Table 2. Standard Gibbs Energy and Ethalpy of Formation

Compound name	Composition	ΔG_f^{01} (kJ/mol)	ΔH_f (kJ/mol)
Biomass	$CH_{1.8}O_{0.5}N_{0.2}$	−67	−91
Water	H_2O	−237.18	−286
Bicarbonate	HCO_3^-	−586.85	−692
CO_2 (g)	CO_2	−394.359	−394.1
Ammonium	NH_4^+	−79.37	−133
Proton	H^+	−39.87	0
O_2 (g)	O_2	0	0
Oxalate^{2-}	$C_2O_4^{2-}$	−674.04	−824
Carbon monoxide	CO	−137.15	−111
Formate	CHO_2^-	−335	−410
Glyoxylate$^-$	$C_2O_3H^-$	−468.6	—
Tartrate^{2-}	$C_4H_4O_6^{2-}$	−1,010	—
Malonate^{2-}	$C_3H_2O_4^{2-}$	−700	—
Fumarate^{2-}	$C_4H_2O_4^{2-}$	−604.21	−777
Malate^{2-}	$C_4H_4O_5^{2-}$	−845.08	−843
Citrate^{3-}	$C_6H_5O_7^{3-}$	−1,168.34	−1,515
Pyruvate$^-$	$C_3H_3O_3^-$	−474.63	−596
Succinate^{2-}	$C_4H_4O_4^{2-}$	−690.23	−909
Gluconate$^-$	$C_6H_{11}O_7^-$	−1,154	—
Formaldehyde	CH_2O	−130.54	—
Acetate	$C_2H_3O_2^-$	−369.41	−486
Dihydroxyacetone	$C_3H_6O_3$	−445.18	—
Lactate	$C_3H_5O_3^-$	−517.18	−687
Glucose	$C_6H_{12}O_6$	−917.22	−1,264
Mannitol	$C_6H_{14}O_6$	−942.61	—
Glycerol	$C_3H_8O_3$	−488.52	−676
Propionate$^-$	$C_3H_5O_{2+}$	−361.08	—
Ethylene glycol	$C_2H_6O_2$	−330.50	—
Acetoine	$C_4H_8O_2$	−280	—
Butyrate	$C_4H_7O_2^-$	−352.63	−535
Propanediol	$C_3H_8O_2$	−327	—
Butanediol	$C_4H_{10}O_2$	−322	—
Methanol	CH_4O	−175.39	−246
Ethanol	C_2H_5O	−181.75	−288
Propanol	C_3H_8O	−175.81	−331
n-Alkane	$C_{15}H_{32}$	+60	−439
Propane	C_3H_8	−24	−104
Ethane	C_2H_6	−32.89	−85
Methane	CH_4	−50.75	−75
H_2 (g)	H_2	0	0
N_2 (g)	N_2	0	0
Nitrite ion	NO_2^-	−37.2	−107
Nitrate ion	NO_3^-	−111.34	−173
Iron II	Fe^{2+}	−78.87	−87
Iron III	Fe^{3-}	−4.6	−4
Hydrogen sulfide (g)	H_2S	−33.56	−20
Sulfide ion	HS^-	+12.05	−17
Sulfate ion	SO_4^{2-}	−744.63	−909
Thiosulfate ion	$S_2O_3^{2-}$	−513.2	−608

Note: pH = 7, 1 atm, 1 mol/L, 298 K.

enthalpy, and the Gibbs energy balance). This means also that there must exist mathematical relations between Y_{DX}, Y_{AX}, Y_{CX}, Y_{QX}, and Y_{GX} (see Fig. 2b). These relations are addressed in a later section (see equations 9a–9e). It is obvious that this knowledge of the complete growth stoichiometry provides essential engineering information with respect to reactor design on the amount of O_2 that must be transferred (aeration capacity), the amount of carbon dioxide that must be removed (ventilation), the amount of heat to be removed (cooling capacity), or the amount of fermentation products (in anaerobic growth). The amounts of the required N source and HCO_3^- (autotrophic growth) also follow from these stoichiometric calculations.

