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BIOENERGETICS OF MICROBIAL GROWTH

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Biomass yield
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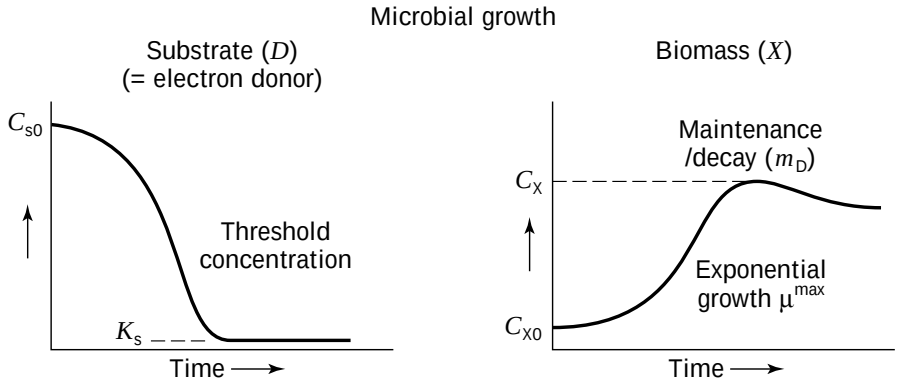
OUTLINE

INTRODUCTION

The growth of microorganisms occurs within a wide range of pHs and temperatures, and on a wide variety of nutrients. Figure 1 shows a typical batch experiment where a substrate (starting at concentration C_{so}) is converted by a microorganism (C_{xo} at $t = 0$). The microorganism grows exponentially at a specific growth rate μ^{max} and with yield Y^m_{DX}. After depletion of the substrate, which is characterized by the substrate affinity constant K_s and a threshold concentration, the biomass concentration reaches $C_x = C_{xo} + Y^m_{DX}C_{so}$. Subsequently the biomass concentration decreases due to maintenance and/or biomass decay, which is characterized by the maintenance coefficient m_D (or the decay coefficient k_d). The relevant substrate always acts as electron donor, and therefore it is proper to define the biomass yield and maintenance on donor (D).

In the design of processes with growing microorganisms (fermentation processes and biological waste-treatment processes) the key parameters that need to be considered are the maximal biomass yield on substrate (Y^m_{DX}), the substrate maintenance coefficient m_D, the maximal growth rate (μ_{max}), and the substrate affinity constant (K_s). These four key parameters are sufficient to describe growth of



Four basic parameters

	Units	Range
Stoichiometric		
Y_{DX}^{m}	[C-mol X/(C)-mol D]	0.010–0.7
m_D	[(C)-mol D/C-mol X/h]	0.01–3
Kinetic		
μ^{max}	(h^{-1})	0.005–2
K_s	(mol D/L)	10^{-6}–10^{-3}

Figure 1. A typical microbial growth batch profile of biomass concentration and substrate concentration.

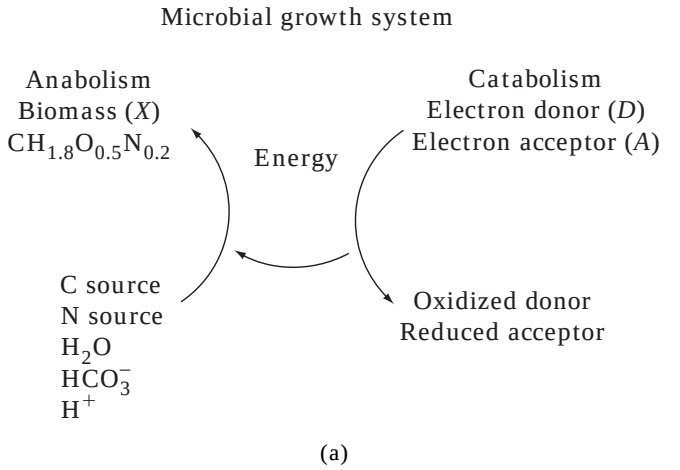
microorganisms in a standard mathematical model (1–4). A practical problem is, however, that the values of these parameters can vary by more than two orders of magnitude for different electron donors or acceptors used by different microorganisms, as indicated in Figure 1. It should be realized that conventionally stoichiometric parameters are expressed with C-mol of biomass, C-mol of electron donor for organic, and mol of donor for inorganic donors (C-mol X/(C)-mol D). It is therefore of interest to provide a general method to estimate values of these parameters for any chemotrophic growth system. Such methods have been provided by, for example, Battley (5), Roels (1), and Westerhoff (6). Recently (2,9) these methods have been critically evaluated with respect to general applicability and internal consistency. It was concluded (2,7,8) that none of these methods was satisfying. However a new method was proposed that is generally applicable and lacks the mentioned problems (2,8). Further, it should be recognized that in growth processes not only biomass production (r_x in C-mol biomass per m^3 reactor/h) and electron donor (substrate) consumption r_D in C-mol substrate (for carbon compounds), or mol substrate (for noncarbon compounds) per m^3 reactor/h are important. Also, the other conversions, such as O_2 consumption, N source consumption, heat production, and CO_2 production are highly relevant for the process design to calculate, for example, the required O_2 and heat transfer. Clearly, the full stoichiometry of the growth process should also be calculated and methods to achieve this are of major interest.

A STANDARD DESCRIPTION OF MICROBIAL GROWTH STOICHIOMETRY

The stoichiometry of microbial growth is most easily understood from Figure 2. Figure 2a introduces the biomass composition of 1 C-mol biomass (the ash-free organic fraction). The composition shown is fairly typical and is taken from Roels (1). One C-mol ash-free organic biomass is the amount of organic dry biomass that contains 12 g of carbon. The indicated biomass organic fraction corresponds to an elemental composition of 48.8% carbon, 7.3% hydrogen, 32.5% oxygen, and 11.4% nitrogen (w/w).

In practice, total dry biomass, which includes the organic fraction and the ash fraction (S, P, K, Mg, etc.), is measured. In general, the organic and ash fraction are obtained by combusting the organic biomass at 500 to 600 °C and weighing the ashes. Recently Battley (9) has indicated that this simple procedure underestimates the real organic biomass weight by 5 to 6%. This is due to the formation of P, S, and metal oxides in the ash during combustion, whereas such oxides are not present in the dry biomass. The composition formula follows directly from the elemental analysis of the biomass. In Figure 2a only the four major elements (C, H, O, N) are shown; however, it is straightforward to include P, S, and metals such as K or Mg in this composition formula, and also in the stoichiometric/energetic calculation. Figure 2a also shows that in the formation of biomass for all chemotrophic growth systems a C source, N source, H_2O, CO_2, and H^+ are always involved. These five compounds provide the building elements for making biomass. This is also called anabolism. For heterotrophic organisms the C source is organic; for autotrophic organisms the C source is CO_2.

