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 pathways 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 PAH-based 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 H₂ 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 C₈ to C₈₀. 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:
Table 1. Change in Gibbs free energy ($\Delta G^\circ$) values for the methanogenic conversion of selected PAHs.\textsuperscript{a}

| Compound    | Substrates          | Products                  | kJ/reaction | kJ mol$^{-1}$ CH$_4$ |
|-------------|---------------------|---------------------------|-------------|----------------------|
| Naphthalene | 4C$_6$H$_8$ + 32H$_2$O $\rightarrow$ 24CH$_4$ + 16CO$_2$ | $\rightarrow$ 24CH$_4$ + 16CO$_2$ | $-385.1$  | $-208.8$  |
| Phenanthrene| 4C$_6$H$_2$ + 46H$_2$O $\rightarrow$ 10CO$_2$ + 24H$_2$ | $\rightarrow$ 10CO$_2$ + 24H$_2$ | $-206.5$  | $-32.3$ |
| Anthracene  | 4C$_2$H$_2$ + 54H$_2$O $\rightarrow$ 33CH$_4$ + 23CO$_2$ | $\rightarrow$ 33CH$_4$ + 23CO$_2$ | $-1166.8$ | $-291.7$  |
| Pyrene      | 4C$_2$H$_2$ + 60H$_2$O $\rightarrow$ 37CH$_4$ + 27CO$_2$ | $\rightarrow$ 37CH$_4$ + 27CO$_2$ | $-1001.2$ | $-250.3$  |
| Chrysene    | 4C$_2$H$_2$ + 60H$_2$O $\rightarrow$ 42CH$_4$ + 30CO$_2$ | $\rightarrow$ 42CH$_4$ + 30CO$_2$ | $-1325.7$ | $-331.4$  |

\textsuperscript{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:

\[
\begin{align*}
C_6H_6 + 20H_2O & \rightarrow 10CO_2 + 24H_2 \quad \text{(reaction 1)} \\
24H_2 + 6CO_2 & \rightarrow 6CH_4 + 12H_2O \quad \text{(reaction 2)} \\
\text{sum} \quad C_6H_6 + 8H_2O & \rightarrow 4CO_2 + 6CH_4 \quad \text{(reaction 3)}
\end{align*}
\]

(ii) oxidation of PAHs to acetate and H$_2$, linked to aceto-clastic methanogenesis and CO$_2$ reduction:

\[
\begin{align*}
C_6H_6 + 10H_2O & \rightarrow 5C_2H_4COO^- + 5H^+ + 4H_2 \quad \text{(reaction 4)} \\
5CH_2COO^- + 5H^+ & \rightarrow 5CO_2 + 5CH_4 \quad \text{(reaction 5)} \\
4H_2 + CO_2 & \rightarrow CH_4 + 2H_2O \quad \text{(reaction 6)} \\
\text{sum} \quad C_6H_6 + 8H_2O & \rightarrow 4CO_2 + 6CH_4
\end{align*}
\]

or (ii) oxidation of PAHs to acetate and H$_2$, linked to syntrophic acetate oxidation and methanogenesis from CO$_2$ reduction:

\[
\begin{align*}
C_6H_6 + 10H_2O & \rightarrow 5C_2H_4COO^- + 5H^+ + 4H_2 \quad \text{(reaction 7)} \\
5CH_2COO^- + 5H^+ & \rightarrow 10CO_2 + 20H_2 \\
24H_2 + 6CO_2 & \rightarrow 6CH_4 + 12H_2O \quad \text{(reaction 8)} \\
\text{sum} \quad C_6H_6 + 8H_2O & \rightarrow 4CO_2 + 6CH_4
\end{align*}
\]

(iii) oxidation of PAHs to acetate alone, linked to aceto-clastic methanogenesis:

\[
\begin{align*}
C_6H_6 + 8H_2O + 2CO_2 & \rightarrow 6CH_2COO^- + 6H^+ \quad \text{(reaction 9)} \\
6CH_2COO^- + 6H^+ & \rightarrow 6CO_2 + 6CH_4 \quad \text{(reaction 10)} \\
\text{sum} \quad C_6H_6 + 8H_2O & \rightarrow 4CO_2 + 6CH_4
\end{align*}
\]

and (iii) oxidation of PAHs to acetate alone, linked to syntrophic acetate oxidation and methanogenesis from CO$_2$ reduction.