MEASUREMENT OF GROWTH STOICHIOMETRY

As shown earlier, the measurement of one stoichiometric coefficient suffices, in general, to calculate all the other stoichiometric coefficients using the conservation relations. This measured stoichiometric coefficient requires the measurement of two conversion rates because, by definition, a stoichiometric coefficient is the ratio of two conversion rates. For example, $Y_{DX} = r_X/-r_D$. The most simple growth system contains eight conversion rates (biomass, N source, H^+, H_2O, CO_2, electron donor, electron acceptor, heat production) and six conservation equations (C, H, O, N, enthalpy, charge). Measurement of two conversion rates is then sufficient to calculate all other rates and, hence, the complete growth stoichiometry. Currently, the most common measurements are biomass production and substrate (equal to electron donor) consumption. For aerobic growth the on-line measurement of O_2 consumption and CO_2 production by the analysis of O_2 and CO_2 in the off gas in air-sparged fermentors is becoming more and more routine. Especially for autotrophic growth, the on-line measurement of CO_2 consumption by off-gas analysis gives direct and highly accurate information on microbial growth (because all consumed CO_2 appears as biomass). This method was very successfully applied to study the growth stoichiometry and kinetics of solid pyrite oxidation by Fe^{2+}-oxidizing bacteria (12,13) and of *Methanobacterium thermoautotrophicum* on H_2/CO_2 (14).

Most recently, it was also shown that on-line measurement of heat production during microbial growth can be used to explore growth stoichiometry and kinetics (15–17).

However, such a simple approach of measuring only two conversion rates often makes certain assumptions:

- Each chosen pair of measured conversion rates will allow the complete calculation of all other conversion rates.
- All measurements are reliable within a certain statistical error but without a systematic deviation.
- The assumed description of the growth system is correct, which means that by-products or additional substrates are assumed to be absent.

All these assumptions are subject to critical considerations, which are dealt with extensively in a recent series of publications (18–21). Here, simple examples are provided to illustrate the points of interest. The reader is referred to Refs. 18–21 for a more elaborate introduction, including the full mathematical and statistical aspects.

Noncalculability of Stoichiometry

Suppose that in Example 1a the chosen two conversion rates to be measured are biomass production (r_X) and

Table 3. Calculated γ, ΔG_e^{01}, and ΔH_e^0 Values for Chemical Compounds under Standard Conditions

	γ Degree of reduction per C-mole for organic and per mole for inorganic compounds in electrons/(C)-mole	ΔG_e^{01} (kJ/e-mol)	ΔH_e^0 (kJ/e-mol)
biomass/NH_4^+ − N source	+4.2	+33.840	−26.1
Biomass/NO_3 − N source	+5.8	+14.820	−44.2
Biomass/N_2 − N source	+4.8	+32.948	−26.3
N source for growth	0	0	0
HCO_3^-	0	0	0
Oxalate	+1	+52.522	−20
Formate	+2	+39.186	−15.50
Glyoxylate	+2	+48.229	—
Tartrate	+2.5	+39.577	—
Malonate	+2.67	+28.976	—
Fumarate	+3	+33.662	−31.60
Malate	+3	+33.354	−32.20
Citrate	+3	+32.282	−33.90
Pyruvate	+3.33	+34.129	−23.60
Succinate	+3.50	+28.405	−36.30
Gluconate	+3.67	+39.106	—
Formaldehyde	+4	+45.326	−0.10
Acetate	+4	+26.801	−33.50
Lactate	+4	+31.488	−28.90
Glucose	+4	+39.744	−25.75
Mannitol	+4.33	+38.777	—
Glycerol	+4.67	+37.625	−24.30
Propionate	+4.67	+26.939	−33.80
Ethylene glycol	+5	+37.292	—
Acetoin	+5	+32.625	—
Butyrate	+5	+27.000	−33.30
Propanediol	+5.33	+33.177	—
Acetone	+5.33	+28.718	−30.90
Butanediol	+5.50	+31.374	—
Methanol	+6	+36.032	−23
Ethanol	+6	+30.353	−28.90
Propanol	+6	+29.144	−32.50
n-Alkane	+6.13	+26.694	—
Propane	+6.66	+25.948	−31.90
Ethane	+7	+25.404	−31.40
Methane	+8	+22.925	−31.50
CO	+2	+47.477	−1.5
H_2	+2	+39.870	0
SO_4^{2-}	0	0	0
SO_3^{2-}	+2	+50.296	—
S^0	+6	+19.146	−55.2
$S_2O_3^{2-}$	+8	+23.584	−27.5
HS^-	+8	+20.850	−43.9
NO_3^-	0	0	0
NO_2^-	+2	−41.650	−108.5
NO(g)	+3	−96.701	—
N_2O(g)	+8	−57.540	−124.55
NH_4^+	+8	−35.109	−101.9
N_2	+10	−72.194	−136.4
Fe^{3+}	0	0	0
Fe^{2+}	+1	−74.270	−46.8
H_2O	0	0	0
O_2	−4	−78.719	−143

Note: pH = 7, 1 mol/L, 1 atm, 298 K.

