Energetic and Other Quantitative Aspects of Microbial Hydrocarbon Utilization: An Introduction

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Abstract

Hydrocarbons represent "energy-rich" growth substrates for aerobic microorganisms and in principle allow high growth yields. In contrast, the energy gain with hydrocarbons in many anaerobic microorganisms is very low. The maximum gain of energy per mol of hydrocarbon degraded in the catabolism is predicted from calculated ΔG values. Some anaerobic degradation reactions of hydrocarbons with very low-energy gain as well as anaerobic activation reactions of hydrocarbons deserve particular attention from a bioenergetic point of view.

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1 Introduction

The study of microbial growth with hydrocarbons and their degradation often gets into energetic aspects, even though at a glance the metabolism of hydrocarbons is not basically different from that of other organic compounds. The overall metabolism in a chemotrophic organism follows the universal bifurcate carbon flow: One part of the carbon substrate together with sources of other elements (N, P, S, Fe, etc.) is used for synthesis of cell components, a process referred to as anabolism (synthetic metabolism, assimilation). The anabolic "upgrading" of the substrate requires and dissipates much energy, which is usually provided in the form of ATP and derived from another part of the carbon substrate. This part of the substrate necessarily undergoes degradation; the degradative substrate flow is referred to as catabolism (energy metabolism, dissimilation). Still, there are some energetic peculiarities in the metabolism of hydrocarbons which deserve attention. (1) First, even though flammability of hydrocarbons at the air implies "energy richness," they are not energy rich under all circumstances. In the absence of oxygen, hydrocarbons are less energy rich than for instance the less flammable glucose. Whereas the latter provides energy for various modes of fermentative growth, fermentation of saturated, aromatic, and many other unsaturated nonaromatic hydrocarbons is energetically not feasible[1]; this is one reason why they tend to be preserved in deep reservoirs. (2) Second, hydrocarbons are chemically unreactive at room temperature. Their use in the metabolism has to begin with an activation reaction, the introduction of a functional group, which may require and "waste" energy from the overall energy budget of the microorganism. Also energies of transition states in the activation reactions have been of interest for a mechanistic understanding. (3) Third, for the theoretical treatment of energy conservation with hydrocarbons as well as for the estimation of microbial cell mass involved in hydrocarbon (petroleum) bioremediation, growth yields (cell mass produced per amount of hydrocarbon utilized) are of interest. This chapter briefly addresses some of these energetic peculiarities and quantitative aspects of hydrocarbon metabolism (Fig. 1).

2 Some Basic Thermodynamic Aspects of Hydrocarbons

Hydrocarbons, the main constituents of oil and gas, are the major source of energy in our industrialized society. A prominent property of hydrocarbons is thus their "energy richness." More precisely, this term expresses that energy is released if they are oxidized with oxygen and that the amount of energy released per unit mass (the gravimetric energy density) of a liquid or solid hydrocarbon is higher than that from the oxidation of many other chemical compounds or elements (Appendix Table 5). In the case of gaseous hydrocarbons, a high volumetric energy density is

[1] A fermentable hydrocarbon is, for instance, the unsaturated acetylene. Also some other unsaturated hydrocarbons are, at least theoretically, fermentable.

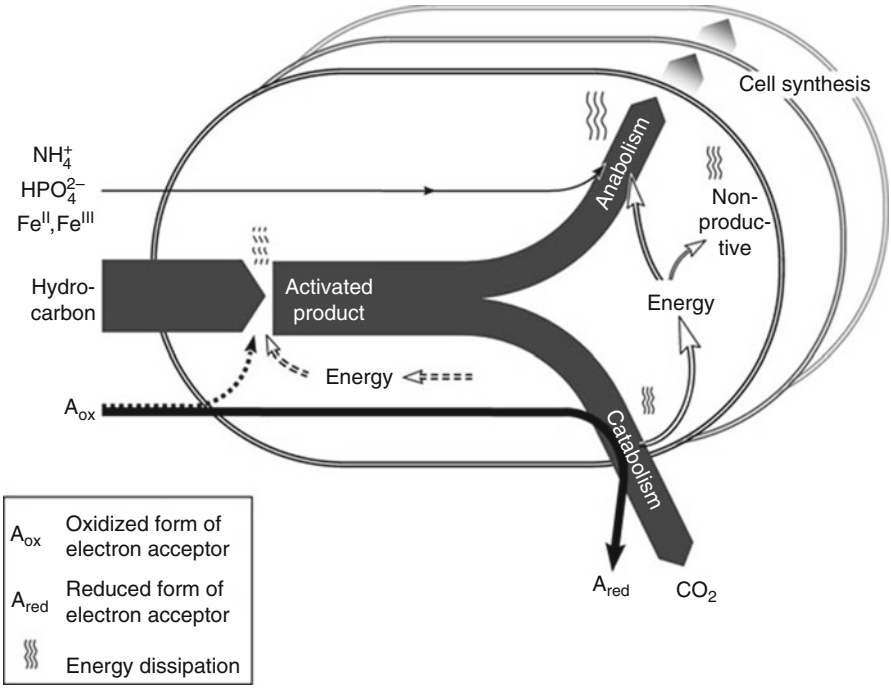

Fig. 1 The metabolism of hydrocarbons in chemotrophic microorganisms follows the universal bifurcate substrate flow into cell synthesis and degradation. A peculiarity in comparison to the metabolism of most non-hydrocarbon substrates is the activation which may require and dissipate energy

obvious if compared to that of other gases (Appendix Table 5). This "energy richness" is due to the high affinity of the two constituents, hydrogen and carbon, for oxygen and to the absence of oxidized carbon groups (such as $C-OH$ or $C=O$ groups). The low atomic masses of hydrogen and carbon[2] is another factor that contributes to the high gravimetric energy density. It is the high gravimetric energy density which, together with the abundance of hydrocarbons in the form of petroleum, has made them ideal fuels for vehicles and aircrafts. Another technical advantage is the formation of volatile products (CO_2, H_2O vapor).

Feasibility and maximum energy gains of formulated stoichiometric reactions are expressed by their free energy changes, the ΔG -values. If a reaction is feasible under the given conditions (exergonic reaction), the ΔG-value is negative by convention. A positive value necessarily indicates that the reaction can in principle not occur under the given conditions (endergonic reaction), and a value of zero indicates that reactants and products are in equilibrium. Most reactions in chemistry and biology are associated with liberation of heat to the surroundings (exothermic reactions),

[2]H, 1.008; C, 12.011.

which is expressed by their heat or enthalpy[3] changes (ΔH-values). Some reactions consume heat from the surroundings (endothermic reactions), and a few of such type also occur in microorganisms. Free energy or enthalpy changes are calculated from free energies of formation (ΔG_f°, sometimes also termed G_f°) or enthalpies of formation (ΔH_f°, sometimes also termed H_f°), respectively, which are given for standard conditions[4] and for which there is a broad data basis. Appendix Table 6 compiles the values under standard conditions for several hydrocarbons and a number of other compounds which often appear in catabolic reactions. For a reaction

$$\mathrm{a\ A + b\ B \rightarrow c\ C + d\ D} \tag{1}$$

(with a, b, c, d being the stoichiometric factors), the standard free energy change (viz., for all compounds at standard conditions) is the difference

$$\Delta G^\circ = \left(\mathrm{c}\ \Delta G_f^\circ \mathrm{C} + \mathrm{d}\ \Delta G_f^\circ \mathrm{D}\right) - \left(\mathrm{a}\ \Delta G_f^\circ \mathrm{A} + \mathrm{b}\ \Delta G_f^\circ \mathrm{B}\right) \tag{2}$$

Calculation of the free energy change ΔG for nonstandard activities (a, in case of gases termed fugacity; a must not be confused with the stoichiometric factor a) considers the "nonchemical" energy change associated with dilution or concentration ("volume work") of each component. These are logarithmic functions involving the gas constant and absolute temperature, the sum of which modifies the free energy change for standard activities, $\Delta G^{Standard}$, according to

$$\Delta G = \Delta G^{Standard} + RT \ln \frac{a_\mathrm{C}^c \cdot a_\mathrm{D}^d}{a_\mathrm{A}^a \cdot a_\mathrm{B}^b} \tag{3}$$

T in this equation must be the temperature for which the underlying $\Delta G^{Standard}$ value has been given (viz., usually 298.15 K), and ΔG values at other temperatures cannot be calculated by this equation.[5] The activities (effective concentrations) of solutes, a, can be usually substituted with acceptable precision by the actual concentrations in mol l^{-1}; similarly, the fugacities (effective pressures) of gases can be substituted by

[3]Heat change of reaction under constant pressure.

[4]T = 298.15 K (25°C); standard activity of solutes, $a = 1$; standard (partial) pressure of gases = 101 kPa (standard fugacity = 1).

[5]$\Delta G^{Standard}$ values at temperatures other than 298.15 K can be calculated via the integrated "Delta-version" of the Gibbs-Helmholtz equation $\left(\frac{\partial}{\partial T}\frac{\Delta \mathrm{G}}{T}\right)_p = \frac{\Delta H}{T^2}$. Assuming that temperature dependence of ΔH within the range of physiologically relevant temperatures is negligible, the free energy change at temperatures other than 298.15 K (but at standard activities) is

$$\Delta G_T^{Standard} = \frac{T}{298.15}\Delta G^\circ + \left(1 - \frac{T}{298.15}\right)\Delta H^\circ$$

The same result is obtained from $\Delta G = \Delta H - T\Delta S\, S$ by assuming that ΔH and ΔS are essentially constant within the range of physiologically relevant temperatures.

their pressures in atm, an otherwise obsolete unit.[6] With such simplification, as well as with $R = 8.315 \times 10^{-3}\text{kJ K}^{-1}\text{mol}^{-1}$, $T = 298.15$ K (25°C), the common use of kJ as energy unit, and $\ln x = 2.303 \lg x$, (3) converts to

$$\Delta G = \Delta G^{\circ} + 5.71 \lg \frac{[\text{C}]^{\text{c}}\ [\text{D}]^{\text{d}}}{[\text{A}]^{\text{a}}\ [\text{B}]^{\text{b}}} (\text{at } 298.15\,\text{K}) \tag{4}$$

Hydrocarbons in the aqueous surroundings of microorganisms can be often considered with good approximation to have the activities of their gaseous, liquid, or solid standard states, viz., $a_{\text{Hydrocarbon}} = 1$, or [Hydrocarbon] = 1. For instance, if a gaseous hydrocarbon at standard pressure dissolves in water and reaches the dissolution equilibrium (ΔG of transfer = 0), it is thermodynamically treated like the gas, even though the dissolved concentration is in the range of 10^{-3}M (Appendix Fig. 6). The same holds true for liquid hydrocarbons: Despite the extremely low saturation concentration of long-chain alkanes in water, the hydrocarbon dissolved in water has the activity (strictly speaking the chemical potential) of the pure liquid hydrocarbon phase. If inorganic (fully oxidized) Carbon is involved, also acid-base dissociation has to be Considered (Appendix Fig. 7).

The free energy data (Appendix Table 6) reveal some basic and sometimes "counterintuitive" thermodynamic properties of hydrocarbons. Many hydrocarbons are metastable (thermodynamically unstable; ΔG_f° positive) with respect to the elements, even though decay into the elements is usually "kinetically inhibited." In the case of acetylene (ethyne), however, compression at room temperature can trigger the release of the energy in a violent decay into the elements. For this reason, compressed welding acetylene in steel bottles must be stabilized by adsorption to a carrier such as acetone. But also hydrocarbons that are stable with respect to the elements (even the rather stable ethane) are metastable with respect to decay into native carbon and methane, the most stable hydrocarbon:

$$\begin{gathered} 2\,\text{C}_2\text{H}_6 \rightarrow \text{C}_{\text{Graphite}} + 3\,\text{CH}_4 \\ \Delta G^{\circ} = -43.3\,\text{kJ}(\text{mol}\,\text{C}_2\text{H}_6)^{-1} \end{gathered} \tag{5}$$

In the presence of CO_2 or bicarbonate, even methane is metastable:

$$\begin{gathered} \text{CH}_4 + \text{CO}_2 \rightarrow 2\,\text{C}_{\text{Graphite}} + 2\,\text{H}_2\text{O} \\ \Delta G^{\circ} = -29.2\,\text{kJ}(\text{mol}\,\text{CH}_4)^{-1} \end{gathered} \tag{6}$$

$$\begin{gathered} \text{CH}_4 + \text{CO}_2 \rightarrow 2\,\text{C}_{\text{Graphite}} + 2\,\text{H}_2\text{O} \\ \Delta G^{\circ} = -29.2\,\text{kJ}(\text{mol}\,\text{CH}_4)^{-1} \end{gathered} \tag{7}$$

[6]The apparent correctness of the old unit atm is due to the fact that it is numerically equivalent with standard fugacity = 1. Activities and fugacities are by definition without units, and the formally correct approximated substitution would $a_{\text{A}} = \frac{[\text{A}]_{Actual}}{[\text{A}]_{Standard}}$, etc. Here, the use of the modern unit Pa or kPa for [A], etc. is coherent.