\[
\begin{align*}
C_6H_6 + 8H_2O + 2CO_2 & \rightarrow 6CH_2COO^- + 6H^+ \quad \text{(reaction 11)} \\
6CH_2COO^- + 6H^+ & \rightarrow 12CO_2 + 24H_2 \\
24H_2 + 6CO_2 & \rightarrow 6CH_4 + 12H_2O \quad \text{(reaction 12)} \\
\text{sum} \quad C_6H_6 + 8H_2O & \rightarrow 4CO_2 + 6CH_4
\end{align*}
\]

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 $\Delta G^\circ$ values in the range of $-208.8$ to $-331.4$ kJ mol$^{-1}$ (Table 1). Calculated on a per mole CH$_4$ produced basis this range collapsed to $-27.1$ to $-34.8$ kJ mol$^{-1}$. The change in Gibbs free energy values per mole of CH$_4$ 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 H$_2$ and CO$_2$ is an endergonic reaction under standard conditions, with $\Delta G^\circ$ values ranging between 575.7 and 1041.4 kJ mol$^{-1}$ PAH
Table 2. Change in Gibbs free energy ($\Delta G'$) values for the complete oxidation of selected PAHs to $H_2$ and $CO_2$ and the hydrogen partial pressure below which the reaction becomes exergonic.\textsuperscript{a}

| Compound     | Substrates       | Products                | $kJ$/reaction | $kJ$ mol$^{-1}$ $H_2$ | $H_2$ threshold\textsuperscript{b} | $H_2$ threshold\textsuperscript{c} |
|--------------|------------------|-------------------------|---------------|-----------------------|--------------------------------------|--------------------------------------|
| Naphthalene  | $C_10H_8 + 20H_2O$ → $10CO_2 + 24H_2$ | 575.7                  | 24.0          | –4.20                 | –4.35                                |                                      |
| Phenanthrene | $C_14H_10 + 28H_2O$ → $14CO_2 + 33H_2$ | 812.5                  | 24.6          | –4.31                 | –4.47                                |                                      |
| Anthracene   | $C_14H_10 + 28H_2O$ → $14CO_2 + 33H_2$ | 787.0                  | 23.8          | –4.18                 | –4.37                                |                                      |
| Pyrene       | $C_16H_12 + 32H_2O$ → $16CO_2 + 37H_2$ | 959.1                  | 25.9          | –4.54                 | –4.71                                |                                      |
| Chrysene     | $C_{18}H_{12} + 36H_2O$ → $18CO_2 + 42H_2$ | 1041.4                 | 24.8          | –4.34                 | –4.53                                |                                      |

\textsuperscript{a} Data for standard conditions (25°C, solutes at 1 M concentrations, and gases at a partial pressure of 1 atm, pH = 7).
\textsuperscript{b} log $H_2$ (atm).
\textsuperscript{c} $H_2$ threshold (log $H_2$ in atm) when the PAH is present at its aqueous solubility.

Table 3. Change in Gibbs free energy ($\Delta G'$) values for the incomplete oxidation of selected PAHs to acetate and $H_2$.\textsuperscript{a}

| Compound     | Substrates       | Products                  | $kJ$/reaction | $kJ$ mol$^{-1}$ acetate | $kJ$ mol$^{-1}$ $H_2$ |
|--------------|------------------|---------------------------|---------------|-------------------------|-----------------------|
| Naphthalene  | $C_10H_8 + 10H_2O$ → $5CH_3COO^- + 5H^+ + 4H_2$ | 101.1                  | 20.2          | 25.3                    |                                      |
| Phenanthrene | $C_{14}H_{12} + 14H_2O$ → $7CH_3COO^- + 7H^+ + 5H_2$ | 148.1                  | 21.2          | 29.6                    |                                      |
| Anthracene   | $C_{14}H_{12} + 14H_2O$ → $7CH_3COO^- + 7H^+ + 5H_2$ | 122.5                  | 17.5          | 24.5                    |                                      |
| Pyrene       | $C_{16}H_{14} + 18H_2O$ → $8CH_3COO^- + 8H^+ + 5H_2$ | 199.8                  | 25.0          | 40.0                    |                                      |
| Chrysene     | $C_{18}H_{12} + 18H_2O$ → $9CH_3COO^- + 9H^+ + 6H_2$ | 187.2                  | 20.8          | 31.2                    |                                      |

