2O$	\rightarrow	$9CH_3COO^- + 9H^+ + 6H_2$	187.2	20.8	31.2

a. Data for standard conditions (25°C, solutes at 1 M concentrations, and H₂ at a partial pressure of 1 atm, pH = 7).

(Table 2). Per mole of hydrogen produced the range is 23.8–25.9 kJ mol⁻¹. Methanogenic hydrogen removal has a ΔG° of 32.7 kJ mol⁻¹ H₂. Thus coupling complete oxidation of PAHs to H₂ and CO₂ to methanogenic CO₂ reduction makes the total reaction exergonic, as long as ΔG° for oxidation of PAHs to H₂/CO₂ is less than 32.7 kJ mol⁻¹ H₂ produced.

When the H_2 concentration is taken into account, the energy yield from complete oxidation of PAHs decreases with increasing H_2 concentration, whereas methanogenic CO_2 reduction becomes more exergonic with increasing H_2 concentration. Figure 2 presents the 'window of opportunity' for naphthalene, defined by the H_2 concentrations where both processes are exergonic.

The H₂ threshold values below which complete oxidation of PAHs becomes exergonic under otherwise standard conditions are between log H₂ = -4.18 atm and log H₂ = -4.54 atm (i.e. between 2.9 and 6.6 Pa) (Table 2). When calculated for PAHs at their aqueous solubility the picture is essentially the same with H₂ threshold values between 2.0 and 4.5 Pa (Table 2).

Oxidation of PAHs to acetate and H₂

Under standard conditions, oxidation of PAHs to acetate and H₂ is an exergonic process and costs between 101.1 and 199.9 kJ mol⁻¹ depending on the PAH degraded (Table 3). Per mole of acetate produced the costs are between 17.5 and 25.0 kJ mol⁻¹, while the costs per mole of H₂ produced are between 25.3 and 40.0 kJ mol⁻¹. The stoichiometry of the reaction $2C_aH_b + 2aH_2O \rightarrow$ $aCH_3COO^- + aH^+ + bH_2$ implies that the molar ratio of the amounts of acetate and H₂ produced from PAH degradation are identical to the C/H ratio of the parent compound. For the five PAHs evaluated here these ratios range between 1.25 for naphthalene and 1.60 for pyrene. This implies that the actual change in Gibbs free energy for PAH degradation to acetate and H₂ under *in situ* conditions is more strongly dependent on the acetate concentration than on the H₂ concentration.



Fig. 2. Effect of hydrogen partial pressure on the change in Gibbs free energy for oxidation of naphthalene to H₂ and CO₂ (open symbols) and for stoichiometric methanogenesis of the hydrogen produced (closed symbols). The arrows delineate the 'window of opportunity' where both reactions are exergonic. Reactions considered: $C_{10}H_8 + 20 H_2O \rightarrow 24H_2 + 10CO_2$; $24H_2 + 6CO_2 \rightarrow 6CH_4 + 12H_2O$. The dotted line illustrates that the sum of the Gibbs free energy changes is constant and equal to the change in Gibbs free energy for methanogenic naphthalene degradation (-208.8 kJ mol⁻¹; see Table 1).

Table 4. Change in Gibbs free energy ($\Delta G^{o'}$) values for the complete oxidation of selected PAHs to acetate, and the acetate concentration below which the reaction becomes exergonic.^a

Compound	Substrates		Products	kJ/reaction	kJ mol⁻¹	kJ mol ⁻¹ acetate	Acetate threshold ^b
Naphthalene	4C ₁₀ H ₈ + 32H ₂ O + 8CO ₂	\rightarrow	24CH₃COO ⁻ + 24H ⁺	24.8	6.2	1.0	-0.18
Phenanthrene	$4C_{14}H_{10} + 46H_2O + 10CO_2$	\rightarrow	33CH ₃ COO ⁻ + 33H ⁺	117.9	29.5	3.6	-0.63
Anthracene	$4C_{14}H_{10} + 46H_2O + 10CO_2$	\rightarrow	33CH ₃ COO ⁻ + 33H ⁺	15.6	3.9	0.5	-0.08
Pyrene	$4C_{16}H_{10} + 54H_2O + 10CO_2$	\rightarrow	37CH ₃ COO ⁻ + 37H ⁺	324.5	81.1	8.8	-1.54
Chrysene	$4C_{18}H_{12}+60H_2O+12CO_2$	\rightarrow	$42CH_3COO^-+42H^+$	179.2	44.8	4.3	-0.75

a. Data for standard conditions (25°C, solutes at 1 M concentrations, and CO₂ at a partial pressure of 1 atm, pH = 7).
 b. log acetate (M).