Nevertheless, formation of elemental carbon by reactions (5, 6, and 7) is kinetically strongly inhibited and has not been observed in abiotic or biotic systems at room temperature. But once the element has been formed by geothermal metamorphism of buried biomass or petroleum (Tissot and Welte 1984), it is the thermodynamically stable species of carbon as long as additional reducing or oxidizing components are absent. In the presence of a mild oxidant, not the element but rather CO_2 and its ionic forms, HCO_3^- and CO_3^{2-}, are the thermodynamically stable forms of carbon. Other forms or intermediate oxidation states ($H_2C=O$, $CO, HCOOH, C_2$ -compounds, etc.), like all natural organic compounds, are metastable[7] with respect to a conversion to CH_4, CO_2 and H_2O, even without involvement of an oxidant or reductant. If on the other hand a reductant with negative enough redox potential is present, the only stable form of carbon is CH_4. Again, intermediate oxidation states (CH_3OH, reduced C_2-compounds, etc.) are metastable. The stability "regions" of the mentioned carbon species are elegantly illustrated in a plot of the redox potential versus the pH (*E*-pH-diagram, Pourbaix diagram; Fig. 2).

Another thermodynamically interesting principle is revealed in the homologous series of *n*-alkanes. *n*-Alkanes become increasingly unstable with increasing chain length, whereas the heat of formation shows an opposite trend (Fig. 3).[8] The heat is the energy released during C−H bond formation from the elements (even though such alkane formation is not observed in reality). Hence, the thermodynamically feasible disintegration of a long-chain alkane into its elements would consume heat:

$$\begin{gathered} C_{16}H_{34} \rightarrow 16\,C_{\text{Graphite}} + 17\,H_2 \\ \Delta G^\circ = -49.8\,\text{kJ}\,(\text{mol}\,C_{16}H_{34})^{-1} \\ \Delta H^\circ = +454.4\,\text{kJ}\,(\text{mol}\,C_{16}H_{34})^{-1} \end{gathered} \tag{8}$$

This thermodynamically "allowed" cooling of the surroundings (and the system), which is a decrease in the entropy of the surroundings, is explained by the numerically higher entropy increase of the reacting system; the molecules of the gaseous H_2 that are formed in high number carry a high amount of "hidden heat."[9] Furthermore, the homologous *n*-alkane series reveals the transition from gaseous to liquid hydrocarbons (*n*-butane/*n*-pentane), which is mirrored by a discontinuity of the ΔH_f°

[7]The extremely low hypothetical equilibrium concentrations of these species can be calculated.

[8]Linearity in the series of the higher alkanes may be a "pre-assumption" and basis for calculation of ΔG_f° or ΔH_f° values of compounds in homologous series via incremental additions. In the numerous sources of thermodynamic data, the original basis underlying such data is often difficult to trace back.

[9]Also, the highly ordered ("improbable") structure of the long-chain alkane contributes to thermodynamic instability.

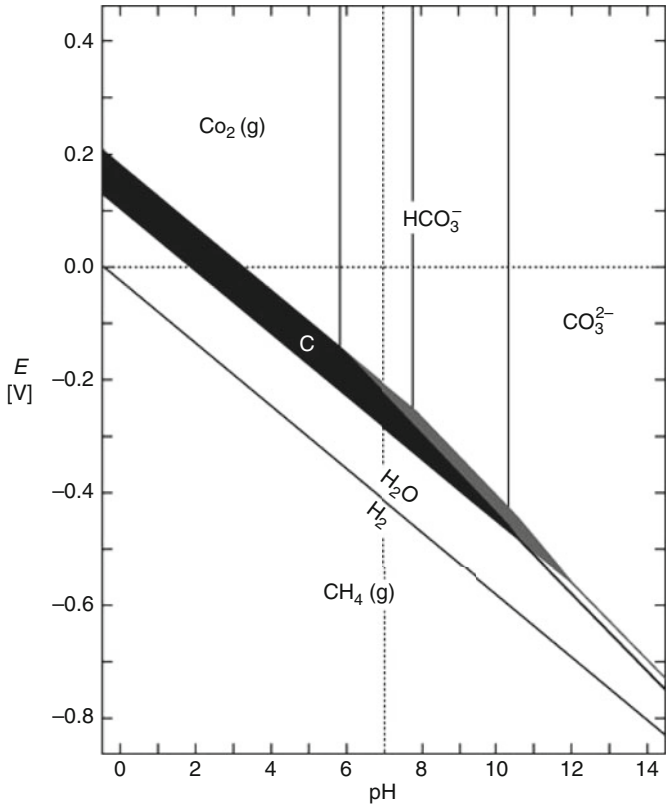

Fig. 2 Stability diagram (Pourbaix diagram) of carbon species. The equilibrium (*borderline*) redox potential E (in V) as a function of pH was calculated from ΔG_f° values (in J) according to $E = \frac{\sum \Delta G^o_{f,\ \mathrm{ox}} - \sum \Delta G^o_{f,\ \mathrm{red}}}{n\ F} + \frac{0.0592}{n} \lg \frac{\prod a_{\mathrm{ox}}}{\prod a_{\mathrm{red}}}$ with n = number of electrons; $F = 96,485$ C mol^{-1}; a = activity. Πa_{ox} includes the H^+-activity, the negative logarithm of which is the pH. Activities or fugacities: CO_2, CH_4, 1.0; HCO_3^- and CO_3^{2-}, 10^{-2} (*black*) or 1.0 (*gray*). The borderlines between CO_2(g), HCO_3^- and CO_3^{2-} in their standard states represent the pK_a values. Note that the pK_{a1} value for CO_2(g) is 7.8 (*vertical gray line*), whereas that of CO_2 (aq) is 6.35 (not shown here), the more commonly known one. The system H_2O/H_2 (electrochemically the same as $2H^+/H_2$) is indicated for comparison

values. This is because liquid pentane has "given off" the heat of condensation to the surroundings (liquid *n*-pentane, the real standard state: $\Delta H_f^\circ = -173$ kJ mol^{-1}; hypothetical gaseous standard state: $\Delta H_f^\circ = -146$ kJ mol^{-1}). The discontinuity of ΔG_f° is less pronounced. Liquid pentane as a highly volatile compound (boiling point, 36.2 °C) is almost in equilibrium with the gaseous state (liquid *n*-pentane: $\Delta G_f^\circ = -9.21$ kJ mol^{-1}; hypothetical gaseous standard state: $\Delta G_f^\circ = -8.11$ kJ mol^{-1}).

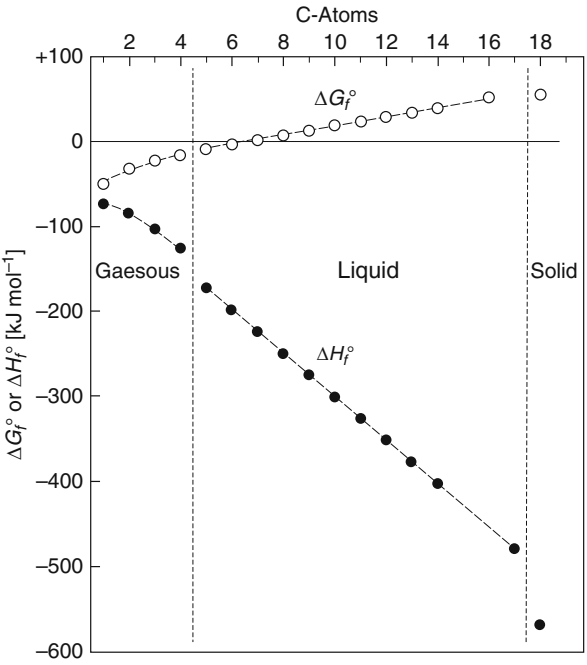

Fig. 3 Free energy and enthalpy (heat) of formation of alkanes from C_1 to C_{18}. The free energy of formation of C_{16} includes a literature value and the value extrapolated in this graph

3 Energetics of Hydrocarbon Utilization by Microorganisms

The biological utilization of a hydrocarbon can be examined bioenergetically (1) on the level of the net reaction performed by a microorganism and (2) on the level of individual enzymatic reactions. Among the latter, those of hydrocarbon activation are usually of highest interest and therefore briefly addressed in this overview.

3.1 Catabolic Net Reactions of Hydrocarbons from the Energetic Perspective

The ΔG of a reaction (the "system") is the maximum amount of energy that a second system can theoretically conserve via coupling to this reaction under full reversibility. However, coupling can only proceed outside of the equilibrium, viz., if the overall reaction of the two systems is more or less irreversible and dissipates free energy. The actual amount of useful energy provided by the catabolic reactions is therefore always less than the calculated ΔG. The subsequent anabolism with many highly irreversible reactions then dissipates most of the free energy. Table 1 lists generalized equations for the degradation of hydrocarbons and Table 2 several particular reactions with naturally important electron acceptors and the associated energy changes.

Table 1 Generalized equations for the catabolism (dissimilation, degradation) of hydrocarbons with various electron acceptors and for the anabolism (assimilation) of hydrocarbons into cell mass

Catabolism
$4\ C_cH_h + (4c + h)\ O_2 \rightarrow 4c\ CO_2 + 2h\ H_2O$
$10\ C_cH_h + (8c + 2h)\ NO_3^- + (8c + 2h)\ H^+ \rightarrow 10c\ CO_2 + (4c + h)\ N_2 + (4c + 6h)\ H_2O$
$C_cH_h + (4c + h)\ Fe(OH)_3 + (3c + h)\ CO_2 \rightarrow (4c + h)\ FeCO_3 + (6c + 2h)\ H_2O$[a]
$8\ C_cH_h + (4c + h)\ SO_4^{2-} + (8c + 2h)\ H^+ \rightarrow 8c\ CO_2 + (4c + h)\ H_2S + 4h\ H_2O$
$8\ C_cH_h + (8c - 2h)\ H_2O \rightarrow (4c - h)\ CO_2 + (4c + h)\ CH_4$
Anabolism
$4\ C_cH_h + h\ CO_2 + (4c - h)\ H_2O \rightarrow (4c + h)\langle CH_2O\rangle$ $d_{ass} = 0.133/(4c + h)\ \text{mol g}^{-1}$ $Y_{ass} = (4c + h)/0.133\ \text{g mol}^{-1}$
$17\ C_cH_h + (4h - c)\ CO_2 + (14c - 5h)\ H_2O \rightarrow (4c + h)\ C_4H_7O_3$ $d_{ass} = 0.165/(4c + h)\ \text{mol g}^{-1}$ $Y_{ass} = (4c + h)/0.165\ \text{g mol}^{-1}$
$17\ C_cH_h + (4h - c)\ CO_2 + (4c + h)\ NH_3 + (10c - 6h)\ H_2O \rightarrow (4c + h)\ C_4H_8O_2N$ $d_{ass} = 0.166/(4c + h)\ \text{mol g}^{-1}$ $Y_{ass} = (4c + h)/0.166\ \text{g mol}^{-1}$

[a]Because many reactions take place in environments containing inorganic carbon, reactions with $Fe(OH)_3$ are for convenience written with the relatively insoluble $FeCO_3$ (siderite) as a product

The aerobic oxidation with O_2 as electron acceptor provides biochemically the highest amount of energy, methanogenic degradation the lowest. Reactions with NO_2^- or N_2O are even more exergonic than those with O_2 (Table 2 includes methane oxidation with nitrite as an investigated example; Ettwig et al. 2008). However, there is no evidence that the higher energy available with NO_2^- or N_2O in comparison to O_2 as electron acceptor is conserved; biochemically, O_2 allows conservation of even more energy from the same amount of organic substrate. The theoretically higher energy gain is due to the "extra energy content" of NO_2^- and N_2O with respect to O_2 : $4\ NO_2^- + 4\ H^+ \rightarrow 2\ N_2 + 3\ O_2 + 2\ H_2O$, $\Delta G^{\circ\prime} = -116\ \text{kJ}(\text{mol}\ NO_2^-)^{-1}$; $2\ N_2O \rightarrow 2\ N_2 + O_2$, $\Delta G^{\circ} = -104\ \text{kJ}(\text{mol}\ N_2O)^{-1}$.