Although it is possible to establish a stoichiometrically correct description to make biomass from these five building compounds, it is easily shown that this is not acceptable from the point of view of the second law of thermodynamics. It has been calculated that the Gibbs energy of such a hypothetical reaction, depending on the C source used, is often positive (7), although sometimes small negative values can also be calculated (5). In addition, it is



(a)

Relevant stoichiometry

$$-\frac{1}{Y_{DX}}\text{(C-)mol electron donor} - (\ldots)\text{ N-source} - \frac{1}{Y_{AX}}\text{ mol electron acceptor} + 1\text{ C-mol biomass} + \frac{1}{Y_{QX}}\text{(heat)} + \frac{1}{Y_{GX}}\text{ Gibbs energy} + (\ldots)\,H_2O + (\ldots)\,HCO_3^+ + (\ldots)\,H^+$$

Many different possibilities

Donor	Acceptor	C-source
Organic	Organic	Organic
Inorganic	Inorganic	Inorganic

$Y_{DX}0.01 - 0.7$ C-mol$X$/(C)mol donor

(b)

Figure 2. (**a**) System definition of microbial growth. (**b**) Macrochemical reaction equation of microbial growth.

known that to convert the five compounds into biomass, microorganisms use a large amount of biochemical energy in the form of ATP (10). Clearly the production of biomass from the five building compounds requires input of large quantities of Gibbs energy. The amount of energy needed to make biomass depends on the type of C source used. Intuitively, one expects that making 1 C-mol biomass from CO_2 requires more Gibbs energy than making 1 C-mol biomass from an organic compound. A quantitative relation for this energy need is presented later (equations 2, 3a, and 3b). The required energy, which must be taken as Gibbs energy and not as enthalpy, is delivered by a redox reaction between an electron donor and an electron acceptor. This redox reaction is called catabolism (Fig. 2a). Examples are the aerobic combustion of glucose ($C_6H_{12}O_6 + 6O_2 \rightarrow 6HCO_3^- + 6H^+$) and the anaerobic formation of ethanol from glucose ($C_6H_{12}O_6 + 2H_2O \rightarrow 2HCO_3^- + 2H^+ + 2C_2H_5OH$). Obtaining the required Gibbs energy is as essential for micro-organisms as it is for higher organisms, and even for human society. Therefore it should not be surprising that during evolution a wide diversity of microorganisms developed that are mainly different in the applied redox reaction for catabolism to obtain Gibbs energy (Fig. 2b). Electron donor or acceptor couples can be organic and inorganic compounds. This microbial variety in catabolic possibilities for generating Gibbs energy has led to the use of a classification system for naming microorganisms (Table 1). This system is understandably based on the source of Gibbs energy (light or chemical energy), the source of electron donor (inorganic or organic), and the source of biomass carbon (CO_2 or organic).

In addition, microorganisms may employ a wide variety of electron acceptors, as reflected in their class names. These class names are related to the electron acceptor used in catabolism (O_2, aerobic; NO_3^-, denitrification; SO_4^{2-}, sulphate reduction) fermentation (absence of external electron acceptor), or to the product of the catabolic reaction (CH_4, methanogenic; acetate, acetogenic; H_2S, sulphidogenic, etc.). The C source also functions often as electron donor, except in autotrophic microorganisms, where the C source is CO_2. For example, a microorganism growing aerobically in the dark on H_2S as the electron donor (inorganic compound) using CO_2 as the C source is called an aerobic chemolithoautotrophic organism. In summary, in each realistic chemotrophic microbial growth system there must be present the five compounds of anabolism and an electron donor/acceptor combination for catabolism.

These considerations bring us then to Figure 2b, which shows the macrochemical reaction equation containing all the stoichiometric information of the growth process. The macrochemical equation of Figure 2b should not be considered a mathematical equation but is a chemical reaction where substrates and products have negative and positive stoichiometric coefficients, respectively. Therefore for $a = 0$, sign is absent. In addition, the stoichiometric involvement of enthalpy and Gibbs energy is expressed in their respective stoichiometric coefficients (Y_{QX}, and Y_{GX}, which have units of C-mol X/kJ). The macrochemical reaction equation is therefore a compact, but exact, form of notation of the relevant stoichiometry of growth. This macrochemical equation shows that for the formation of +1 C-mol of biomass an amount of $-1/Y_{DX}$ of electron donor is required. The minus sign shows that the electron donor is consumed. Y_{DX} is in C-mol biomass per C-mol electron donor (in case of an organic donor) or per mol donor (in case of an inorganic donor). Its units are written as C-mol X/(C) mol D. An amount of $-1/Y_{AX}$ mol electron acceptor is consumed (minus sign) per 1 C-mol biomass produced and, in addition, $1/Y_{QX}$ kJ of heat and $1/Y_{GX}$ kJ of Gibbs energy are involved in the production of 1 C-mol of biomass. $1/Y_{QX}$ and $1/Y_{GX}$ are found as the conventionally calculated enthalpy of reaction and Gibbs energy of reaction of the macrochemical reaction equation, which produces 1 C-mol of biomass. Finally, certain amounts of H_2O, CO_2, (or HCO_3^-), H^+, and N source are involved. It is important that in each macrochemical reaction equation in which biomass is grown, HCO_3^-, H_2O, H^+, and N source be present. The differences between different organisms occur mostly in the electron acceptor/donor combinations used. The N source is often NH_4^+ and sometimes NO_3^-, N_2, or something else. The most important point in stoichiometry is to recognize that it is nearly always sufficient to measure one stoichio-

Table 1. Microbial Classification System

Source of Gibbs energy	Source of electron donor	C-source
Light (phototrophic)	Inorganic (lithotrophic)	CO_2 (autotrophic)
Chemical (chemotrophic)	Organic (organotrophic)	Organic (heterotrophic)

metric coefficient, that is, Y_{DX}, which is the traditional biomass yield on substrate (equal to carbon source and electron donor). All the other stoichiometric coefficients then follow from the so-called conservation equations (elements, electric charge, and enthalpy) (Example 1a) and the Gibbs energy balance (Example 1b).

EXAMPLE 1a

Calculation of stoichiometric coefficients in the macrochemical equation

Consider the aerobic growth of *Pseudomonas oxalaticus* on oxalate using NH_4^+ as the N source. The relevant chemical compounds in this growth system are the five compounds [biomass ($CH_{1.8}O_{0.5}N_{0.2}$), NH_4^+, HCO_3^-, H^+, H_2O], the electron donor oxalate ($C_2O_4^{2-}$), and the electron acceptor O_2. In total there are seven compounds and four elements (C, H, O, N). The conversion rates of these compounds are mathematically related by the conservation relations of C, H, O, N, and electric charge. In total there are five independent relations. This means that seven conversion rates are related by five conservation equations, and that the measurement of two rates (e.g., biomass production r_x and consumption of the electron donor oxalate r_D), which is equivalent to the measurement of $Y_{DX} = r_X/-r_D$, allows the calculation of all other yields.

Suppose that from measurement the biomass yield Y_{DX} is found to be +0.086 C-mol biomass produced per C-mol oxalate consumed. The proper macrochemical reaction equation can be written in a general form, without knowing all the stoichiometric coefficients but one (+1 for biomass), as

$$f C_2O_4^{2-} + a NH_4^+ + b H^+ + c O_2 + d H_2O \rightarrow 1 CH_{1.8}O_{0.5}N_{0.2} + e HCO_3^-$$

The following conservation equations can now be written:

C conservation	$2f + 1 + e = 0$
H conservation	$4a + b + 2d + 1.8 + e = 0$
O conservation	$4f + 2c + d + 0.5 + 3e = 0$
N conservation	$a + 0.2 = 0$
Charge conservation	$-2f + a + b - e = 0$

Clearly there are six unknown stoichiometric coefficients (a–f) that are related by five conservation equations. (Biomass has been assigned a convenient, yet arbitrary coefficient +1.) Having one measured coefficient allows the calculation of all other coefficients. Y_{DX} was measured as 0.086. This means that 1/0.086 = 11.63 C-mol oxalate are consumed to produce 1 C-mol biomass. The previously defined macrochemical equation contains f mol of oxalate, which was two carbon atoms. The stoichiometric coefficient f therefore has the value −11,63/2 = −5.815 (remember the minus sign). Using this f value and the five conservation equations, one can calculate the whole chemical growth stoichiometry. The result is

$$-5.815 C_2O_4^{2-} - 0.2 NH_4^+ - 0.8 H^+ - 1.857 O_2 - 5.42 H_2O \rightarrow 1 CH_{1.8}O_{0.5}N_{0.2} + 10.63 HCO_3^-$$

All the different biomass yields can be read from this reaction equation; thus, $Y_{AX} = 1/1.857 = 0.538$ C-mol biomass/mol O_2 or $Y_{CX} = 1/10.63 = 0.094$ C-mol biomass per mol CO_2.