Table 2.2 Typical values for daily energy expenditure in humans

Activity	Time (min)	Energy cost (kJ min^{-1})	Total energy expenditure (kJ)
lying	540	5.0	2700
sitting	600	5.9	3540
standing	150	8.0	1200
walking	150	13.4	2010
TOTAL	1400	–	9450

The values were recalculated from measurements derived by indirect calorimetry of students by Haslam and Banner (1991, *Biochem. Soc. Trans.* **19**, 433S). A value of around 10 000 kJ is usually taken to be the daily energy requirement for humans, of which 6000 kJ will be the contribution by the basal metabolic rate. The calorific equivalent of the daily energy requirement will be 2400 kcal. (Note that the latter units are kilocalories not calories. How many times have you seen a diet in the press recommending daily requirements in calories, with a small 'c'? The dieter would soon starve to death.)

and can be up to a third higher in growing young children. The basal metabolic rate is also higher, by around 10%, than the sleeping metabolic rate because of the additional energy expenditure of wakefulness.

2.2.1 *Energy expenditure and size*

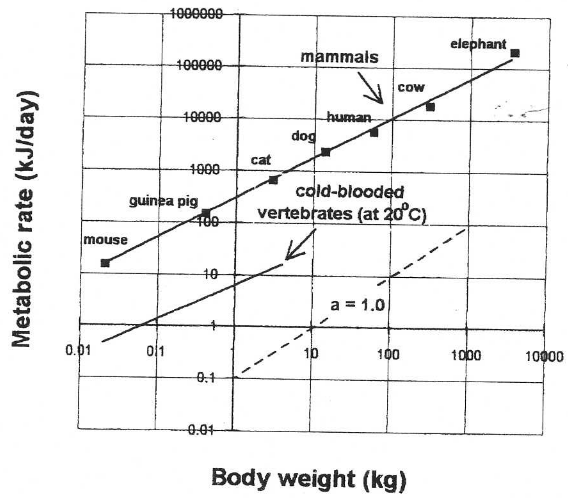
Many attempts have been made to relate the basal metabolic rate to body size. For aerobic organisms the relationship seems to be proportional to body mass raised to the power 0.75:

$$\text{basal metabolic rate} = a \cdot M^{0.75}$$

The constant of proportionality a varies with phylum and even class, although little change is seen in the value of the exponent. For example, the basal metabolic rates for mammals are higher than for cold-blooded vertebrates such as reptiles when compared at the same body mass, but the slopes of the lines relating metabolic rate with mass are similar (see Figure 2.1). Even the metabolic rates for trees and microorganisms fall on lines with similar slopes but with different values of a. The results suggest some common mechanism to explain the rate–mass relationship.

The separate contributions of different metabolic reactions to steady-state heat production are not yet known. Brand (1990) has proposed that mitochondrial activity is important to the basal metabolic rate in aerobic organisms. He has related the total mitochondrial inner membrane area to the

Figure 2.1
Daily metabolic rates of various organisms in relation to body weight



Note that both scales are logarithmic. The slopes of the lines for aerobic organisms are the same ($a \approx 0.75$) although the position of the regression lines are different for warm-blooded as opposed to cold-blooded animals. (Plotted using data from Kleiber (1947) and Schmidt-Nielsen, K., 1970, *Fedn. Proc.* **29**, 1524–1532.)

basal metabolic rate over a wide range of body masses in both warm and cold blooded animals. Mitochondria conserve the energy of oxidation of metabolites such as pyruvate by the creation of a gradient of protons across the inner mitochondrial membrane. The gradient can then be used to catalyse the movement of other ions across the membrane and also for the synthesis of ATP (see Chapter 7). The free energy stored in the gradient can be lost if the protons simply leak back across the inner membrane. Brand suggests that a significant contribution to heat production by mitochondria is the leak of protons across the mitochondrial inner membrane. In fact, the total number of liver mitochondria in mammals has been shown to be proportional to $M^{0.72}$. As might be expected from this argument, anaerobic organisms have a relationship more directly proportional to body mass.

The ability to store food (for example carbohydrate or triglyceride) is generally proportional to the mass, M, of an organism. Since energy expenditure relates to $M^{0.75}$, problems of size can arise. A large animal such as a camel has plenty of body size to store food for its energy needs. As size decreases, the difference between the two mass functions, food storage and energy expenditure, converges (Figure 2.2). A mouse has relatively smaller energy stores to meet