One of the least exergonic catabolic reactions is the anaerobic oxidation of methane (Table 2). Under certain environmental conditions, the net free energy change under in situ concentrations of the reactants may be only around $\Delta G = -20\ \text{kJ mol}(CH_4)^{-1}$ (Nauhaus et al. 2002). The fact that this minute amount is further shared between two organisms with different metabolism challenges the energetic understanding of energy conservation under "low-energy" conditions (viz., life at low chemical potential), a topic developed in the study of other syntrophic associations (Jackson and McInerney 2002; Schink 1997, 2002). Another anaerobic reaction of a hydrocarbon of thermodynamic interest is the conversion of alkanes

Table 2 Thermodynamic characteristics of observed and some hypothetical reactions of selected hydrocarbons. The degradation of hydrocarbons to methane and carbon dioxide is often endothermic

Reactants[a]	Products[a]	Free energy change of reaction per mol hydrocarbon[b], ΔG° or $\Delta G^{\circ\prime}$ (kJ mol^{-1})	Enthalpy change of reaction per mol hydrocarbon, ΔH° (kJ mol^{-1})	Entropy change of reaction per mol hydrocarbon[b,c], ΔS° or $\Delta S^{\circ\prime}$ (J K^{-1}mol^{-1})
Methane				
$CH_4(g) + 2\ O_2(g)$	$\rightarrow CO_2(g) + 2\ H_2O$	−818.0[d]	−890.4	−243
$CH_4(g) + 2\ O_2(g)$	$\rightarrow HCO_3^- + H^+ + H_2O$	−813.3[d]	−903.0	−301
$5\ CH_4(g) + 8\ NO_3^- + 8\ H^+$	$\rightarrow 5\ CO_2(g) + 4\ N_2(g) + 14\ H_2O$	−766.2	−788	−73
$3\ CH_4(g) + 8\ NO_2^- + 8\ H^+$	$\rightarrow 3\ CO_2(g) + 4\ N_2(g) + 10\ H_2O$	−928.3	−993	−215
$CH_4(g) + 8\ Fe(OH)_3 + 7\ CO_2(g)$	$\rightarrow 8\ FeCO_3 + 14\ H_2O$	−344[d]	−559	−723
$CH_4(g) + SO_4^{2-} + 2\ H^+$	$\rightarrow CO_2(g) + H_2S(aq) + 2\ H_2O$	−21.2[d]	−20.9	1
$CH_4(g) + SO_4^{2-} + H^+$	$\rightarrow HCO_3^- + H_2S\ (aq) + H_2O$	−16.5[d,e]	−33.5	−57
$CH_4(g) + SO_4^{2-}$	$\rightarrow HCO_3^- + HS^- + H_2O$	−16.6[d,e]	−11.3	18
Ethane				
$2\ C_2H_6(g) + 7\ O_2(g)$	$\rightarrow 4\ CO_2(g) + 6\ H_2O$	−1,467	−1,560	−310
$4\ C_2H_6(g) + 7\ SO_4^{2-} + 14\ H^+$	$\rightarrow 8\ CO_2(g) + 7\ H_2S(aq) + 12\ H_2O$	−73	−38	117
$4\ C_2H_6(g) + 2\ H_2O$	$\rightarrow 7\ CH_4(g) + CO_2(g)$	−36	−2	115
Propane				
$C_3H_8(g) + 5\ O_2(g)$	$\rightarrow 3\ CO_2(g) + 4\ H_2O$	−2,108	−2,220	−375
$2\ C_3H_8(g) + 5\ SO_4^{2-} + 10\ H^+$	$\rightarrow 6\ CO_2(g) + 5\ H_2S(aq) + 8\ H_2O$	−117	−46	236
$2\ C_3H_8(g) + 2\ H_2O$	$\rightarrow 5\ CH_4(g) + CO_2(g)$	−63	6	232

***n* -Hexane**				
$2\ C_6H_{14}(l) + 19\ O_2(g)$	$\rightarrow 12\ CO_2(g) + 14\ H_2O$	−4,023	−4,163	−471
$4\ C_6H_{14}(l) + 19\ SO_4^{2-} + 38\ H^+$	$\rightarrow 24\ CO_2(g) + 19\ H_2S(aq) + 28\ H_2O$	−238	−33	688
$4\ C_6H_{14}(l) + 10\ H_2O$	$\rightarrow 19\ CH_4(g) + 5\ CO_2(g)$	−137	66	682
***n* -Hexadecane**				
$2\ C_{16}H_{34}(l) + 49\ O_2(g)$	$\rightarrow 32\ CO_2(g) + 34\ H_2O$	−10,392	−10,701	−1,036
$5\ C_{16}H_{34}(l) + 98\ NO_3^- + 98\ H^+$	$\rightarrow 80\ CO_2(g) + 49\ N_2(g) + 134\ H_2O$	−9,757	−9,445	1,047
$4\ C_{16}H_{34}(l) + 49\ SO_4^{2-} + 98\ H^+$	$\rightarrow 64\ CO_2(g) + 49\ H_2S\ (aq) + 68\ H_2O$	−632	−50	1,952
$4\ C_{16}H_{34}(l) + 30\ H_2O$	$\rightarrow 49\ CH_4(g) + 15\ CO_2(g)$	−372	206	1,937
Benzene				
$2\ C_6H_6(l) + 15\ O_2(g)$	$\rightarrow 12\ CO_2(g) + 6\ H_2O$	−3,202	−3,268	−220
$5\ C_6H_6(l) + 30\ NO_3^- + 30\ H^+$	$\rightarrow 30\ CO_2(g) + 15\ N_2(g) + 30\ H_2O$	−3,008	−2,883	419
$C_6H_6(l) + 30\ Fe(OH)_3 + 24\ CO_2(g)$	$\rightarrow 30\ FeCO_3 + 48\ H_2O$	−1,423	−2,026	−2,024
$4\ C_6H_6(l) + 15\ SO_4^{2-} + 30\ H^+$	$\rightarrow 24\ CO_2(g) + 15\ H_2S(aq) + 12\ H_2O$	−214	−7	696
$4\ C_6H_6(l) + 18\ H_2O$	$\rightarrow 15\ CH_4(g) + 9\ CO_2(g)$	−135	71	691
Toluene				
$C_7H_8(l) + 9\ O_2(g)$	$\rightarrow 7\ CO_2(g) + 4\ H_2O$	−3,823	−3,910	−293
$5\ C_7H_8(l) + 36\ NO_3^- + 36\ H^+$	$\rightarrow 35\ CO_2(g) + 18\ N_2(g) + 38\ H_2O$	−3,590	−3,449	473
$C_7H_8(l) + 36\ Fe(OH)_3 + 29\ CO_2(g)$	$\rightarrow 36\ FeCO_3 + 58\ H_2O$	−1,689	−2,421	−2,456
$2\ C_7H_8(l) + 9\ SO_4^{2-} + 18\ H^+$	$\rightarrow 14\ CO_2(g) + 9\ H_2S\ (aq) + 8\ H_2O$	−238	2	806
$2\ C_7H_8(l) + 10\ H_2O$	$\rightarrow 9\ CH_4(g) + 5\ CO_2(g)$	−142	96	801

(*continued*)

Table 2 (continued)

Reactants[a]	Products[a]	Free energy change of reaction per mol hydrocarbon[b], ΔG° or $\Delta G^{\circ\prime}$ (kJ mol^{-1})	Enthalpy change of reaction per mol hydrocarbon, ΔH° (kJ mol^{-1})	Entropy change of reaction per mol hydrocarbon[b,c], ΔS° or $\Delta S^{\circ\prime}$ (J K^{-1}mol^{-1})
Naphthalene				
$C_{10}H_8(c) + 12\ O_2(g)$	$\rightarrow 10\ CO_2(g) + 4\ H_2O$	−5,093	−5,156	−212
$5\ C_{10}H_8(c) + 48\ NO_3^- + 48\ H^+$	$\rightarrow 50\ CO_2(g) + 24\ N_2(g) + 44\ H_2O$	−4,782	−4,541	808
$C_{10}H_8(c) + 48\ Fe(OH)_3 + 38\ CO_2(g)$	$\rightarrow 48\ FeCO_3 + 76\ H_2O$	−2,247	−3,171	−3,098
$C_{10}H_8(c) + 6\ SO_4^{2-} + 12\ H^+$	$\rightarrow 10\ CO_2(g) + 6\ H_2S\ (aq) + 4\ H_2O$	−313	61	1,253
$C_{10}H_8(c) + 8\ H_2O$	$\rightarrow 6\ CH_4(g) + 4\ CO_2(g)$	−186	186	1,245

[a]Indicated standard states: g, gaseous; l, liquid; aq, aqueous, dissolved in water; c, crystalline

[b]If protons are involved, $\Delta G^{\circ\prime}$ (viz., for $[H^+] = 10^{-7}$M, pH = 7) is given

[c]Here calculated via $\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T}$

[d]Standard free energy changes of reactions formulated with CO_2 differ from those formulated with HCO_3^- because the reaction $HCO_3^- + H^+ \rightarrow CO_2(g) + H_2O$ is exergonic under standard conditions at pH = 7, with $\Delta G^{\circ\prime} = -4.7$ kJ mol^{-1}

[e]Reactions with H_2S and HS^- as products are energetically equivalent because both sulfide species are essentially in equilibrium under standard conditions

to methane (Anderson and Lovley 2000; Jones et al. 2008; Zengler et al. 1999), an endothermic reaction (for explanation, see remark on (8)):

$$\begin{aligned} 4\,C_{16}H_{34} + 30\,H_2O &\rightarrow 49\,CH_4 + 15\,CO_2(g) \\ \Delta G^{\circ} &= -372\,\mathrm{kJ}(\mathrm{mol}\,C_{16}H_{34})^{-1} \\ \Delta H^{\circ} &= +206\,\mathrm{kJ}(\mathrm{mol}\,C_{16}H_{34})^{-1} \end{aligned} \tag{9}$$

The Gibbs-Helmholtz equation predicts that the reaction becomes increasingly exergonic with increasing temperature (Dolfing et al. 2008). The process involves three organisms, (1) the hexadecane-degrading syntrophs ($C_{16}H_{34} + 16\,H_2O \rightarrow 8CH_3COO^- + 8\,H^+ + 17\,H_2$), (2) acetate-cleaving microorganisms which are either methanogens ($CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$) or additional syntrophs ($CH_3COO^- + H^+ + 2\,H_2O \rightarrow 2\,CO_2 + 4\,H_2$), and (3) H_2-utilizing methanogens ($CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O$). The available energy per transferred acetate, the "metabolic unit" in this syntrophism, is only around -47 kJ mol^{-1}; this amount is shared between three organisms. The thermodynamic constraints of this reaction with respect to petroleum hydrocarbon conversion to methane have been examined (Dolfing et al. 2008).

3.2 Hydrocarbon Activation from the Energetic Perspective

As any chemical or biochemical reaction, the activation reaction of hydrocarbons involves two energetic aspects. These are the net ΔG of the reaction (and its share in the overall catabolic ΔG), and the energy level which during the activation reaction is attained by the energy-rich short-lived transition state in the active site of the hydrocarbon-activating enzyme; an apparent transition state may further resolve into elementary reactions upon closer examination (Fig. 4). Net free energy changes of several activation reactions of hydrocarbons are listed in Table 3.