In the Example 1a, only the chemical stoichiometry was calculated. However, there are two additional biomass yields of interest that relate the heat production and Gibbs energy dissipation occurring during the growth process to biomass production. These yields can be simply calculated if the full chemical stoichiometry is known by using tabulated ΔH_f^0 and ΔG_f^{01} values (at pH = 7 and standard conditions) and calculating the enthalpy and Gibbs energy of reaction (Example 1b).

Table 2 contains all the required thermodynamic information as taken from Thauer et al. (11). The values for biomass are taken from Roels (1). Although there is some discussion about the value of ΔG_f^{01} for biomass, its value is not very important in thermodynamic calculations, as shown by Heijnen (3).

EXAMPLE 1b

Calculation of the yield of biomass on enthalpy and Gibbs energy (Y_{QX} and Y_{GX})

The chemical stoichiometry from Example 1a and the appropriate ΔH_f^0 and ΔG_f^{01} values from Table 2 can be used to obtain the heat (enthalpy) and Gibbs energy of reaction.

The enthalpy of reaction, using ΔH_f^0 from Table 2, is calculated as

$$(10.63)(-692) + 1(-91) - (5.42)(-286) - (1.857)(0) - (0.8)(0) - (0.2)(-133) - (5.815)(-824) = -1078.7 \text{ kJ}$$

For the Gibbs energy of reaction using ΔG_f^{01} values there follows a value of −1052.4 kJ. Because in the macrochemical reaction 1 C-mol of biomass is produced, this means that for each 1 C-mol biomass produced there is a heat production of 1078.7 kJ and a Gibbs energy dissipation of 1052.4 kJ, showing that $Y_{QX} = 1/1078.7 = 0.00093$ C-mol biomass produced per kilojoule heat produced and that $Y_{GX} = 1/1052.4 = 0.0095$ C-mol biomass produced per kilojoule of Gibbs energy dissipated. The complete chemical and energetic stoichiometry now can be written as

$$-5.815 C_2O_4^{2-} - 0.2 NH_4^+ - 0.8 H^+ - 1.857 O_2 - 5.42 H_2O \rightarrow 1 CH_{1.8}O_{0.5}N_{0.2} + 10.63 HCO_3^- + 1078.7 \text{ kJ heat} + 1052.4 \text{ kJ Gibbs energy}$$

Example 1 shows that the complete chemical and energetic stoichiometry of microbial growth can be calculated from one measured yield using conservation equations and the Gibbs energy and enthalpy balance (elements, charge,

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

NH_4^+ consumption (r_N). Measurement of these two rates would not lead to a calculation of the other rates because r_X and r_N occur in the nitrogen-conservation equation in such a way that r_X uniquely determines r_N, and vice versa. It is then said that r_X and r_N are redundant. The N balance gives a constraint for these two measured conversion rates that can be used to calculate the statistically best estimate of r_X and r_N, which also exactly satisfies the N balance.

Clearly the choice of the two measured rates must be such that calculability of all other conversion rates is assured. In example 1a, suitable combinations would be the oxygen consumption rate (r_O) and biomass production rate (r_X), r_O and the carbondioxide production rate (r_C), or r_X and the heat production rate (r_Q).

Ill-Conditioned Calculability of Stoichiometry (Error Propagation)

It is well known that measured conversion rates have a certain measurement error. The subsequently calculated conversion rates, from combining the conservation relations and the two measurements, have an error due to error propagation. It is obviously of great practical importance to choose two measured conversion rates where this error propagation is minimal. A simple example to illustrate this problem is the aerobic growth of biomass on the donor glucose. If oxygen consumption ($-r_O$) and carbon dioxide production (r_C) are the measured rates, then the following relations (using conservation relations and the standard biomass composition) to calculate r_X and $-r_D$ (in C-mol glucose/m^3 h) from the measured r_C and ($-r_O$) can be derived:

$$r_X = 20r_C - 20(-r_O)$$
$$(-r_D) = 20(-r_O) - 21r_C$$

Due to the large multiplication factors of 20 and 21 in these equations, the propagation of the measurement errors in r_C and r_O into r_X and $-r_D$ is enormous.

If the donor conversion rate ($-r_D$) and the carbon dioxide production rate (r_C) were chosen as the measured rates r_X and ($-r_O$) would be calculated as

$$r_X = (-r_D) - r_C$$
$$(-r_O) = -0.05(-r_D) + 1.05r_C$$

The error propagation now is much lower and, therefore, from the measured ($-r_D$) and (r_C), r_X and ($-r_O$) can be calculated, as can be the other conversion rates involved. Clearly the aspect of error propagation is of major importance, and this propagation can be significantly decreased by a proper choice of the conversion rates to be measured.

Redundancy of Measurements

As stated earlier, in general two well-chosen measured conversion rates are usually sufficient to reliably calculate the complete stoichiometry. However, it is advantageous (Example 2) to measure more conversion rates than the minimum requirement of two. This leads to so-called redundant measurements, which can be used for two purposes error diagnosis and data reconciliation.

Error Diagnosis

- To check the validity of the defined growth systems with respect to the absence of by-products or possible second substrates
- To check the measured conversion rates for systematic errors

Data Reconciliation

- To decrease the measurement error in the calculated and measured conversion rates, provided that the statistically based checks (error diagnosis) on the validity of the growth system and the systematic errors in the measured conversion rates are passed

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EXAMPLE 2

Use of redundant measurements to establish the presence of errors in the definition of the growth system or in the measurements

Consider the microbial growth system of Example 1a, where the following four conversion rates have been measured. The biomass has the standard elemental composition.

Biomass production	$r_X = +1$ C-mol/h
O_2 consumption	$-r_O = 1.2$ mol/h
HCO_3^- production	$r_C = 10.5$ mol/h
Oxalic acid ($C_2O_4^{2-}$ consumption)	$-r_D = 5.8$ mol/h

We know that a minimum of two rate measurements are needed to calculate the full stoichiometry. Therefore there are two redundant measurements. We can now establish the conservation equations (with r_W, r_H, and r_N as the water, proton, and NH_4^+ conversion rates, respectively) based on conversion rates as

C conservation	$2r_D + r_X + r_C = 0$
H conservation	$4r_N + r_H + 2r_W + 1.8r_X + r_C = 0$
O conservation	$4r_D + 2r_O + r_W + 0.5r_X + 3r_C = 0$
N conservation	$r_N + 0.2r_X = 0$
Charge conservation	$-2r_D + r_N + r_H - r_C = 0$

By eliminating the three nonmeasured rates (r_W, r_H, r_N) from these five conservation equations, one obtains $5 - 3 = 2$ equations, which relate the measured conversion rates only. The result is as follows:

$$2r_D + r_X + r_C = 0$$
$$2r_D - 4r_O + 4.2r_X = 0$$

The first relation can be recognized as the carbon balance, and the second is so-called electron balance or the balance of degree of reduction (1) (see also a later section). With respect to the C balance, one finds from the measurements:

$$\text{C-in} = 2 \times 5.8(\text{oxalate}) = 11.6 \text{ C-mol/h}$$
$$\text{C-out} = 1(\text{biomass}) + 10.5(CO_2) = 11.5 \text{ C-mol/h}$$

Clearly the C balance seems satisfying (0.86% gap).