\textsuperscript{a} Data for standard conditions (25°C, solutes at 1 M concentrations, and $H_2$ 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 $\Delta G'$ of 32.7 $kJ$ mol$^{-1}$ $H_2$. Thus coupling complete oxidation of PAHs to $H_2$ and $CO_2$ to methanogenic $CO_2$ reduction makes the total reaction exergonic, as long as $\Delta G'$ for oxidation of PAHs to $H_2/CO_2$ is less than 32.7 $kJ$ mol$^{-1}$ $H_2$ 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 $H_2$ threshold values below which complete oxidation of PAHs becomes exergonic under otherwise standard conditions are between log $H_2 = –4.18$ atm and log $H_2 = –4.54$ 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 $H_2$ threshold values between 2.0 and 4.5 Pa (Table 2).

**Oxidation of PAHs to acetate and $H_2$**

Under standard conditions, oxidation of PAHs to acetate and $H_2$ 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 $H_2$ produced are between 25.3 and 40.0 $kJ$ mol$^{-1}$. The stoichiometry of the reaction $2C_\alpha H_\beta + 2aH_2O \rightarrow aCH_3COO^- + aH^+ + bH_2$ implies that the molar ratio of the amounts of acetate and $H_2$ produced from PAH degradation is 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 $H_2$ under *in situ* conditions is more strongly dependent on the acetate concentration than on the $H_2$ concentration.

![Fig. 2. Effect of hydrogen partial pressure on the change in Gibbs free energy for oxidation of naphthalene to $H_2$ and $CO_2$ (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: $C_\alpha H_\beta + 20H_2O \rightarrow 24H_2 + 10CO_2$; $24H_2 + 6CO_2 \rightarrow 6CH_4 + 12H_2O$. 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).](image-url)
Anthracene 4C 14H10
Phenanthrene 4C 14H10
Chrysene 4C 18H12

b. log acetate (M).

The one naphthalene degraders. The route via incomplete oxidation of acetate is the energetically most favourable route for the 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 H₂ and acetate concentrations complete oxidation either to H₂/CO₂ or to acetate is the energetically most favourable route for the naphthalene degraders. The route via incomplete oxidation to H₂ plus acetate is always second best. The one exception to this rule is for the conditions where acetate and H₂/CO₂ are in thermodynamic equilibrium, i.e. where ΔG° = 0 for CH₃COO⁻ + H⁺ + 2H₂O → 4H₂ + 2CO₂ (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...

Table 4. Change in Gibbs free energy (ΔG°) 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 acetate | Acetate thresholdb |
|--------------|-----------------------------------|---------------------------|-------------|-----------------|-------------------|
| Naphthalene  | 4C₆H₈ + 32H₂O + 5CO₂              | 24CH₃COO⁻ + 24H₂⁺         | 24.8        | 6.2             | 1.0               | -0.18             |
| Phenanthrene | 4C₆H₈ + 46H₂O + 10CO₂             | 33CH₃COO⁻ + 33H₂⁺         | 117.9       | 29.5            | 3.6               | -0.63             |
| Anthracene   | 4C₁₄H₁₀ + 46H₂O + 10CO₂            | 33CH₃COO⁻ + 33H₂⁺         | 15.6        | 3.9             | 0.5               | -0.08             |
| Pyrene       | 4C₁₄H₁₀ + 54H₂O + 10CO₂            | 37CH₃COO⁻ + 37H₂⁺         | 324.5       | 81.1            | 8.8               | -1.54             |
| Chrysene     | 4C₁₈H₁₂ + 60H₂O + 12CO₂            | 42CH₃COO⁻ + 42H₂⁺         | 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 ΔG 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 H₂ 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.
Fig. 3. Hydrogen and acetate as thermodynamic constraints on methanogenic naphthalene degradation.