The activation of a hydrocarbon by introduction of a functional group to allow further metabolic processing is usually not a problem from a merely thermodynamic point of view. For instance, an O_2-independent hydroxylation of methane by dehydrogenation at a hypothetical "methane dehydrogenase" employing a mildly oxidizing biological agent such as cytochrome c($\mathrm{Cyt\ c_{ox}}/\mathrm{Cyt\ c_{red}}, E^{\circ} = E^{\circ\prime} = +0.245$ V) would be thermodynamically allowed:

$$\begin{aligned} CH_4 + 2\,\mathrm{Cyt}\ c[Fe^{3+}] + H_2O &\rightarrow CH_3OH + 2\,\mathrm{Cyt}\ c[Fe^{2+}] + 2\,H^+ \\ \Delta G^{\circ\prime} &= -16\,\mathrm{kJ}(\mathrm{mol}\,CH_4)^{-1} \end{aligned} \tag{10}$$

The problem lies in the high energy barrier, mainly due to the apolar and very stable C−H bond that must be attenuated by an appropriate biocatalyst. Despite the astounding capabilities of enzymes to decrease energy barriers of chemically difficult reactions, there is not always the ideal biochemical solution to any activation

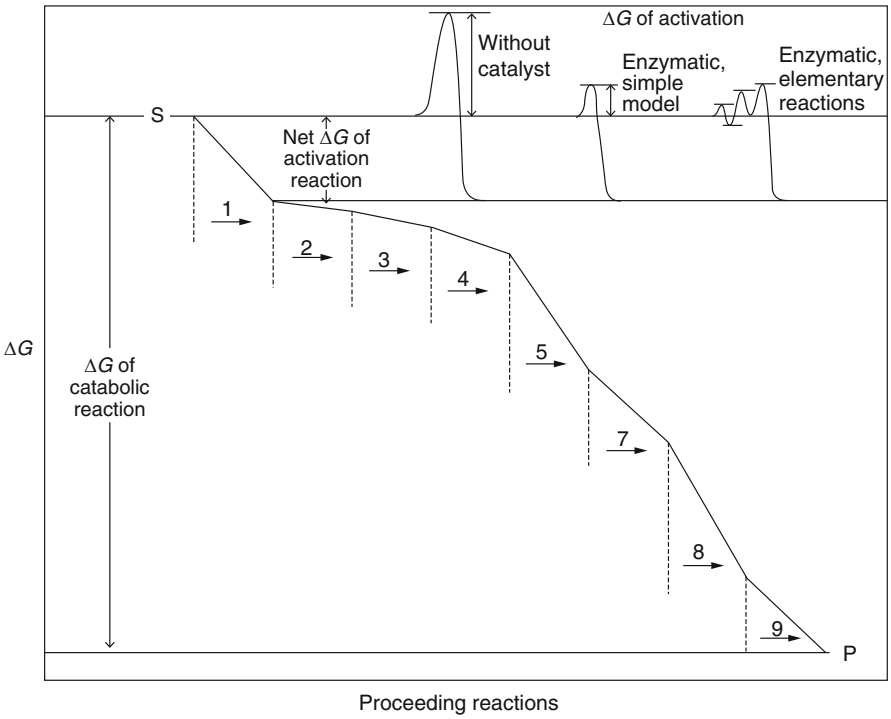

Fig. 4 Free energy changes during a fictive reaction of a hydrocarbon (S; in principle any other substrate) converted to an end product (P), free energy change of the activation reaction, and free energies of transition states at the activating enzyme. The scheme is a simplification because it does not display any electron acceptor that allows oxidation and the indicated free energy changes

problem. Not every thermodynamically possible but kinetically inhibited reaction can be catalyzed to take place at any rate.[10] To overcome the activation barrier and to reach high rates in such cases, activating enzymes invest an extra input of energy that is not conserved and makes the activation highly irreversible. Oxygenases which involve a strong oxidant (O_2/2 H_2O, $E^{\circ\prime} = +0.818$ V) and produce water besides the organic activation product are such energy-"wasting" catalysts that achieve high rates. The reaction with methane is

$$\begin{gathered} CH_4 + O_2 + NADH + H^+ \rightarrow CH_3OH + NAD^+ + H_2O \\ \Delta G^{\circ\prime} = -344\,\mathrm{kJ\,mol^{-1}} \end{gathered} \tag{11}$$

The sacrifice of energy to achieve activation via oxygenases is also reflected by the consumption of reducing equivalents detained from the energy-conserving

[10]A prominent example is nitrogenase: Despite the long evolution of nitrogen fixation, an enzyme type has not evolved that catalyzes the thermodynamically feasible N_2 reduction with H_2 or energetically equivalent electron donors without an investment of energy.

Table 3 Free energies of activation reactions of saturated, monounsaturated, and aromatic hydrocarbons. Also purely hypothetical reactions have been included. Values are given for standard conditions (pH = 7, if protons are involved)

Type of activation; compound	Reaction[a]	Free energy change[b] ΔG° or $\Delta G^{\circ\prime}$ (kJ mol^{-1})	Reference
Mono- and dioxygenation			
Methane	$CH_4 + \mathbf{O_2} + NADH + H^+ \rightarrow CH_3\mathbf{O}H + H_2\mathbf{O} + NAD^+$	−344[c]	Widdel et al. (2007)
n-Hexadecane[d]	$C_{15}H_{31} - CH_3 + \mathbf{O_2} + NADH + H^+ \rightarrow C_{15}H_{31} - CH_2\mathbf{O}H + H_2\mathbf{O} + NAD^+$	−368	This article
Benzene	$C_6H_6 + \mathbf{O_2} \rightarrow$ [Intermediate; NADH − recycling] $\rightarrow$ *o*-$C_6H_4(\mathbf{O}H)_2$	−335	Widdel et al. (2007)
Methyl-coenzyme M reductase			
Methane	$\mathbf{CH_4} + CoM - S - S - CoB \rightarrow CoM - S - \mathbf{CH_3} + \mathbf{H}S - CoB$	+30	Shima and Thauer (2005)
Addition to fumarate			
Methane[e]	$\mathbf{CH_4} + {}^-OOC - CH = CH - COO^- \rightarrow {}^-OOC - [\mathbf{CH_3}]CH - \mathbf{H}CH - COO^-$	−27 to −31	Rabus et al. (2001)
n-Alkane	$\mathbf{R} - \mathbf{CH_2} - \mathbf{CH_3} + {}^-OOC - CH = CH - COO^- \rightarrow {}^-OOC - [(\mathbf{R}) - \mathbf{CH} - (\mathbf{CH_3})]CH - \mathbf{H}CH - COO^-$	−35 to −39	Rabus et al. (2001)
Toluene	$\mathbf{C_6\,H_5\,CH_3} + {}^-OOC - CH = CH - COO^- \rightarrow {}^-OOC - [\mathbf{C_6\,H_5\,CH_2}]CH - \mathbf{H}CH - COO^-$	−31 to −35	Rabus et al. (2001)

(*continued*)

Table 3 (continued)

Type of activation; compound	Reaction[a]	Free energy change[b] ΔG° or $\Delta G^{\circ\prime}$ (kJ mol^{-1})	Reference
Addition of water to isolated double bond			
Butene[e]	$CH_3 - CH_2 - CH = CH_2 + \mathbf{H_2O} \rightarrow CH_3 - CH_2 - HC\mathbf{H} - CH_2\mathbf{OH}$	−7	Widdel et al. (2007)
Carboxylation[f]			
Benzene	$CH_3 - CH_2 - CH = CH_2 + \mathbf{H_2O} \rightarrow CH_3 - CH_2 - HC\mathbf{H} - CH_2\mathbf{OH}$	−4	Widdel et al. (2007)
Benzene[g]	$\mathbf{C_6H_6} + Carrier - COO^- \rightarrow \mathbf{C_6H_5} - COO^- + Carrier - \mathbf{H}$	−31	Widdel et al. (2007)

[a]Fate of one reactant is visualized in bold

[b]$\Delta G^{\circ\prime}$ is indicated if protons are involved

[c]Would be less exergonic with an electron donor of less negative redox potential than that of $NAD^+/NADH$ (−0.320 V)

[d]Sum of the following formal reactions: $C_{15}H_{31} - CH_3 + 0.5\mathbf{O_2} \rightarrow C_{15}H_{31} - CH_2\mathbf{OH}$, $\Delta G^\circ = -148.5$ kJ mol^{-1}; $0.5\mathbf{O_2} + NADH + H^+ \rightarrow \mathbf{H_2O} + NAD^+$, $\Delta G^{\circ\prime} = -219.6$ kJ mol^{-1} (calculated from $\Delta E^{\circ\prime} = 1.138$ V; Thauer et al. 1977)

[e]Hypothetical reaction

[f]Carboxylations have been suggested on the basis of chemical analyses

[g]A carboxyl carrier and donor have not been suggested or identified. The present free energy change is based on a calculation with oxaloacetate as a purely hypothetical carboxyl donor that may be energetically comparable to potent carboxyl donors such as carboxy-biotin

respiratory chain: The oxygenase reaction consumes two reducing equivalents from the metabolism, and the insertion of the oxygen atom to yield the alcohol "cancels" two additional reducing equivalents; hence, four reducing equivalents are consumed. Despite the significant amount of free energy dissipated and reducing power consumed by oxygenase reactions, this drain is not critical. The total free energy from the aerobic oxidation in this example is

$$\begin{aligned} &CH_4 + 2\,O_2 \rightarrow CO_2 + 2\,H_2O \\ &\Delta G^\circ = -818\,\mathrm{kJ(mol\,CH_4)^{-1}} \end{aligned} \tag{12}$$

From the totally available 8 [H] per methane, 4 [H] are still available for the respiratory chain. With higher hydrocarbons, the drain of energy and reducing equivalents are even less relevant.

An activation of hydrocarbons under anoxic conditions excludes oxygen[11] and in the case of many catabolic net reactions with low-energy gain strongly restricts the energy that can be dissipated to achieve activation. A reaction with particularly low net energy gain is the anaerobic oxidation of methane with sulfate (Table 2). The activation reaction is most likely a reversal of the methyl-coenzyme M reductase (Mcr) reaction, the final step in methanogenesis which is exergonic under standard conditions ($CoM-S-CH_3 + HS-CoB \rightarrow CoM-S-S-CoB + CH_4$, $\Delta G^\circ = -30\ [\pm 10]$ kJ mol^{-1}; Shima and Thauer 2005; Thauer and Shima 2008). For methane activation, the standard free energy of the reverse Mcr (rMcr) reaction in methane oxidizing archaea would thus be $+30\ [\pm 10]$ kJ mol^{-1}. Methane activation with the disulfide $CoM-S-S-CoB$ can therefore only take place if the products $CoM-S-CH_3$ and $HS-CoB$ are kept at very low concentration by effective scavenge in subsequent reactions. With respect to energy conservation in the total process, such a highly "concentration-controlled" reaction would be advantageous because it would be always very close to the equilibrium and not dissipate much energy.

For the activation of the strong $C-H$-bond of methane (absolute value, -440 kJ mol^{-1}; McMillen and Golden 1982) by a thiyl radical or a Ni^{III} center, which may be the most critical step, a decrease of the activation energy by a "dual-stroke engine" mechanism was proposed (Thauer and Shima 2008). rMcr has presumably two active sites, like Mcr. The release of the products from one site may transfer conformational energy to the other site where the substrates enter the reaction. However, this does not influence the equilibrium of the net activation reaction.

Methane activation via a reversal of the Mcr reaction is not only of mechanistic but also of kinetic interest. The positive standard free energy change of the rMcr reaction sets severe limits to the rate of the formation of the initial intermediates.

[11]The utilization of chlorate by facultatively anaerobic bacteria for hydrocarbon metabolism (Chakraborty and Coates 2004; Tan et al. 2006) involves O_2 that is generated from an intermediate ($ClO_2^- \rightarrow Cl^- + O_2$).

Using the Haldane equation,[12] which connects the catalytic efficiencies of the forward and back reactions through an enzyme with the thermodynamic equilibrium constant of the reaction, the first step in AOM was estimated to be slower by a factor between 10^{-3} and 10^{-7} than the final step in methanogenesis (Shima and Thauer 2005; Thauer and Shima 2008). Also, the rate of the subsequent enzymatic step may be drastically limited by the low near-equilibrium concentrations of methyl-coenzyme M and coenzyme B. The high content of the apparent rMcr in naturally enriched anaerobic methane oxidizers (Krüger et al. 2003) may be a means to compensate for the slowness of the enzyme.

The carbon–carbon addition of non-methane hydrocarbons at their methyl or methylene group to fumarate is slightly exergonic (Table 3) and to our present knowledge not associated with energy conservation. However, in view of the net energy gain with non-methane hydrocarbons under anoxic conditions, such a loss is "affordable." Only methane activation in an analogous way to yield methylsuccinate would be critical in an oxidation of methane with sulfate. The suggested mechanistic steps are an abstraction of a specific glycyl hydrogen in the polypeptide chain by a protein-activating enzyme (yielding $-\mathrm{Gly}^{\bullet}-$), subsequent hydrogen abstraction from a cysteyl group by the glycyl radical (yielding $-\mathrm{CysS}^{\bullet}-$), abstraction of a methyl hydrogen from toluene (yielding $\mathrm{C_6H_5}-{}^{\bullet}\mathrm{CH_2}$), addition of the benzyl radical to fumarate (yielding the benzylsuccinyl radical), and quenching of the radical to yield free benzylsuccinate and regenerate the cysteyl radical for the next catalytic round (Boll et al. 2018). Quantum chemical modeling of this reaction, for which a crystal structure of the enzyme was not available, supported the feasibility of the suggested steps (Himo 2002, 2005). The rate-limiting step was calculated to be the addition of the benzyl radical to fumarate.