For the balance of degree of reduction one obtains

$$\text{electrons in} = 2 \times 5.8 = 11.6 \text{ mol electrons/h}$$

$$\text{electrons out} = -1.2(-4) + 1(4.2) = 9 \text{ mol electrons/h}$$

Clearly there is a large gap of 2.6 mol electrons/h.

Because the C balance fits, it is reasonable to assume that the measured values of r_D, r_X, and r_C are reliable. The balance of degree of reduction can therefore be wrong for two reasons:

1. A very inaccurate measurement of r_O.
2. If the measurement of r_O is found to be correct, then the only other possibility is the presence of an additional electron acceptor (e.g., NO_3^-). This would be an error in the defined growth system.

A Mathematically Complete Analysis of Calculability, Analysis of Redundancy, Error Diagnoses, and Data Reconciliation

In the preceding section simple examples were provided to highlight the problems in accurately establishing the full growth stoichiometry from measurements. Because all these calculations are based on linear conservation relations, it is highly appropriate to use matrix algebra. Basic to these calculations is the "elemental" matrix, which specifies the element, charge, and enthalpy information for each compound in the growth system. Recently, an extensive and coherent mathematical description has been provided for calculability, redundancy analysis, error diagnosis, statistical aspects, and data reconciliation using involved matrix algebra (18–21). The developed mathematical theory has been put in a user-friendly computer program called Macrobal (22).

THE EFFECT OF GROWTH RATE ON GROWTH STOICHIOMETRY

Maintenance Energy Concept

In his pioneering work, Monod (23) found that in exponential growth the amount of biomass formed increased in proportion to the amount of substrate consumed. This led to the definition of growth yield Y_{DX} [amount of biomass produced per amount of electron donor (substrate) consumed]. We have seen that Y_{DX} usually determines the complete growth stoichiometry. With the introduction of the chemostat in the early 1950s, microbial growth could be studied at a range of growth rates, and it became clear that Y_{DX} decreased at lower growth rates μ, as shown in Figure 3 (24). This phenomenon was explained by two different concepts (24–26):

- Endogenous respiration or microbial decay, determined by the parameter k_d
- Electron donor (substrate) requirements for maintenance, determined by the parameter m_D

The basic idea is, however, similar in recognizing that a microorganism is a complex structure where the polymers (proteins, etc.) are subject to slow thermal denaturation

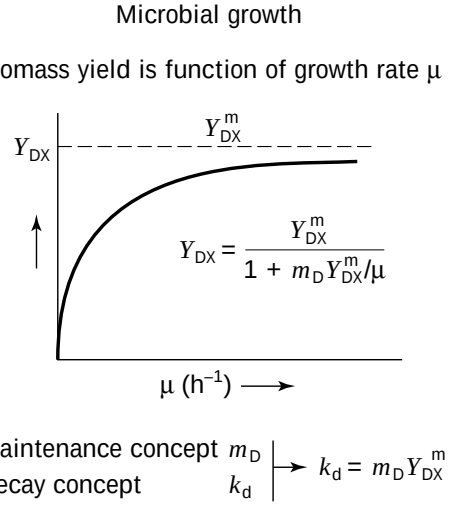


Figure 3. Dependence of Y_{DX} on growth rate μ.

and where there are numerous small leaks associated with the many transmembrane gradients (e.g., Na^+ leaking into the microorganism). These leaking substances must be pumped out, and the degraded polymers must be rebuilt at the expense of Gibbs energy. This results in a small, but finite, need of Gibbs energy to maintain the biomass structure and the transmembrane gradients (maintenance Gibbs energy). In the concept of endogeneous respiration or microbial decay, this energy is produced by catabolism of biomass itself. In the concept of maintenance this energy is produced by catabolism of a part of the substrate (electron donor).

Mathematically, the dependence of Y_{DX} on growth rate μ is described by equation 1a and shown in Figure 3.

$$1/Y_{DX} = 1/Y^m_{DX} + m_D/\mu \qquad (1a)$$

This equation contains two model parameters, Y^m_{DX} and m_D, where m_D is the rate of consumption of electron donor (substrate) that is catabolized to generate the necessary Gibbs energy flow for maintenance in C-mol electron donor per C-mol biomass per hour. Y^m_{DX} is the maximal biomass yield. Figure 3 shows that Y_{DX}, using equation 1a, decreases with decreasing growth rate. Clearly, at higher growth rates Y_{DX} comes close to Y^m_{DX}. Using typical values for m_D it can be shown that only for $\mu < 0.01$ to 0.05 h^{-1}, Y_{DX} starts dropping significantly below Y^m_{DX}. This means that in exponential growth, as occurs in batch fermentation where μ is high, the stoichiometry is properly covered by Y^m_{DX}. However, in many industrial-fed batch-production processes, maintenance is extremely important due to the low growth rates applied. For example, in penicillin fermentation $\mu \approx 0.01$ h^{-1} and about 70% of all consumed glucose is spent for maintenance (27). Similarly, in wastewater-treatment processes, where low growth rates are also applied, the maintenance effects are very relevant. However, in this area one often uses the biomass decay coefficient k_d. This coefficient is however related to m_D according to $k_d = m_D\, Y^m_{DX}$. In general, it can be shown that all biomass yields, Y_{iX} as defined in Figure 2a, decrease with decreasing growth rate μ.

Measuring m_D

Equation 1a shows that Y^m_{DX} and m_D can be obtained directly by measuring Y_{DX} at different growth rates μ. Using equation 1a to plot $1/Y_{DX}$ versus $1/\mu$ as a straight line to obtain $1/Y^m_{DX}$ and m_D is, however, not desirable, because the error distribution of the measurements Y_{DX} and μ is completely distorted due to the use of $1/Y_{DX}$ and $1/\mu$.

It is more proper to directly use the nonlinear equation 1a in combination with the measured Y_{DX} and μ, and to use an algorithm for nonlinear parameter estimation. It is stressed that for accurate m_D values one should measure Y_{DX} at low growth rates (0.005–0.03 h^{-1}).

EXAMPLE 3a

Calculating Y^m_{DX} and m_D from measured Y_{DX} as function of growth rate μ

Consider aerobic growth on glucose and that there are two measurements available. At $\mu = 0.5\ h^{-1}$, $Y_{DX} = 0.49$ C-mol biomass per C-mol glucose, and at $\mu = 0.02\ h^{-1}$, $Y_{DX} = 0.33$.

Applying equation 1a will show that $Y^m_{DX} = 0.50$ and $m_D = 0.02$ C-mol glucose/C-mol biomass/h.

Other Maintenance Quantities

Microorganisms require Gibbs energy for maintenance. This is obtained by catabolizing the required amount of electron donor m_D. It is then obvious that other quantities, such as electron acceptor, heat, Gibbs energy, oxidized electron donor, and reduced electron acceptor, are also involved in maintenance, to catabolize the m_D electron donor. These maintenance-related quantities are directly obtained from the stoichiometry of the catabolic reaction (Example 3b).