Oxidation of PAHs to acetate alone

The Δ G values for the oxidation of PAHs to acetate range between 3.9 kJ mol⁻¹ (for anthracene) and 81.1 kJ mol⁻¹ (for pyrene). Expressed per mole of acetate produced the range is 0.5–8.8 kJ mol⁻¹ (Table 4). The acetate thresholds below which the reaction becomes exergonic range between 29 and 827 mM. This implies that in most methanogenic ecosystems oxidation of PAHs to acetate alone will be an exergonic reaction.

The thermodynamic landscape of methanogenic PAH degradation

The windows of opportunity with respect to acetate and H_2 have been summarized for the range of processes that are presumably involved in methanogenic PAH degradation (Figs 3-5). Figure 3 elaborates the case for methanogenic naphthalene degradation. This analysis allows identification of clear zones where different methanogenic phenanthrene degradation pathways can occur. For example, the window of opportunity linking conversion of naphthalene to acetate with acetoclastic methanogenesis (domain I to IV and VI in Fig. 3) is much larger than the equivalent window for linking incomplete oxidation of naphthalene to both acetoclastic methanogenesis and methanogenic CO2 reduction (domain II, III and IV and VI in Fig. 3), or complete oxidation of naphthalene linked to methanogenic CO₂ reduction (domain II, IV and V in Fig. 3). This is summarized in Fig. 4. A comparison of Fig. 3 and Fig. 5 shows that this observation holds for all five PAHs evaluated here.

While Fig. 3 gives information on the domains where the various routes of naphthalene degradation are exergonic, it does not give information on the actual energy yield in the various domains. This information is depicted in Fig. 6. The graph shows that depending on the actual H_2 and acetate concentrations complete oxidation either to H_2/CO_2 or to acetate is the energetically most favourable route for the naphthalene degraders. The route via incomplete oxidation to H_2 plus acetate is always second best. The one exception to this rule is for the conditions where acetate

and H_2/CO_2 are in thermodynamic equilibrium, i.e. where $\Delta G' = 0$ for $CH_3COO^- + H^+ + 2H_2O \rightarrow 4H_2 + 2CO_2$ (the line separating domains III and IV in Figs 3 and 4). In Fig. 6 this is the line where the planes representing the energy yields of the various routes intersect.

Discussion

Microbial degradation of PAHs under methanogenic conditions is potentially of global significance given that the bulk of the world's oil reserves are biodegraded and that this degradation process involves the removal of PAHs (Head et al., 2003). In addition, PAHs are widespread environmental contaminants and due to their low water solubility and high octanol-water partition coefficients they tend to accumulate in anaerobic environments such as sediments and soils. It has long been thought that these compounds are inert in the absence of molecular oxygen, especially under methanogenic conditions, but there are scattered reports that this is not necessarily the case (Chang et al., 2002; 2003; 2005; 2006; Christensen et al., 2004; Foght, 2008; Fuchedzhieva et al., 2008). The thermodynamic calculations presented here indicate that thermodynamics is not an impediment to the biodegradation of PAHs under methanogenic conditions: energy yields of about 30 kJ mol⁻¹ CH₄ indicate that methanogenic PAH degradation is an exergonic process. However, it should also be taken into consideration that these 30 kJ mol-1 CH₄ have to be shared by at least two and probably three organisms. Against this background, i.e. as a strategy to minimize energy sharing, it is tempting to speculate that PAH degradation proceeds via complete oxidation to H₂ or via complete conversion to acetate coupled to the conversion of these substrates to methane. The free energy calculations indicate that conversion of PAHs to acetate is already exergonic at rather high acetate concentrations. This would allow methanogenic PAH degradation to proceed as an exergonic process under widely varying acetate concentrations. The caveat here though is that the PAH degraders would have to use CO₂ as an external electron acceptor. In this sense, the PAH degraders would simultaneously act as PAH degrader and 'acetogen' by



Fig. 3. Hydrogen and acetate as thermodynamic constraints on methanogenic naphthalene degradation.