4 Quantitative Aspects of Cell Synthesis

4.1 ATP and Growth Yields

The more exergonic a catabolic reaction and the higher the efficiency of ATP synthesis (proportion of total free energy conserved in ATP), the more cell mass can be synthesized from a given substrate. The quantitative treatment of the efficiency of free energy conservation in the form of ATP and the amounts of cell mass formed with various substrates are subjects of an own area of research in microbiology. In this research, the measurable molar growth yield is of central interest,

[12]The Haldane equation describes the connection between the equilibrium concentrations of the reactants and products and their kinetic constants k_{cat} and K_m. The equilibrium constant is also thermodynamically given by the concentrations at $\Delta G = 0$. In case of the reaction $\mathrm{S} \rightarrow \mathrm{P}$, the connection is $\left(\frac{[\mathrm{P}]}{[\mathrm{S}]}\right)_{\mathrm{eq}} = \frac{k_{cat}^{\mathrm{S}}/K_m^{\mathrm{S}}}{k_{cat}^{\mathrm{P}}/K_m^{\mathrm{P}}} = \mathrm{e}^{-\Delta G^{\circ}/(RT)}$.

besides calculated free energy changes and ATP yields known from well-established pathways such as glycolysis.

The molar growth yield, Y, is defined as the amount of cell dry mass, X (in g) per amount of totally consumed substrate, S_{tot} (in mol). On the other hand, for indication of the energy gain from the catabolism, a growth yield with respect to the dissimilated (viz., the energy yielding) proportion of the substrate, S_{diss}, would be a more meaningful definition:

$$Y = \frac{X}{S_{tot}} \left(\mathrm{g\,mol^{-1}}\right) \qquad Y_{diss} = \frac{X}{S_{diss}} \left(\mathrm{g\,mol^{-1}}\right) \tag{13a, b}$$

However, the latter definition and distinctive subscripts are not very common. S_{diss} can be determined experimentally by quantifying the biomass, X, and the consumed electron acceptor (O_2, NO_3^-, Fe^{III}, or SO_4^{2-}) or at least one of the products (CO_2, N_2, $Fe^{II/III}$, or H_2S). The chemically formulated stoichiometric relationship between substrate and product (Table 1) then reveals S_{diss}, which leads to Y_{diss} (13b). The fraction of the dissimilated substrate as part of the totally consumed substrate in anaerobic bacteria is usually much higher than in aerobic bacteria:

$$\left(\frac{S_{diss}}{S_{tot}}\right)_{anaerobic} > \left(\frac{S_{diss}}{S_{tot}}\right)_{aerobic} \tag{14}$$

Some measured growth yields of aerobic and anaerobic hydrocarbon utilizing microorganisms are listed in Table 4.

If consumption of the electron acceptor or formation of the catabolic product has not been quantified, or if only a Y value (13a) has been reported, Y_{diss} can be calculated. With S_{ass} for the assimilated amount of substrate, the totally consumed substrate is

$$S_{tot} = S_{diss} + S_{ass} \tag{15}$$

Division by the obtained cell mass yields

$$\frac{S_{tot}}{X} = \frac{S_{diss}}{X} + \frac{S_{ass}}{X} \tag{16}$$

and with definitions (13a, b)

$$\frac{1}{Y} = \frac{1}{Y_{diss}} + \frac{S_{ass}}{X} \tag{17}$$

The expression S_{ass}/X may be termed the assimilatory substrate demand, d_{ass} (in mol g^{-1}). The reciprocal term X/S_{ass} can be defined as another type of yield, the amount of cell mass (in g) obtained per assimilated amount of substrate (in mol), and designated Y_{ass}. The connection is thus $d_{ass} = 1/Y_{ass}$. This leads to

Table 4 A selection of growth yields[a] of aerobic and anaerobic microorganisms on hydrocarbons and calculated fraction of the dissimilated substrate

Microorganism	Hydrocarbon and electron acceptor	Growth yield[b] (cell dry mass per amount of hydrocarbon)			Fraction of substrate catabolized[d], S_{diss}/S_{tot}	Reference
		by mass ($g\ g^{-1}$)	molar, Y ($g\ mol^{-1}$)	molar[c], Y_{diss} ($g\ mol^{-1}$)		
Aerobic						
Pseudomonas sp.	*n*-Octane, O_2	1.1*	125	214	0.58 (58%)	Wodzinski and Johnson (1968)
Nocardia sp.	Long-chain *n*-alkane mixture, O_2	0.60*,[e] to 1.32*	127[f] to 280[f]	164[f] to 563[f]	0.77 (77%) to 0.50 (50%)	Wagner et al. (1969)
Micrococcus cereficans	*n*-Hexadecane, O_2	0.9*	204	310	0.66 (66%)	Einsele (1983)
Candida tropicalis	*n*-Hexadecane, O_2	1.0*	226	366	0.62 (62%)	Einsele (1983)
Ideal aerobe	*n*-Hexadecane, O_2	1.77	401	1240	0.32 (32%)	This article (see text)
Pseudomonas nautica	*n*-Heptadecane, O_2	0.50	120*,[g]	148	0.81 (81%)	Bonin et al. (1992)
Pseudomonas putida	Benzene, O_2	1.20*	94.7	197	0.48 (48%)	Reardon et al. (2000)
Pseudomonas putida	Toluene, O_2	1.28*	100	185	0.54 (54%)	Reardon et al. (2000)
Pseudomonas putida	Toluene, O_2	1.0*	92.1	159	0.58 (58%)	Bordel et al. (2007)

Pseudomonas putida	Toluene, O_2	0.70*	64.5	91.6	0.70 (70%)	Dinkla et al. (2001)
Pseudomonas sp.	Naphthalene, O_2	0.50*	64.1	82	0.78 (78%)	Wodzinski and Johnson (1968)
Anaerobic						
Betaproteobacterium, strain EbN1	Ethylbenzene, NO_3^-	0.83	88*	129*	0.68 (68%)	Rabus and Widdel (1995)
Archaea (ANME-2) Deltaproteobacteria	Methane, SO_4^{2-}	0.037	0.59	0.6*	0.99 (99%)	Nauhaus et al. (2007)
Deltaproteobacterium, strain AK-01	*n*-Hexadecane, SO_4^{2-}	0.059	13.5*,[h]	13.8	0.97 (97%)	So and Young (1999)
Desulfobacula toluolica	Toluene, SO_4^{2-}	0.31	29*	33*	0.88 (88%)	Rabus et al. (1993)

[a]Only directly measured ("real") growth yields are listed and not Y_{max} values obtained from extrapolation
[b]Original value from the reference is indicated by asterisk; other values were calculated for this chapter (see text). Y_{diss} was calculated via (19). The needed Y_{ass} was calculated according to Table 3, assuming the biomass bulk formula $C_4H_7O_3$; the Y_{ass} values (g mol^{-1}) are as follows: methane, 48.5; *n*-octane, 303; *n*-pentadecane, 558; *n*-hexadecane, 594; *n*-heptadecane, 630; benzene, 182; toluene, 218; ethylbenzene, 255
[c]Relative to dissimilated substrate
[d]Calculated according to $S_{diss}/S_{tot} = Y/Y_{diss}$
[e]Additional assimilation of added yeast extract is likely
[f]For convenience calculated with pentadecane (which was part of the mixture)
[g]With 2% O_2 in gas phase; with more O_2 the yield decreased
[h]Estimated by assuming that 55% of cell dry mass is protein

$$\frac{1}{Y} = \frac{1}{Y_{\text{diss}}} + \frac{1}{Y_{\text{ass}}} \tag{18}$$

Rearrangement leads to

$$Y_{\text{diss}} = \frac{Y_{\text{ass}} \cdot Y}{Y_{\text{ass}} - Y} \tag{19}$$

The values for d_{ass} or Y_{ass} are calculated from chemically formulated stoichiometries. This requires the assumption of bulk formulas for cell dry mass. The simplest bulk formula is that of carbohydrates, $\langle CH_2O \rangle$. For aerobic methanotrophs, the formula $\langle C_4H_8O_2N \rangle$ was used (van Dijken and Harder 1975). A simpler N-free variant with the same bulk oxidation state of carbon is $\langle C_4H_7O_3 \rangle$ (Pfennig and Biebl 1976). A precise yet more complicated formula, $\langle C_{4.36}H_{8.24}O_{1.87}N \rangle$, was determined for an aerobic bacterium grown with heptadecane (Bonin et al. 1992). Considering the oxidation state of carbon is more important than including nitrogen. Because in the case of hydrocarbons the substrate carbon is more reduced than cell mass carbon, CO_2 is included in the assimilation equations (Table 1).

Now, also the fraction of the dissimilated substrate as part of the totally consumed substrate can be calculated even if only a Y value is available from the literature:

$$\frac{S_{\text{diss}}}{S_{\text{tot}}} = \frac{Y_{\text{ass}} - Y}{Y_{\text{ass}}} \tag{20}$$

For instance, for aerobic growth with hexadecane (M = 226.45), a growth yield by mass of 1 g $(\text{g } C_{16}H_{34})^{-1}$ has been reported, which equals a molar growth yield of $Y = 226$ g $(\text{mol } C_{16}H_{34})^{-1}$. The assimilation equation is

$$\begin{gathered} 17\,C_{16}H_{34} + 120\ CO_2 + 54\,H_2O \rightarrow 98\,C_4H_7O_3 \\ d_{\text{ass}} = 1.68 \times 10^{-3}\ \text{mol g}^{-1};\ Y_{\text{ass}} = 594\,\text{g mol}^{-1} \end{gathered} \tag{21}$$

Equation (19) yields $Y_{\text{diss}} = 366$ g mol^{-1}. The fraction of the dissimilated substrate is

$$\frac{S_{\text{diss}}}{S_{\text{tot}}} = 0.62\,(\text{or } 62\%) \tag{22}$$

Above all, Y values are expected to provide information about the ATP yield as a parameter of high relevance to understand the efficiency of or losses in the energy flow:

Free energy of catabolic reaction
↓
Free energy in formed ATP
↓
Free energy (or ATP) consumed for cell synthesis

The ATP yield or q_{ATP} is the amount of ATP (in mol) formed per amount of dissimilated substrate (in mol). At first glance, the concept appears straightforward. From anaerobic pathways with biochemically known q_{ATP}, as for instance the homolactic fermentation of glucose ($q_{ATP} = 2$), the amount of cell mass obtained per mol ATP, the so-called Y_{ATP}, can be calculated from the determined growth yield via $Y_{ATP} = Y_{diss}/q_{ATP}$. If for another bacterium of interest, the q_{ATP} is unknown but Y_{diss} has been determined, this should in principle allow to calculate the desired q_{ATP} parameter via $q_{ATP} = Y_{diss}/Y_{ATP}$.[13] However, there is a serious drawback in that determined Y_{ATP} values, viz., the energy expenses for biomass synthesis, vary enormously for different growth substrates and among various bacteria. This is not surprising because synthesis of an amount of biomass for instance from free acetate as the growth substrate needs more ATP than synthesis from carbohydrates and amino acids added to the medium. But even with the same substrate for biosynthesis, determined Y_{ATP} values among bacteria vary significantly.

These problems are treated by the calculation of theoretical ATP demands for the synthesis of biomass with its diverse fractions (polysaccharides, protein, etc.) from starting substrates and by consideration of the fractions of energy or ATP that do not lead to productive growth. This nonproductive consumption of energy or ATP is interpreted as maintenance energy (Pirt 1965; Tempest and Neijssel 1984), an uncoupling of the anabolism from the catabolism at varying extent, or an extra "spill" of energy (Russell 2007) in addition to the "regular" dissipation. In the concept of Pirt (1965), the proportion of the substrate consumed per time for maintenance rather than for productive growth is regarded as a constant that is independent of the growth rate, μ. Hence, the slower the growth of a bacterium and the lower the biomass production per time, the higher the proportion of the substrate consumed for maintenance. If therefore growth yields of an organism at different growth rates are extrapolated to a theoretical infinitely high growth rate (no time required for growth) in a plot of $1/Y_{diss}$ versus $1/\mu$, the proportion of the substrate consumed for maintenance should become zero. At $1/\mu = 0$ ($\mu = \infty$) the theoretically highest growth yield, Y^{max} (more precisely $Y_{diss}{}^{max}$) is obtained that is used to gain information about q_{ATP} and Y_{ATP}. Such concepts have been applied to vast series of non-hydrocarbon substrates (Heijnen and van Dijken 1992; Stouthamer 1988). In the case of hydrocarbons, aerobic methanotrophs (Leak and Dalton 1985; van Dijken and Harder 1975) and degraders of long-chain alkanes (Erickson 1981; Ferrer and Erickson 1979) have been of interest for such mainly theoretical studies.