EXAMPLE 3b

Calculating other maintenance rates using the catabolic reaction

In Example 3a it was found that $m_D = 0.02$ C-mol glucose/C-mol biomass/h.

In the growth system being considered, the catabolic reaction is the aerobic oxidation of glucose according to

$$C_6H_{12}O_6 - 6O_2 + 6HCO_3^- + 6H^+ + 2{,}814\ \text{kJ heat} + 2{,}843\ \text{kJ Gibbs energy}$$

Using the stoichiometry of this catabolic reaction and the known m_D, it is now easy to calculate the other maintenance rates:

$$\text{maintenance glucose } m_D = 0.02\ \text{C-mol glucose/C-mol Xh} = 0.02/6\ \text{mol glucose/C-mol biomass/h}$$

$$\text{maintenance oxygen } m_A = \frac{0.02}{6} \times 6\ \text{mol } O_2\text{/C-mol biomass/h}$$

$$\text{maintenance } HCO_3^- \ m_C = \frac{0.02}{6} \times 6\ \text{mol } CO_2\text{/C-mol biomass/h}$$

$$\text{maintenance heat } m_q = \frac{0.02}{6} \times 2{,}814\ \text{kJ/C-mol biomass/h}$$

$$\text{maintenance Gibbs energy } m_G = \frac{0.02}{6} \times 2{,}843\ \text{kJ/C-mol biomass/h}$$

Complete Growth Stoichiometry as a Function of Growth Rate

Equation 1a shows how $1/Y_{DX}$ depends on the growth rate μ and the two parameters Y^m_{DX} and m_D. Completely similar equations can be derived for growth yields on acceptor (A), carbon dioxide (C), heat (Q), and Gibbs energy (G) according to equation 1b:

$$1/Y_{iX} = 1/Y^m_{iX} + \frac{m_i}{\mu} \qquad (1b)$$

Here i can be A, C, Q, or G. The maintenance coefficients for the different compounds are related to m_D according to Example 3b. The maximal yields Y^m_{AX}, Y^m_{CX}, Y^m_{QX} and Y^m_{GX} are related to Y^m_{DX}, and can be found by solving the macrochemical equation, using the available Y^m_{DX} value, according to Example 1.

A THERMODYNAMICALLY BASED METHOD TO ESTIMATE GROWTH STOICHIOMETRY

In the previous paragraphs the methods for accurate measurement of a growth stoichiometric coefficient, as, for example, the biomass yield on electron donor Y_{DX} and the subsequent calculation of all the nonmeasured stoichiometric coefficients of the macrochemical equation (using the conservation principles) have been provided. In past decades, the value of Y_{DX} for many different microorganisms, different electron donors, C sources, and electron acceptors has been measured under C- and energy-limited growth conditions. Many methods have been proposed to predict Y_{DX} because of its obvious importance. Recently, a critical evaluation of these methods has been performed (2). The following criteria were used for the evaluation:

- The method should be generally applicable to all chemotrophic growth systems.
- The method should relate directly to the second law of thermodynamics.
- No detailed knowledge of metabolism is required; only the identity of the electron donor, C source, and electron acceptor is known.
- Methodological problems are absent.

The conclusion of this evaluation was that none of the published methods satisfied these simple criteria. Therefore, an alternative method that satisfies the mentioned criteria has been proposed (2). This method is based on $1/Y_{GX}$, which is the amount of Gibbs energy (in kilojoules) that must be dissipated for the production of 1 C-mol biomass.

The Gibbs energy stoichiometric parameter $1/Y_{GX}$ has already been introduced as one of the stoichiometric coefficients in the macrochemical reaction equation (Fig. 2b). Therefore, it is obvious that this energetic parameter can be calculated directly if only one of the chemical stoichio-

metric coefficients has been measured and if the electron donor, electron acceptor, and C source are known (see Example 1b, where $1/Y_{GX}$ = 1,052 kJ/C-mol biomass).

Furthermore it is well known that the value of growth yields depends on the growth rate (μ) due to the Gibbs energy that must be used for maintenance (see earlier section). This means that the Gibbs energy needed to produce biomass should be divided into two parts:

1. A growth-related part
2. A maintenance-related part

Mathematically this can be expressed as

$$\underset{\text{Total needed Gibbs energy kJ/C-mol biomass}}{1/Y_{GX}} = \underset{\text{Gibbs energy for new biomass}}{\frac{1}{Y^{m}_{GX}}} + \underset{\text{Maintenance Gibbs energy for existing biomass}}{\frac{m_G}{\mu}} \quad (1c)$$

where $1/Y^{m}_{GX}$ is the Gibbs energy needed to make 1 C-mol of biomass (kJ/C-mol X) and m_G is the Gibbs energy needed for biomass maintenance (kJ/C-mol biomass h). The biomass specific growth rate (h^{-1}) is μ.

Clearly, at high growth rate μ, the m_G/μ term becomes negligible and $1/Y_{GX}$ becomes practically equal to Y^{m}_{DX}. At low growth rates Y_{GX} becomes much lower than Y^{m}_{GX}.

Equation 1c shows that in order to calculate Y_{GX} as a function of growth rate μ we need information about Y^{m}_{GX} and m_G. In the past years two simple correlations have been found with which to estimate Y^{m}_{GX} and m_G (2,4). These correlations were established using a very large body of experimental growth yields, which covered carbon- and energy-limited growth for the following:

- Many different microorganisms (bacteria, fungi, plant cells)
- Many different C sources, including CO_2 and a wide variety of organic substrates
- Different electron acceptors (aerobic, anaerobic, denitrifying)
- Electron donors that need reversed electron transport (RET)

The resulting correlations are given in equations 2 and 3. Figure 4 shows the m_G data used to establish equation 2; Figure 5 shows the $1/Y^{m}_{GX}$ data used to establish the correlations 3a and 3b.

Maintenance Gibbs Energy Need m_G

The data for m_G as a function of temperature shown in Figure 4 can be correlated with an Arrhenius type of relation:

$$m_G = 4.5 \exp\left[\frac{-69{,}000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right] \quad (2)$$

This correlation was found to hold (with ± 40% accuracy) for a very wide variety of organisms, for different electron donors, for aerobic and anaerobic conditions, and for a temperature range of 5–75 °C (4). Obviously, the main influencing factor is the temperature, which behaves as an Arrhenius function with an activation energy of 69,000 J/mol. The type of electron donor (organic or inorganic), the microorganism, and the electron acceptor are of minor importance. This seems logical, because maintenance is a biomass-linked Gibbs-energy-requiring process that counteracts the biomass-deteriorating processes (protein degradation, leakage over cell membranes, etc.).

Gibbs Energy for Growth

The data for Y^{m}_{GX} shown in Figure 5a (heterotrophic growth) and Figure 5b (autotrophic growth) can be correlated by equations 3a and 3b as shown in Ref. 2. For autotrophic growth it was found to be important to distinguish electron donors for which reversed electron transport (RET) was necessary. Such electron donors (e.g., Fe^{2+}/Fe^{3+}, NH_4^+/NO_2^-) provide electrons that have insufficient Gibbs energy to reduce the C source CO_2 to biomass. Microorganisms using such electron donors first have to increase the Gibbs energy level of the donor electrons by the biochemical process RET.