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virtue of an ability to form acetate from bicarbonate and H_2 . There are, however, reports in the literature that state that there is probably an optimal pathway length for individual organisms, and combining these two functions (e.g. PAH degrader and acetogen) in one organism may result in suboptimal allocation of resources (Dolfing, 2001; Costa *et al.*, 2006). A similar line of reasoning applies to the complete oxidation of PAHs to H_2 and CO₂. Here the presumed PAH degrader would not merely produce the typical fermentation products H_2 and acetate, but would have to go through an extra steps to oxidize the acetate that is a typical intermediate in most anaerobic degradation pathways to H_2 and CO₂. Thus it seems most likely that PAHs are converted to acetate and H_2 .

For the routes where PAHs degradation is a hydrogenogenic process H_2 removal is a prerequisite for sustained PAH degradation, which implies syntrophy. When acetate is the sole product, methanogenic activity would not be necessary to sustain PAH degradation. Observations that bromoethanesulfonic acid, a selective inhibitor of methanogenesis, inhibited the degradation of 200 μ M naphthalene and phenanthrene in methanogenic PAH degrading enrichment cultures suggest that a hydrogenogenic pathway operated in these enrichment cultures (Chang *et al.*, 2006).

The general sequence of removal of PAHs during crude oil and natural gas biodegradation is naphthalenes, phenanthrenes, chrysenes (Head *et al.*, 2003). Interestingly the Gibbs free energy yields per mole of methane produced for methanogenic degradation of PAHs follows exactly the same order. This mirrors observations that in natural environments the electron acceptor with the highest redox potential and therefore the highest energy yield is used preferentially, followed by those of decreasing redox potential, and, although the actual yield differences are quite small, makes it tempting to speculate that degradation of PAHs in subsurface oil reservoirs is under thermodynamic control.

Experimental procedures

Background

The amount of free energy available from a reaction depends on the Gibbs free energies of formation of substrates and products as given by the relationship $\Delta G^{\circ} = \Sigma \Delta G_{\ell}^{\circ}(products) - \Sigma \Delta G_{\ell}^{\circ}(substrates)$. ΔG° is the increment in free energy for the reaction under standard conditions. For biological systems the conventional standard conditions are 25°C and a pressure of 1 atm. In aqueous solutions the standard condition of all solutes is 1 mol kg⁻¹ activity, that of water is the pure liquid (Thauer *et al.*, 1977). Under environmentally relevant conditions the concentrations of substrates and products are not 1 mol kg⁻¹. This is considered in $\Delta G'$ values. For

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Fig. 5. Hydrogen and acetate as thermodynamic constraints on methanogenic degradation of phenanthrene, anthracene, pyrene and chrysene.

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a hypothetical reaction $aA + bB \rightarrow cC + dD$, $\Delta G'$ values are calculated by using the mass equation

$$\Delta G' = \Delta G^{o'} + RT \ln \frac{[C]^{c}[D]^{a}}{[A]^{a}[B]^{b}}$$
⁽¹⁾

The $\Delta G^{o'}$ value is obtained from the ΔG^{o} value by making the appropriate corrections for pH = 7.

Notation

The format of the ΔG values used in the present paper is similar to that used by Thauer and colleagues (1977) in that the values are given for aqueous solutions of 1 mol kg⁻¹ activity. For the ΔG_{t}^{ρ} values of the dissolved species at unit molality, the ΔG_{t}^{ρ} values of the liquid phase are corrected by using the equation

$$\Delta G^o_{f \ dissolved \ species} = \Delta G^o_{f \ liquid} - RT \ln C^{sat}_w \tag{2}$$

where C_{wat}^{ext} is the aqueous solubility of the liquid. The main advantage of this way of presenting free energy of formation data is that extrapolation to *in situ* conditions can be easily done by simply substituting the actual concentration for the standard concentration in Eq. 1 (Dolfing and Harrison, 1992).