If the catabolism of a substrate is likely to involve conventional biochemical reactions (β-oxidation, citric acid cycle, dehydrogenations with NAD^+ and flavoenzymes, etc.) and an aerobic respiratory chain, a q_{ATP} value can be also predicted from the ATP-yielding reactions. Via Y_{ATP} values determined in other

[13]The q_{ATP} is conceptually related to the $P/2e^-$ ratio in aerobic and anaerobic respiration which indicates the number of ATP molecules formed per electron pair transported in the respiratory chain (in aerobes also P/O ratio). However, the q_{ATP} also includes ATP from substrate level phosphorylation.

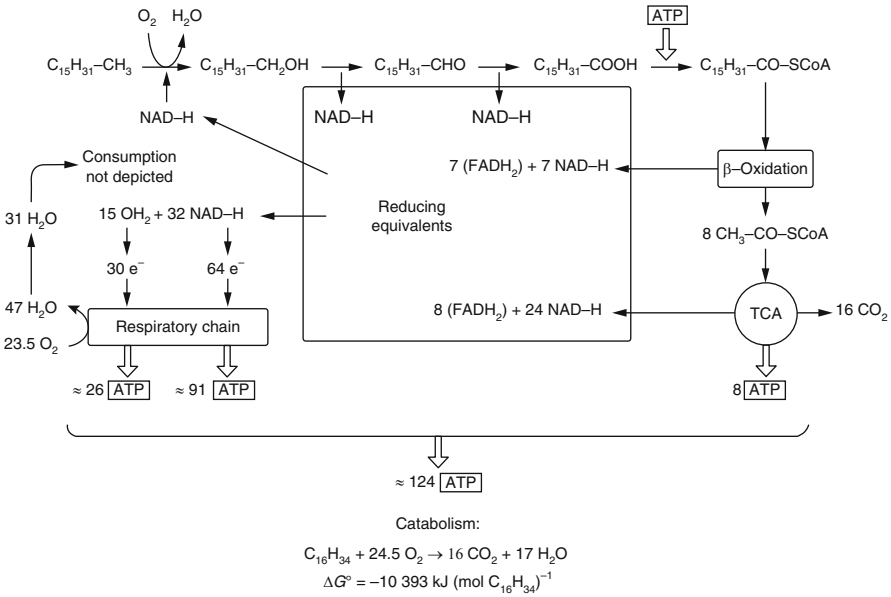

Fig. 5 Reducing equivalents and ATP synthesis in the aerobic catabolism of hexadecane ($C_{16}H_{32}$, M = 226.45). Reducing equivalents from enzyme-bound $FADH_2$ enter the respiratory chain at the quinone (Q) level. The assumed proton translocation in the respiratory chain underlying this scheme is 10 H^+/NADH and 6 H^+/QH_2. A phosphorylation yield of 1 ATP per 3.5 H^+ was arbitrarily assumed here (based on the commonly assumed range of 1 ATP per 3 to 4 H^+). The resulting net ATP yield is thus 124 mol ATP per mol $C_{16}H_{34}$, 0.55 mol ATP per g $C_{16}H_{34}$, or 5.3 mol ATP per mol O_2. For comparison, glucose ($C_6H_{12}O_6$, M = 180.16) would yield 10 NADH and 2 QH_2 allowing formation of 32 ATP via respiration; with 4 ATP from glycolysis and the tricarboxylic acid cycle, the net yield is 36 mol ATP per mol $C_6H_{12}O_6$, 0.20 mol ATP per g $C_6H_{12}O_6$, or 6.0 mol ATP per mol O_2

studies, a Y^{max} can be subsequently predicted and compared to an experimentally determined one. As an example, Fig. 5 presents the catabolic scheme for aerobic degradation of hexadecane with $q_{ATP} = 124$ (mol/mol). According to the free energy change of the reaction (−10 392 kJ mol^{-1}; Fig. 5), the average energy need for ATP synthesis would be 100 kJ $(mol\ ATP)^{-1}$. If a Y_{ATP} of 10 g cell dry mass $(mol\ ATP)^{-1}$ is assumed that is likely for cell synthesis from the hexadecane-derived acetate units (Erickson 1981; Stouthamer 1988), this would lead to $Y_{diss} = 1240$ g cell mass $(mol\ C_{16}H_{34})^{-1}$. The Y value is obtained via a transformation of (19):

$$Y = \frac{Y_{ass} \cdot Y_{diss}}{Y_{ass} + Y_{diss}} \tag{23}$$

This yields (with the above $Y_{ass} = 594$ g mol^{-1}) a value of $Y = 401$ g mol^{-1}, which would be a yield by mass of 1.77 g cell mass $(g\ C_{16}H_{34})^{-1}$. This may be regarded as an "ideal" yield with hexadecane. The fraction of dissimilated hexadecane would be only

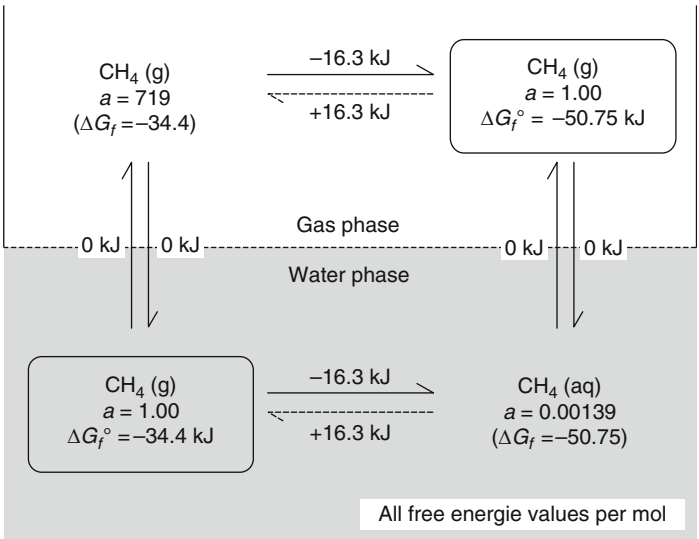

Fig. 6 The two standard states (framed) of methane. Aqueous methane, CH_4(aq), in its standard state which corresponds to a very high partial pressure has a higher energy content than gaseous methane, CH_4(g), in its standard state. Hence, indication of the ΔG° oder $\Delta G^{\circ\prime}$of a formulated reaction involving methane must indicate the applied standard state. Calculation of the free energy change of a reaction for real (measured) pressures or concentrations (according to (4)) must yield the same result with each standard state. Application of the gaseous standard state for calculation is also justified if there is no gas phase. Most natural conditions will be closest to the gaseous standard state. The ΔG_f° of CH_4 (aq) was calculated via the solubility of 0.00139 mol $l^{-1}atm^{-1}$ (Wilhelm et al. 1977), assuming that this concentration is numerically equivalent with the activity of CH_4 (aq) that is in equilibrium with CH_4 (g) of standard pressure. In seawater, the dissolved methane concentration in equilibrium with gaseous methane of standard pressure is lower (Yamamoto et al. 1976), even though this has the same activity as methane in pure water

$$\frac{S_{\text{diss}}}{S_{\text{tot}}} = 0.32\,(\text{or}\,32\%) \tag{24}$$

Most of the substrate is therefore assimilated. The lower yields from experiments (Table 4) indicate significant energy consumption for maintenance or by uncoupling.

4.2 Requirement for Minerals (N, P, Fe)

Growth yields are not only of basic but also of practical interest because they can be used to estimate the amount of essential minerals required for oil-degrading bacteria. Since crude oil has an extremely low content of nitrogen, phosphorous, and iron, these important elements are often the limiting ones in oil biodegradation. Availability of sulfur is usually not a problem, because oil contains organic sulfur and many natural waters are rich in sulfate (seawater, 28 mM). In the environment and in cultures, microorganisms often obtain the limiting elements

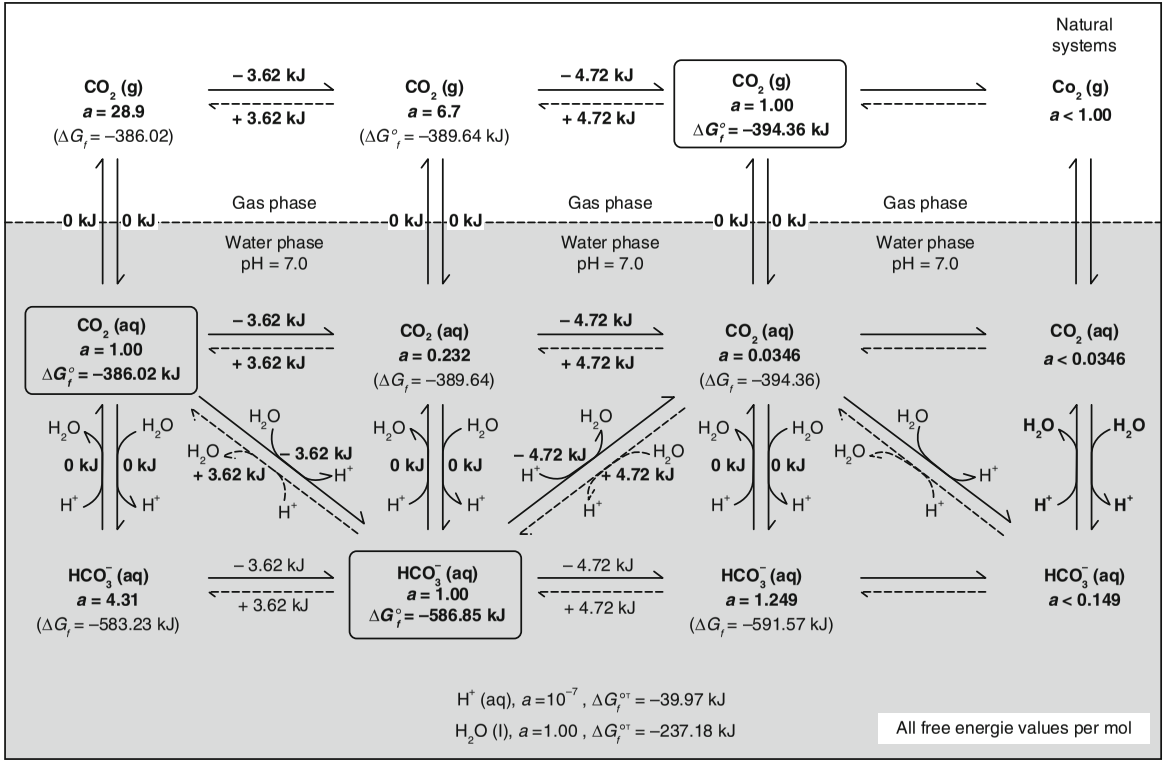

Fig. 7 The three standard states (framed) of inorganic carbon (CO_3^{2-} not included), the product of hydrocarbon oxidation. Most natural conditions will be closest to the gaseous standard state. See also remarks in legend of Appendix Fig. 6. Reactions are indicated for pH = 7

as inorganic species (NH_4^+,NO_3^-, $H_2PO_4^-/HPO_4^{2-}$, Fe^{2+}, Fe^{III} minerals, etc.). The above bulk formula for cell mass which considers nitrogen, $\langle C_4H_8O_2N \rangle$, suggests a content of 14% N by mass; it does not consider phosphorus and iron. The extended Redfield ratio, $(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) = \langle C_{106}H_{263}O_{110}N_{16}P \rangle$, which was derived from the originally determined molar C:N:P ratio of 106:16:1 of marine phytoplankton (Brewer et al. 1997), considers in addition phosphorus. Carbon in this formula has the bulk oxidation state as in carbohydrates, which may not very precisely reflect bacterial cell mass. "Redfield biomass" contains 6.3% N and 0.9% P by mass. With these ratios, 1 g biomass produced aerobically during complete consumption of 1 g (1.3 ml) hexadecane would need 0.24 g $\left(4.5 \times 10^{-3}\text{mol}\right)$ NH_4Cl and 0.04 g $\left(0.3 \times 10^{-3}\text{mol}\right)$ KH_2PO_4. In a marine environment with for instance 1 μM combined nitrogen and 0.06 μM phosphate, the microbial cell mass produced with 1 g hexadecane would consume the nitrogen and phosphorous from roughly 5 m^3 water.