Heterotrophic growth/autotrophic growth (−RET)

$$1/Y^{m}_{GX} = 200 + 18(6 - C)^{1.8} + \exp[((3.8 - \gamma)^2)^{0.16}(3.6 + 0.4C)] \quad (3a)$$

Autotrophic growth (+RET)

$$1/Y^{m}_{GX} = 3{,}500 \quad (3b)$$

It was found (4) that the Gibbs energy dissipation required for the production of 1 C-mol biomass mainly depends on the C source used (equations 3a and 3b) The type of microorganism and the type of electron acceptor have only minor effects, as shown in Figures 5a and 5b. The influence of the C source on Y^{m}_{GX} can be characterized by the following:

- Its number C of carbon atoms (e.g., for CO_2 C = 1 and for glucose C = 6) as shown in Figure 5a
- Its degree of reduction γ (1)

γ is a stoichiometric number of a chemical compound that represents the number of electrons in the compound. For organic compounds γ is per C-mol, for inorganic compounds γ is per mol. For example, for CO_2 γ = 0, for CH_4 γ = 8, and for glucose γ = 4. The concept of degree of reduction will be further elucidated extensively later in this article. For organic compounds (Fig. 5a), γ has a value between 0 and 8. For inorganic compounds (Fig. 5b), only a lower value of 0 holds; a maximal value does not exist because there is no normalization per atom. It is relevant to know that biomass has a degree of reduction of about 4.2. Equation 3a and Figure 5a show that, in the situation that RET is not required for both hetero- and autotrophic growth, the Gibbs energy needed to produce biomass

- Increases if the number of C atoms in the carbon source (the parameter C in equation 3a) decreases

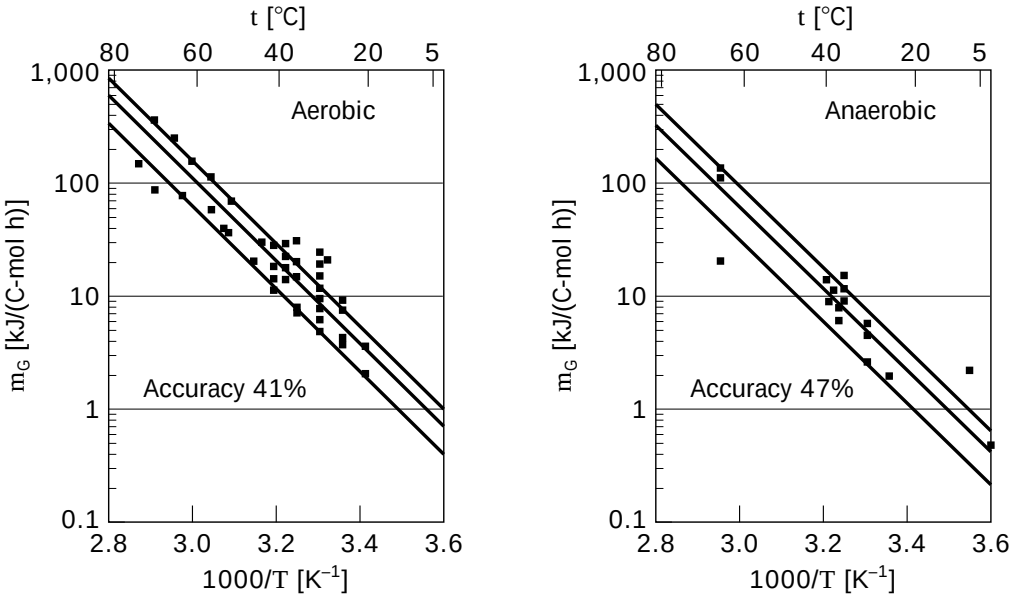


Figure 4. Maintenance Gibbs energy m_G (in kJ/C-mol biomass h) for aerobic (a) and anaerobic (b) growth, shown as an Arrhenius function of temperature. The lines represent equation 2.

- Increases if the degree of reduction of the carbon source (the parameter γ in equation 3a) is smaller or larger than about 3.8

Equation 3a further shows that for heterotrophic growth $1/Y^m_{GX}$ ranges between about 200 and 1,000 kJ/C-mol biomass, for the C sources explored, for which:

- The number of carbon atoms in the carbon source ranges between C = 1 (e.g., CO_2, formate, methane) and C = 6 (e.g., glucose, citrate).
- The degree of reduction of the C source γ ranges between 0 (for CO_2) and 8 (for CH_4).

The effect of the number of C atoms (C) and degree of reduction (γ) of the C source can be simply understood as follows:

- Biomass contains many polymers that contain monomers of four to six C atoms. If the C source contains fewer than four to six C atoms, the microorganism must perform extra biochemical reactions to achieve C–C couplings. This requires extra Gibbs energy, compared to a C source that has six C-atoms, Hence $1/Y^m_{GX}$ increases for C-sources with less carbon atoms.
- Biomass has $\gamma = 4.2$. If the C source is more reduced ($\gamma > 4.2$) or more oxidized ($\gamma < 4.2$), there is a need for additional oxidation reactions or reduction reactions, respectively, as compared to a carbon source (like glucose) with $\gamma = 4$. These additional reactions lead to extra Gibbs energy dissipation, leading to a higher value of $1/Y^m_{GX}$.

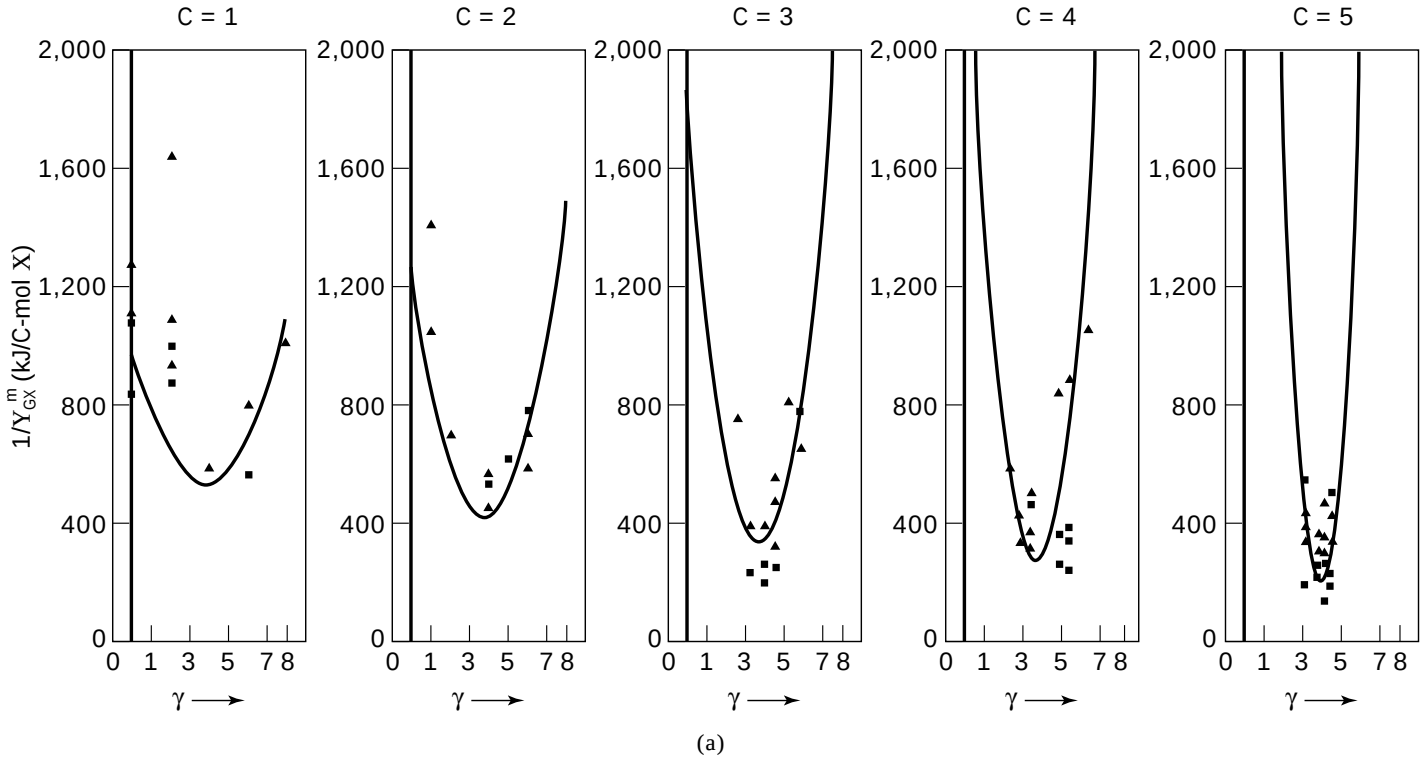
Simply stated, the more biochemical tinkering is needed to convert an organic C source into biomass, the more Gibbs energy is dissipated and the higher $1/Y^m_{GX}$ becomes. Obviously glucose (C = 6, $\gamma = 4$) is a nearly ideal C-source because it requires the least Gibbs energy dissipation for biomass production. According to equation 3a, for glucose $1/Y^m_{GX} = 200 + 0 + 36 = 236$ kJ/C-mol biomass. In contrast, CO_2 is a very poor C source, because it requires about four times as much Gibbs energy ($1/Y^m_{GX} = 200 + 236 + 460 = 986$ kJ/C-mol according to equation 3a). Equation (3b) shows that for autotrophic growth, in the situation where RET is needed (which occurs for many inorganic electron donors), $1/Y^m_{GX}$ has a very high value of 3,500 kJ/C-mol biomass. This value should be compared to autotrophic growth without RET as occurs with, for example, H_2 or CO as electron donor (for which $1/Y^m_{GX} \approx 1{,}000$ kJ/C-mol according to equation 3a).