Gibbs free energy of formation values for PAHs in the aqueous phase

Gibbs free energy of formation $(\Delta G_{f}^{\circ}(I))$ values for naphthalene, phenanthrene, anthracene, pyrene and chrysene were taken from Richard and Helgeson (1998) and converted to $\Delta G_{f}^{\circ}(aq)$ by using Eq. 2, with aqueous solubility data taken from Sverdrup and colleagues (2002). The values are presented in Table 5.

All other Gibbs free energy of formation data used in the present study were taken from Thauer and colleagues (1977).

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Table 5. Aqueous solubility and Gibbs free energy of formation values for selected PAHs in the liquid and the aqueous phase.^a

Compound	$\Delta G_{f}^{o}(I)$	$-\log C_w^{sat}$	$\Delta G_{f}^{o}(aq)$
Naphthalene	203.67	3.61	224.26
Phenanthrene	278.07	5.14	307.24
Anthracene	296.52	6.39	332.99
Pyrene	285.57	6.18	320.83
Chrysene	352.51	8.06	398.52

a. Aqueous solubility at 25°C (mol l⁻¹); data from Sverdrup and colleagues (2002). Gibbs free energy of formation ($_{\Delta G_{\ell}^{\circ}}$, kJ mol⁻¹) under standard conditions at 25°C as liquid (Helgeson *et al.* 1998) and at an aqueous concentration of 1 M.

Sample calculation for threshold values

The change in Gibbs free energy ($\Delta G^{o'}$) for the conversion of acetate into methane and carbon dioxide according to CH₃COO⁻ + H⁺ \rightarrow CH₄ + CO₂ is -35.8 kJ mol⁻¹ CH₄ (Thauer *et al.*, 1977). Hence at pH = 7 $\Delta G'$ = -35.8 – RTIn $\frac{[CH_3COO^-]}{[CO_2][CH_4]}$. Therefore, under otherwise standard conditions, $\Delta G'$ = -35.8 –5.71 log [CH₃COO⁻] (where 5.71 log x equals RT_{298.15}lnx). As the threshold value is the value where $\Delta G'$ = 0 it follows that [CH₃COO⁻] = 10^(-35.8/5.71) = 10^{-6.27}. Thus [acetate]_{crit} = 0.54 µM.

General approach

Change in Gibbs free energy calculations for PAH degradation were made based on the following stoichiometry.

8C _a H _b + 16aH ₂ O	\rightarrow	8aCO ₂ + (16a + 4b)H ₂	(reaction 12)
8C _a H _b + (8a – 2b)H ₂ O	\rightarrow	(4 <i>a</i> + <i>b</i>)CH ₃ COO ⁻ +	(reaction 13)
+ 2 <i>b</i> CO ₂		$(4a + b)H^+$	
8C _a H _b + 8 <i>a</i> H ₂ O	\rightarrow	4 <i>a</i> CH ₃ COO ⁻ + 4 <i>a</i> H ⁺	(reaction 14)
		+ 4 <i>b</i> H ₂	
8C _a H _b + (8a – 2b)H ₂ O	\rightarrow	(4 <i>a</i> + <i>b</i>)CH ₄ +	(reaction 15)
		$(4a - b)CO_2$	

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Based on these equations the H_2 concentration below which hydrocarbon oxidation becomes exergonic is:

log H₂ = $X_1/5.71^*(16a + 4b)$, with H₂ in atm, where $X_1 = \Delta G^{\circ}$ for reaction 12.

Similarly the acetate concentration below which reaction 13 becomes exergonic is:

log [Acetate] = $X_2/5.71^*(4a + b)$, with [Acetate] in M, where $X_2 = \Delta G^{o'}$ for reaction 13.

The combinations of hydrogen and acetate concentrations below which reaction 14 becomes exergonic are given by:

log [Acetate] = $-(b/a)^*\log H_2 + X_3/5.71^*4a$, with H₂ in atm and [Acetate] in M, where $X_3 = \Delta G^{o'}$ for reaction 14.

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