However, such calculations should be applied reservedly in the study of natural hydrocarbon bioremediation. A lower in situ growth yield and N and P release from lysed cells may result in a lower than the calculated need for N and P. On the other hand, oil as a hydrophobic substrate is not distributed like soluble organic carbon in the water body but forms buoyant layers. Cells of hydrocarbon-degrading bacteria largely depend on physical contact with the oil, so that supply of biominerals by advective transport is a severely limiting factor (Harms et al. 2017). The controlled use of environmentally friendly immobilized N and P sources (as well as of iron sources that have not been considered here) that tend to stay in contact with oil may therefore be a justified method to stimulate oil degradation in eutrophic waters (Ron and Rosenberg 2010).

5 Research Needs

The application of thermodynamic data to microbial systems as a whole is a theoretical approach that is basic for the understanding of the overall catabolism of chemotrophic microorganisms (Thauer et al. 1977). Even though it is not regarded as an own field of microbiological research, the underlying formalism accompanies the study of numerous metabolic types of bacteria and may lead to the recognition of scientifically challenging questions that have not been encountered before. One of these is clearly the appropriate understanding of how microorganisms conserve energy at low chemical potential, viz., with low-energy substrates and combinations of electron donors and electron acceptors with marginal differences in their redox potential. Prominent processes of such type are anaerobic reactions involving hydrocarbons, such as the anaerobic oxidation of methane or conversion of non-methane hydrocarbons to methane by microbial consortia which even have to share the low net energy gain. Also individual enzymatic reactions in the anaerobic degradation of hydrocarbons, in particular the activating steps and intermediate energetic states (energy-rich transition states), need a deeper understanding from an energetic and kinetic point of view. There may be even open questions concerning growth yields

and the efficiency of energy conservation during growth with hydrocarbons under various environmental conditions. Their examination could be relevant for the study of hydrocarbon bioremediation in oligotrophic aquatic environments.

Appendix

Table 5 Hydrocarbons (methane, propane, *n*-hexane, and benzene as examples) and other substances as "energy carriers." In a reaction with oxygen, liquid hydrocarbons reveal a high gravimetric energy density in comparison to many other compounds and elements (calculated for the highest oxides in their standard state). Gaseous hydrocarbons reveal a high volumetric energy density

	ΔG° of oxidation with O_2		ΔH° of oxidation with O_2	
Substance	Per mass of substance $(kJ\ kg^{-1})$	Per volume of substance $(kJ\ m^{-3})$		Per mass of substance $(kJ\ kg^{-1})$
Gases (101 kPa)				
H_2	-117.6×10^3	-9.7×10^3	-141.8×10^3	-11.7×10^3
CH_4	-51.0×10^3	-33.5×10^3	-55.5×10^3	-36.4×10^3
C_3H_8	-47.8×10^3	-86.2×10^3 [a]	-50.3×10^3	-90.8×10^3 [a]
NH_3[b]	-19.9×10^3	-13.8×10^3 [a]	-22.5×10^3	-15.6×10^3 [a]
H_2S[c]	-19.3×10^3	-26.8×10^3 [a]	-23.3×10^3	-32.4×10^3 [a]
Solids or liquids				
Li	-40.4×10^3	-21.6×10^6	-43.0×10^3	-23.0×10^6
B	-55.2×10^3	-135.7×10^6	-58.8×10^3	-144.7×10^6
$C_{Graphite}$	-32.8×10^3	-74.4×10^6	-32.8×10^3	-74.4×10^6
C_6H_{14}	-46.7×10^3	-30.8×10^6	-48.3×10^3	-31.9×10^6
C_6H_6	-41.0×10^3	-36.0×10^6	-41.8×10^3	-37.5×10^6
CH_3OH	-21.9×10^3	-17.5×10^6	-22.7×10^3	-18.1×10^6
CH_3CH_2OH	-28.8×10^3	-22.8×10^6	-29.7×10^3	-23.5×10^6
$C_6H_{12}O_6$(α-D-Glucose)	-16.0×10^3	-25.0×10^6	-15.6×10^3	-24.3×10^6
Mg	-23.4×10^3	-40.8×10^6	-24.8×10^3	-43.1×10^6
Al	-29.3×10^3	-79.2×10^6	-31.1×10^3	-84.0×10^6
Si	-30.5×10^3	-71.1×10^6	-32.4×10^3	-75.5×10^6
P_{white}	-21.8×10^3	-39.7×10^6	-24.1×10^3	-43.9×10^6
S	-11.6×10^3	-22.7×10^6	-12.3×10^3	-24.1×10^6
Fe	-6.6×10^3	-52.3×10^6	-7.4×10^3	-58.3×10^6

[a]For convenience, ideal behavior assumed. In reality, the volumetric energy density will be somewhat higher
[b]If N_2(g) is produced
[c]If H_2SO_4(l) (l) is produced

Table 6 Thermodynamic properties of hydrocarbons and other compounds. Data are from other compilations[a]

Compound (Standard states: g, gaseous; l, liquid; c, crystalline; aq, aqueous)	Formula mass ($g\ mol^{-1}$)	Free energy of formation from the elements, ΔG_f° ($kJ\ mol^{-1}$)	Enthalpy of formation from the elements, ΔH_f° ($kJ\ mol^{-1}$)	Entropy[b], S° ($J\ K^{-1} mol^{-1}$)
Alkanes				
Methane (g)	16.043	−50.72[c] −50.8[d] −50.75[f]	−74.81[c] −74.9[d]	186.26[c] 186.2[e]
Methane (aq)	16.043	−34.4[g]		
Ethane (g)	30.069	−32.82[c] −32.6[e] −32.89[f]	−84.68[c] −83.8[d]	229.60[c] 229.1[d]
Propane (g)	44.096	−23.49[c] −23.6[d] −23.4[e]	−103.85[c] −104.7[d]	269.91[c] 270.2[d]
n-Butane (g)	58.123	−17.03[c] −17.2[d] −15.7[e]	−126.15[c] −125.6[d] −125[e]	310.23[c] 310.1[d]
2-Methylpropane (g)	58.123	−20.9[d] −18.0[e]	−134.2[d] −132[e]	294.6[d] 295[e]
n-Pentane (l)	72.150	−9.3[d] −9.21[e]	−173.1[c] −173.5[d]	262.7[d]
2-Methylbutane (l)	72.150	−14.6[e]	−179[e]	260[e]
n-Hexane (l)	86.177	−3.8[d] −4.28[e]	−198.7[c] −198.8[d] −199[e]	296.1[d] 296[e]
2-Methylpentane (l)	86.177	−8.11[e]	−204[e]	291[e]
n-Heptane	100.203	1.0[c] 1.28[e]	−224.4[c]	328.6[c] 328[e]
n-Octane (l)	114.23	6.41[e]	−249.9[c]	361.1[c]

(*continued*)

Table 6 (continued)

Compound (Standard states: g, gaseous; l, liquid; c, crystalline; aq, aqueous)	Formula mass ($g\ mol^{-1}$)	Free energy of formation from the elements, ΔG_f° ($kJ\ mol^{-1}$)	Enthalpy of formation from the elements, ΔH_f° ($kJ\ mol^{-1}$)	Entropy[b], S° ($J\ K^{-1} mol^{-1}$)
2-Methylheptane (l)	114.23	3.85[e]	−255.1[c]	352[e]
3-Methylheptane (l)	114.23	4.68[e]	−252[c]	358[e]
4-Methylheptane (l)	114.23	7.8[e]	−252[c]	350[e]
n-Decane (l)	142.28	17.5[d] 17.4[e]	−300.9[d]	425.5[d] 426[e]
n-Dodecane (l)	170.34	28.1[d] 28.4[e]	−350.9[d] −352[e]	490.6[d]
n-Tridecane (l)	184.36	33.8[e]	−378[e]	523[e]
n-Tetradecane (l)	198.39	38.8[e]	−403[e]	555[e]
n-Hexadecane (l)	226.44	49.8[h] 52.2[i]	−454.4[h]	
n-Heptadecane (l)	240.47		−480[e]	
n-Octadecane (c)	254.50	53.9[e]	−569[e]	497[e]
Cyclopentane (l)	70.134	36.4[d] 36.5[e]	−105.1[d] −106[e]	204.3[d]
Cyclohexane (l)	84.161	26.8[e] 26.7[d]	−156[c] −156.4[d]	204.4[d]
Unsaturated hydrocarbons, nonaromatic				
Ethene (g)	28.054	68.15[c] 68.4[d]	52.26[c] 52.5[d]	219.56[c] 219.3[d]
Propene (g)	42.080	62.78[c] 62.8[d] 74.8[e]	20.42[c] 20.0[d] 20.4[e]	267.05[c] 266.6[d] 227[e]

1-Butene (g)	56.107	71.39[c] 71.3[d] 72.0[e]	−0.13[c] 0.1[d] 1.17[e]	305.71[c] 305.6[d] 307[e]
cis-2-Butene (g)	56.107	65.95[c] 65.9[d] 67.3[e]	−6.99[c] −7.1[d] −5.70[e]	300.94[c] 300.8[d]
trans-2-Butene (g)	56.107	63.06[c] 63[d] 64.3[e]	−11.17[c] −11.4[d] −10.1[e]	296.59[c] 296.5[d] 296[e]
Ethyne (g)	26.038	209.2[c]	226.73[c]	200.94[c]
Aromatic hydrocarbons				
Benzene (l)	78.113	124.3[c] 124.4[d] 124.5[f]	49.0[c]	173.3[c] 173.4[d]
Toluene (l)	92.140	113.8[d] 110[e] 114.22[f]	12.4[d] 8.08[e]	221[d] 219[e]
Ethylbenzene (l)	106.17	120[e]	−12.5[e]	255[e]
o-Xylene (l)	106.17	110.3[d] 111[e]	−24.4[e]	246.5[d] 246[e]
m-Xylene (l)	106.17	107.7[d]	−25.4[e]	252.2[d]
p-Xylene (l)	106.17	110.1[d]	−24.4[d] −24.3[e]	247.4[d]
1,3,5-Trimethylbenzene (l)	120.19	103.9[d]	−63.4[d] −63.5[e]	273.6[d] 273[e]
Naphthalene (c)	128.17	201[d]	78.53[c] 77.9[d]	166.9[d]
1-Methylnaphthalene (l)	142.20	189.4[d]	56.3[d]	254.8[d]
2-Methylnaphthalene (c)	142.20	192.6[d]	44.9[d]	220[d]
Biphenyl (c)	154.21	254.2[d]	99.4[d]	205.9[d]

(*continued*)

Table 6 (continued)

Compound (Standard states: g, gaseous; l, liquid; c, crystalline; aq, aqueous)	Formula mass ($g\ mol^{-1}$)	Free energy of formation from the elements, ΔG_f° ($kJ\ mol^{-1}$)	Enthalpy of formation from the elements, ΔH_f° ($kJ\ mol^{-1}$)	Entropy[b], S° ($J\ K^{-1} mol^{-1}$)
Anthracene (c)	178.23	286.0[d]	129.2[d] 128[e]	207.6[d] 207[e]
Phenanthrene (c)	178.23	268.3[d]	116.2[d] 113[e]	211.7[d] 212[e]
Alcohols, phenolic compounds				
Methanol (l)	32.042	−166.27[c] −166.8[d]	−238.66[c] −239.1[d]	126.8[c] 127.2[d]
Methanol (aq)	32.042	−175.39[f]		
Ethanol (l)	46.069	−174.78[c] −174.2[d]	−277.69[c] −277.0[d]	160.7[c] 161.0[d]
Ethanol (aq)	46.069	−181.75[f]		
1-Propanol (l)	60.096	−170.6[d]	−302.6[d]	194.6[d]
1-Propanol (aq)	60.096	−175.81[f]		
2-Propanol (l)	60.096	−180.3[d] −182[e]	−318.1[d] −319[e]	180.6[d] 180[e]
2-Propanol (aq)	60.096	−185.94[f]		
1-Butanol (l)	74.122	−162.5[d]	−327.3[d]	226.4[d] 252[e]
1-Butanol (aq)	74.122	−171.84[f]		
2-Butanol (l)	74.122	−177.0[d]	−342.6[d]	225.1[d]
1-Hexadecanol (c)	242.44	−98.7[d] −98.8[e]	−686.7[d] −684[e]	451.9[d] 452[e]
Benzyl alcohol (l)	108.14	−27.5[d] −27.3[e]	−160.7[d]	216.7[d]