\[ \Delta G < 0 \]

\[ C_{10}H_8 + 8H_2O + 2CO_2 \rightarrow 6CH_3COO^- + 6H^+ \]

\[ C_{10}H_8 + 10H_2O \rightarrow 5CH_3COO^- + 5H^+ + 4H_2 \]

\[ C_{10}H_8 + 20H_2O \rightarrow 10CO_2 + 24H_2 \]

\[ 4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O \]

\[ CH_3COO^- + H^+ + 2H_2O \rightarrow 4H_2 + 2CO_2 \]

\[ CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \]
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 CO$_2$. 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 CO$_2$. 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 μM 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

$$
\Delta G^\circ = \sum \Delta G^\circ(products) - \sum \Delta G^\circ(substrates)
$$

$\Delta G^\circ$ 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 $\Delta G^\circ$ values. For
Fig. 5. Hydrogen and acetate as thermodynamic constraints on methanogenic degradation of phenanthrene, anthracene, pyrene and chrysene.
a hypothetical reaction $aA + bB \rightarrow cC + dD$. $\Delta G'$ values are calculated by using the mass equation

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

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

**Notation**

The format of the $\Delta G$ 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 $\Delta G^o_s$ values of the dissolved species at unit molality, the $\Delta G^o_s$ values of the liquid phase are corrected by using the equation

$$\Delta G^o_{\text{dissolved species}} = \Delta G^o_{\text{liquid}} - RT \ln C^a_w$$  \hspace{1cm} (2)$$

where $C^a_w$ 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 ($\Delta G^o/(l)$) values for naphthalene, phenanthrene, anthracene, pyrene and chrysene were taken from Richard and Helgeson (1998) and converted to $\Delta G^o/(aq)$ 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).

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

| Compound          | $\Delta G^o/(l)$ | $-\log C^a_w$ | $\Delta G^o/(aq)$ |
|-------------------|------------------|---------------|------------------|
| 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 ($\Delta G^o$, 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 ($\Delta G'$) for the conversion of acetate into methane and carbon dioxide according to $\text{CH}_3\text{COO}^- + \text{H}^+ \rightarrow \text{CH}_4 + \text{CO}_2$ is $-35.8$ kJ mol$^{-1}$ (Thauer et al., 1977). Hence at pH = 7 $\Delta G' = -35.8 - RT \ln \frac{[\text{CO}_2][\text{H}_2]}{[\text{CH}_3\text{COO}^-]}$. Therefore, under otherwise standard conditions, $\Delta G' = -35.8 - 5.71 \log [\text{CH}_3\text{COO}^-]$ (where 5.71 logx equals $RT_{298.15}(\ln x)$). As the threshold value is the value where $\Delta G' = 0$ it follows that $[\text{CH}_3\text{COO}^-] = 10^{(35.8/5.71)} = 10^{-6.27}$. Thus [acetate]$_{\text{crit}} = 0.54$ μM.

**General approach**

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

8$\text{C}_8\text{H}_8 + 16\text{H}_2\text{O} \rightarrow 8\text{aCO}_2 + (16a + 4b)\text{H}_2$ (reaction 12)
8$\text{C}_8\text{H}_8 + (8a - 2b)\text{H}_2\text{O} \rightarrow (4a + b)\text{CH}_4\text{COO}^- + (4a + b)\text{H}^+$ (reaction 13)
8$\text{C}_8\text{H}_8 + 8a\text{H}_2\text{O} \rightarrow 4a\text{CH}_4\text{COO}^- + 4a\text{H}^+ + 42\text{H}_2$ (reaction 14)
8$\text{C}_8\text{H}_8 + (8a - 2b)\text{H}_2\text{O} \rightarrow (4a + b)\text{CH}_4 + (4a - b)\text{CO}_2$ (reaction 15)
Based on these equations the H2 concentration below which hydrocarbon oxidation becomes exergonic is:

\[ \log H_2 = \frac{X_1}{5.71} \left(16a + 4b\right) \]

with \( H_2 \) in atm, where \( X_1 = \Delta G'' \) for reaction 12.

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

\[ \log [\text{Acetate}] = \frac{X_2}{5.71} \left(4a + b\right) \]

with \([\text{Acetate}]\) in M, where \( X_2 = \Delta G'' \) for reaction 13.

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

\[ \log [\text{Acetate}] = -\left(\frac{b}{a}\right) \log H_2 + \frac{X_3}{5.71} \cdot 4a \]

with \( H_2 \) in atm and \([\text{Acetate}]\) in M, where \( X_3 = \Delta G'' \) for reaction 14.

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