Obviously, the use of RET increases the Gibbs energy dissipation needed for biomass production tremendously. The explanation is that, using the RET process, the electrons of the electron donor are increased in energy level, up to the energy level of electrons in NADH in order to make CO_2 reduction to biomass thermodynamically feasible. This "energy-pumping" process (RET) apparently requires a large amount of Gibbs energy, of about 3,500 − 1,000 = 2,500 kJ/C-mol biomass produced.

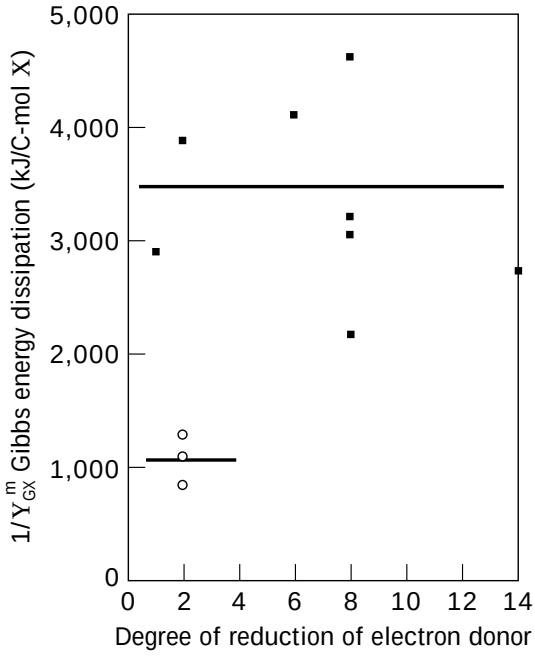
The effect that the type of the available C source has on the Gibbs energy needed for biomass synthesis is well known in biochemistry. Biochemists express the energy need in ATP. Figure 6 compares the calculated Gibbs energy dissipation needed for biomass synthesis ($1/Y^m_{GX}$, in kJ/C-molX) with the theoretically calculated amount of ATP expenditure for biomass synthesis in mol ATP/C-mol X. The points shown are for different C sources, ranging from glucose (28.8) to CO_2 (2.5). The parenthetical numbers are the published (10) biomass yields on ATP in gram-X/mol ATP. It is clear that there is a close correspondence, which is logical. Equations 3a and 3b provide the energy needed for biomass synthesis in kilojoules, whereas the biochemists use mol ATP as the energy measure.

In conclusion, it should be realized that equations 2, 3a and 3b are completely sufficient to estimate a biomass yield and the full macrochemical equation for any arbitrary chemotrophic growth system (Example 4).

The predictive accuracy of this correlation for chemotrophic growth has been shown (2,4) to be ±10 to 20% rela-



(a)



(b)

Figure 5. Reciprocal maximal yield of biomass on Gibbs energy, $1/Y^{m}_{GX}$, (kJ/C-mol X); (a) Heterotrophic growth (triangles, aerobic; squares, fermentation, X's denitrifying systems); C is the number of carbon atoms in the carbon source; γ is the degree of reduction of the carbon source. (b) Autotrophic growth (squares, electron donors where reversed electron transport [RET] is needed; circles, donors without RET). The lines represent equations 3a and 3b.

tive error in a yield range of nearly two orders of magnitudes of 0.01–0.70 C-mol biomass per C-mol organic electron donor or per mol inorganic donor while covering aerobic, anaerobic, denitrifying, autotrophic microbial systems with and without RET (Fig. 7). The measured yield data used were taken from Refs. 2 and 4.

EXAMPLE 4

Calculation of the full macrochemical reaction equation using the correlations of equations 2, 3a, and 3b

It is assumed that a microorganism grows anaerobically on methanol as C source and electron donor with NH_4^+ as the N source

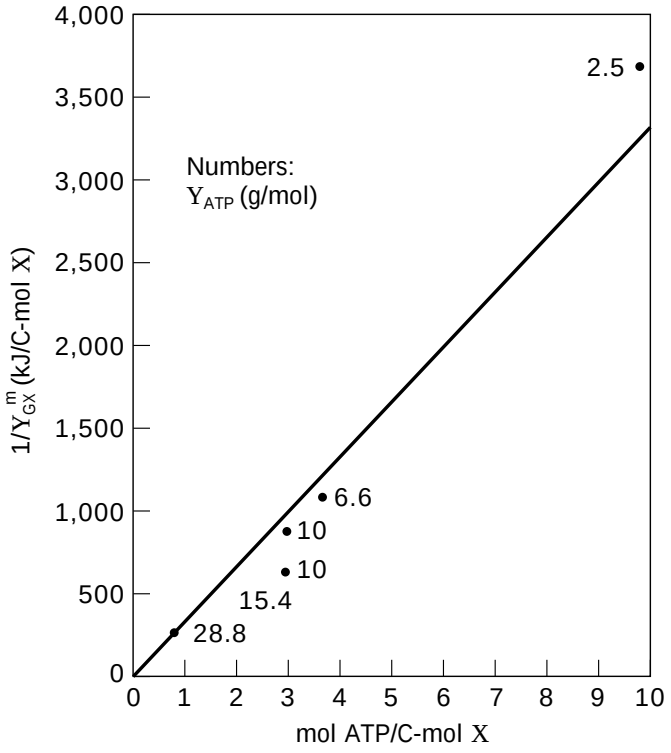


Figure 6. Comparison of energy needed for biomass synthesis on different carbon sources in mol ATP/C-mol biomass and in kJ/C-mol biomass ($1/Y^m_{GX}$). The numbers refer to the conventional biomass yield on ATP in gram biomass/mol ATP for different carbon sources (10).