Phenol (s)	94.113	−50.9[c] −50.4[d] −47.5[e] −47.6[f]	−165[c] −165.1[d] −163[e]	146[c] 144[d] 142[e]
1,2-Dihydroxybenzene (s)	124.14	−210.0[d]	−361.1[d]	150.2[d]
Aldehydes, ketones				
Formaldehyde (g)	30.026	−102.53[c] −109.9[d] −111[e] −112.97[f]	−108.57[c] −116[e]	218.77[c]
Formaldehyde (aq)	30.026	−130.54[f]		
Acetaldehyde (l)	44.053	−128.12[c] −128.3[d]	−192.30[c] −191.8[d]	160.2[c] 160.4[d]
Acetaldehyde (aq)	44.053	−139.9[f]		
Butyraldehyde (l)		−127[e]	−247[e]	247[e]
Acetone (l)	58.080	−155.4[c] −155.8[d]	−248.1[c] −242.1[d]	200.4[c] 200.6[d]
Acetone (aq)	58.080	−161.17[f]		
2-Butanone (l)	72.107	−151.4[d] −156[e]	−273.3[d] −279[e]	238.8[d] 241[e]
Benzaldehyde (l)	106.12	9.4[d]	−87.0[d]	
Carboxylic acids, carboxylates				
Formic acid (l)	46.026	−361.35[c] −360[e]	−424.72[c] −425.1[d] −423[e]	128.95[c]
Formic acid (aq)	46.026	−356.3[j]	−410.3[j]	163.7[j]
$Formate^{-}$(aq)	45.018	−351.04[f] −334.9[j]	−410.3[j]	91.7[j]

(*continued*)

Table 6 (continued)

Compound (Standard states: g, gaseous; l, liquid; c, crystalline; aq, aqueous)	Formula mass ($g\ mol^{-1}$)	Free energy of formation from the elements, ΔG_f° ($kJ\ mol^{-1}$)	Enthalpy of formation from the elements, ΔH_f° ($kJ\ mol^{-1}$)	Entropy[b], S° ($J\ K^{-1} mol^{-1}$)
Acetic acid (l)	60.052	−389.9[c] −390.2[d] −392[e]	−484.5[c] −484.4[d]	159.8[c] 159.9[d]
Acetic acid (aq)	60.052	−396.46[c]	−485.76[c]	178.7[c]
$Acetate^-$ (aq)	59.045	−369.31[c] −369.41[f]	−486.01[c]	86.6[c]
$Propionate^-$ (aq)	73.071	−361.08[f]		
$Butyrate^-$ (aq)	87.098	−352.63[f]		
$Hexanoate^-$ (aq)	115.15	−335.96[f]		
Palmitic acid (c)	256.43	−305.0[f]	−882[e]	452[e]
Benzoic acid (c)	122.12	−245.3[c] −245.6[f]	−385.1[c] −385.2[d]	167.6[c]
$Benzoate^-$ (aq)	121.12	−229.3[k]		
$Fumarate^{2-}$ (aq)	114.06	−604.21[f]		
$Succinate^{2-}$ (aq)	116.07	−690.23[f]		
$Methylsuccinate^{2-}$ (aq)	130.10	−681.6 to −685.5[l]		
(1 − Methylpentyl) $succinate^{2-}$ (aq)	200.23	−644.0 to −647.3[l]		
$Benzylsuccinate^{2-}$ (aq)	206.20	−521.1 to −525.4[l]		
Hydrocarbon-derived nitrogen, oxygen, sulfur, and halocompounds				
Methylamine (g)	31.057	32.3[d] 27.5[e]	−23.0[d] −28[e]	242.6[d] 242[e]
$Methylammonium^+$ (aq)	32.065	−40.0[f]		

Ethylamine (g)	45.084	37.3[d]	−47.4[d] −48.5[e]	289.9[d]
Pyridine (l)	79.101	181.3[d]	100.2[d]	177.9[d]
Pyridine (aq)	79.101	177.1[f]		
Aniline (l)	93.128	149.2[d] 148[e]	31.3[d] 29.7[e]	191.4[d] 192[e]
Dimethyl ether (g)	46.069	−112.9[d] −114[e]	−184.1[d] −185[e]	267.1[d]
Diethyl ether (l)	74.122	−116.7[d]	−279.3[d]	253.1[d]
Methanethiol (g)	48.10	−9.9[d] 0.754[e]	−22.9[d] −12.4[e]	255.1[d]
Dimethyl sulfide (l)	62.13	5.72[e]	−65.4[e]	196[e]
Ethanethiol (l)	62.13	−5.7[e]	−73.7[e]	207[e]
Thiophene (l)	84.14	121.2[d] 122[e]	80.6[d] 81.7[e]	181.2[d]
Thiophenol (l)	110.17	134[d]	64.1[d] 62.8[e]	222.8[d]
Fluoromethane (g)	34.033	−213.8[d] −222[e]	−237.8[d] −247[e]	222.8[d]
Tetrafluoromethane (g)	88.005	−888.3[d] −862[e]	−933.6[d] −908[e]	261.3[d] 262[e]
Chloromethane (g)	50.488	−58.5[d] −58.1[e]	−81.9[d] −82.0[e]	234.2[d] 233[e]
Dichloromethane (l)	96.944	−63.3[e]	−117[e]	179[e]
Trichloromethane (l)	119.38	−71.2[e]	−132[e]	203[e]
Tetrachlorromethane (l)	153.82	−62.6[d] −68.4[e]	−132.8[d] −139[e]	216.2[d] 214[e]
Chloroethane (g)	64.515	−60.5[d] −53.0[e]	−112.1[d] −105[e]	275.8[d] 275[e]

(*continued*)

Table 6 (continued)

Compound (Standard states: g, gaseous; l, liquid; c, crystalline; aq, aqueous)	Formula mass ($g\ mol^{-1}$)	Free energy of formation from the elements, ΔG_f° ($kJ\ mol^{-1}$)	Enthalpy of formation from the elements, ΔH_f° ($kJ\ mol^{-1}$)	Entropy[b], S° ($J\ K^{-1} mol^{-1}$)
Fluorobenzene (l)	96.104	−69.0[e]	−145[e]	206[e]
Chlorobenzene (l)	112.56	89.2[d] 93.7[e]	11.0[d] 10.6[e]	209.2[d] 194[e]
Inorganic compounds				
H_2(g)	2.0158	0	0	130.684[c]
H^+(aq), pH = 0	1.0074	0	0	0
H^+(aq), pH = 7	1.0074	$\left(\Delta G_f^{\circ\prime}\right)$ −39.97[l]	0	($\Delta S^{\circ\prime}$) 134.06
C, graphite (c)	12.011	0	0	5.740[c]
C, diamond (c)	12.011	2.900[c]	1.895[c] 1.897[d]	2.377[c]
CO (g)	28.011	−137.17[c] −137.15[f]	−110.53[c]	197.67[c]
CO_2(g)	44.010	−394.36[c] −394.39[d]	−393.51[c] −393.52[d]	213.74[c] 213.80[d]
CO_2(aq)	44.010	−385.98[c] −386.02[f]	−413.80[c]	117.6[c]
HCO_3^-(aq)	61.017	−586.77[c] −586.85[f]	−691.99[c]	91.2[c]
CO_3^{2-}(aq)	60.009	−527.81[c] −527.90[f]	−677.14[c]	−56.9[c]
N_2(g)	28.0134	0	0	191.61[c]
NH_4^+(aq)	18.038	−79.31[c] −79.37[e]	−132.51[c]	113.4[c]
N_2O (g)	44.013	104.20[c] 104.18[f]	82.05[c]	219.85[c]

NO_2^-(aq)	46.006	−37.2[f,m] −34.54[j]	−106.3[j] −104.6[m]	125.2[j] 140[m]
NO_3^-(aq)	62.005	−108.74[c] −111.34[f] −110.7[j]	−205.0[c] −206.7[j] −207.3[m]	146.4[c] 146.5[j]
O_2(g)	31.999	0	0	205.138[c] 205.147[d]
H_2O (l)	18.015	−237.13[c] −237.14[d] −237.178[f] −237.18[m]	−285.83[c]	69.91[c] 69.95[d]
S, (α, rhombic; c)	32.06	0	0	31.80[c] 32.056[d]
H_2S (g)	34.08	−33.56[c] −33.3[d]	−20.63[c] −20.5[d]	205.79[c] 205.7[d]
H_2S (aq)	34.08	−27.83[c] −27.87[d]	−39.7[c] −39.8[d]	121[c]
HS^-(aq)	33.072	12.08[c] 12.05[m]	−17.6[c]	62.08[c]
SO_4^{2-}(aq)	96.06	−744.53[c] −744.63[f]	−909.27[c]	20.1[c]
F^-(aq)	18.999	−278.79[c]	−332.63[c]	−13.8[c]
Cl^-(aq)	35.453	−131.23[c] −131.3[m]	−167.16[c]	56.5[c]
Mn^{2+}(aq)	54.937	−227.8[j] −228.0[m]	−223.3[j] −220.7[m]	−84[j] −73.6[m]
$MnCO_3$	114.95	−816.0[m]	−889.3[m]	100[m]
MnO_2	86.937	−465.1[m]	−520.0[m]	53[m]
Fe^{2+}(aq)	55.846	−78.90[c] −78.87[m]	−89.10[m]	−137.7[c]

(*continued*)

Table 6 (continued)

Compound (Standard states: g, gaseous; l, liquid; c, crystalline; aq, aqueous)	Formula mass ($g\ mol^{-1}$)	Free energy of formation from the elements, ΔG_f° ($kJ\ mol^{-1}$)	Enthalpy of formation from the elements, ΔH_f° ($kJ\ mol^{-1}$)	Entropy[b], S° ($J\ K^{-1} mol^{-1}$)
Fe^{3+}(aq)	55.845	−4.7[c] −4.60[m]	−48.5[c]	−315.9[c]
$FeCO_3$ (siderite; c)	115.86	−674.3[j] −666.7[m]	−748.2[j] −737.0[m]	92.9[j] 105[m]
$Fe(OH)_3$ (amorphous)	106.87	−695[j] −699[m]	−824.8[j]	96[j]
Fe_2O_3 (α, hematite; c)	159.69	−742.2[c] −742.7[m]	−824.2[c] −824.6[m]	87.40[c]
Fe_3O_4 (magnetite; c)	231.54	−1015.4[c] −1012.6[m]	−1118.4[c] −1115.7[m]	146.4[c]

[a]Original sources cited in the used compilations were not consulted. If a precise and rounded value is given in the compilations, the precise value is indicated here

[b]The absolute entropy values may be used to calculate entropy changes of reactions as well as entropies of formation, ΔS_f°; the latter can be used to prove consistency of literature data via $\Delta G_f^\circ = \Delta H_f^\circ - 298.15 \Delta S_f^\circ$. Example of *n*-hexane (C_6H_{14}): $\Delta S_f^\circ = 291.6 - (6 \times 5.74) - (7 \times 130.68) = -657.6\ J\ K^{-1}\ mol^{-1}$. $\Delta G_f^\circ = -198.8 - (298.15 \times -0.6576) = -2.7\ kJ\ mol^{-1}$. The result is close to the value given in the literature source ($-3.8\ kJ\ mol^{-1}$)

[c]Atkins and de Paula (2006)

[d]Dean (2004)

[e]D'Ans and Lax (1983)

[f]Thauer et al. (1977)

[g]Calculated via solubility of $1.39\ mol\ l^{-1} atm^{-1}$ at 25 °C (from Wilhelm et al. 1977)

[h]Via extrapolation or interpolation of the listed data

[i]Zengler et al. (1999)

[j]Garrels and Christ (1965) (data transformed by using 1 cal = 4.1868 J)

[k]Widdel et al. (2007)

[l]Free energy associated with dilution of 1 mol H^+ from $a = 1$ to $a = 10^{-7}$, which is $R\,T \ln 10^{-7}$

[m]Stumm and Morgan (1981)

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