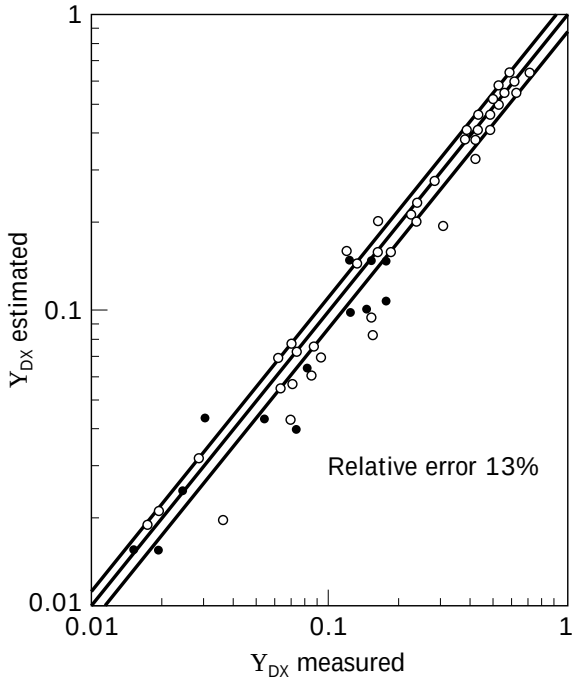


Figure 7. Comparison of measured and predicted biomass yield Y_{DX} (solid circles, fermentative; open circles, aerobic growth systems).

and acetate is produced. The growth system contains biomass, NH_4^+, H^+, HCO_3^-, H_2O, methanol, and acetate as the seven compounds. The general macrochemical reaction equation for the production of 1 C-mol biomass can be written as follows:

$$fCH_3OH + aNH_4^+ + bH^+ + cC_2H_3O_2^- + dH_2O + 1CH_{1.8}O_{0.5}N_{0.2} + eHCO_3^-$$

Clearly there are six unknown stoichiometric coefficients (a–f). However, using equation 3a we can calculate that (C = 1, γ = 6 for methanol, maintenance has been neglected) $1/Y^m_{GX}$ = 200 + 326 + 172 = 698 kJ/C-molX. This means that we know that the Gibbs energy of reaction of the macrochemical reaction equation equals −698 kJ.

We can now write the conservation equations for C, H, O, N, electric charge, and the Gibbs energy balance (taking values of ΔG_f^{01} from Table 2):

C balance	f + 2c + 1 + e = 0
H balance	4f + 4a + b + 3c + 2d + 1.8 + e = 0
O balance	f + 2c + d + 0.5 + 3e = 0
N balance	a + 0.2 = 0
charge balance	a + b − c − e = 0

Gibbs energy balance (−175.39)f + (−79.37)a + (−39.87)b + (−369.41)c + (−237.18)d + (−67)1 + (−586.85)e + 698 = 0

Solving these six equations gives, for a to f,

a = −0,2; b = 2.866; c = 8.898; d = 12.964; e = −6.232; f = −12.564

This gives a biomass yield on methanol of 1/12.564 = 0.08 C-mol biomass/C-mol methanol. The stoichiometric result also shows that the acetate production is 8.898/12.564 = 0.70 mol acetate/mol methanol, showing a C yield of 1.4 acetate carbon/methanol-carbon. This is, of course, due to the CO_2 fixation that occurs (6.232 mol HCO_3^- per 12.564 mol methanol).

If maintenance is not allowed to be neglected m_G must be taken into account. For example, the temperature is assumed to be 50 °C. Equation 2 then shows that m_G = 38.8 kJ/C-mol biomass h. If the growth rate μ = 0.03 h^{-1}, then we can calculate, using equation 1c that $1/Y_{GX}$ = 698 + 38.8/(0.03) = 1,991 kJ/C-mol biomass. Using this number one can solve the six equations to obtain the complete stoichiometry, which holds under these conditions.

Before ending this section, a final warning is relevant. The described thermodynamic method of predicting growth stoichiometry is based on a very wide database of experimentally measured growth systems. No detailed biochemical information is required, because intrinsically a kind of average biochemistry used by most organisms is assumed. This is an attractive feature, but in the end we should consider that, of course, the biochemistry used by microorganisms does have a significant influence. For example, for the anaerobic ethanol fermentation on glucose the mentioned method will give Y_{DX} = 0.15 C-mol biomass/C-mol glucose. This is indeed found for Saccharomyces cerevisae. However, another organism, Zymomonas mobilis, does the same glucose/ethanol process, but with Y_{DX} = 0.07. The explanation is that Z. mobilis uses a completely different biochemical pathway for glucose catabolism than S. cerevisae. From this example we can also

learn that if the predicted biomass yield differs very substantially from the actually measured yield, it might be possible that the microorganism being studied uses a novel pathway for catabolism or anabolism.

A USEFUL REFERENCE SYSTEM TO SIMPLIFY GROWTH STOICHIOMETRIC AND ENERGETIC CALCULATIONS AND TO GAIN INSIGHT

The Growth Reference System

In the preceding sections, the stoichiometric coefficients for the macrochemical reaction equation of biomass formation have been solved by setting up the proper conservation equations (C, H, O, N, charge, enthalpy) and the Gibbs energy balance. Although this is a sufficient and straightforward method, solving these linear equations remains unattractive and does not provide insight. To simplify these calculations and to gain insight, a special reference system has been designed—the growth reference system. This reference system is based on the observation that, in all chemotrophic growth systems, H_2O, HCO_3^-, H^+, and N source (mostly NH_4^+) occur as chemical compounds (see earlier section on growth system definition). In this special reference system each chemical compound is assigned three new numbers.

- γ the degree of reduction, which represents the electron content per C-mol (for organic compounds) or per mol (for inorganic compounds)
- ΔG_e the Gibbs energy per electron present in the compound
- ΔH_e the enthalpy per electron present in the compound

Clearly γ is a stoichiometric quantity and ΔG_e and ΔH_e are energetic parameters.

The reference system is designed such that for H_2O, HCO_3^-, H^+ (pH = 7), N source for growth, HPO_4^{2-}, NO_3^-, SO_4^{2-}, and Fe^{3+}, the values of γ, ΔG_e, and ΔH_e are zero. For ΔG_e, the biochemical standard conditions (1 mol/L, 1 bar, pH = 7,298 K) are assumed, ΔH_e is calculated for CO_2 (gas) because of the large heat effect of HCO_3^- (liq) $\Leftrightarrow$ CO_2 (gas) transfer.

The calculation of γ, ΔG_e, and ΔH_e follows from the reference redox half reaction where 1 C-mol of organic or 1 mol of inorganic compound is converted into the reference chemicals and a number of electrons. The number of electrons is by definition equal to γ (Example 5). From the Gibbs energy and enthalpy of this reference reaction, called ΔG_{ref} and ΔH_{ref} (calculated with the usual thermodynamic ΔG_f^{01} and ΔH_f^{01} values, see Table 2), the values of ΔG_e and ΔH_e follow from equations 4a and 4b.

$$\Delta G_e = \frac{-\Delta G_{ref}}{\gamma} \tag{4a}$$

$$\Delta H_e = \frac{-\Delta H_{ref}}{\gamma} \tag{4b}$$

EXAMPLE 5

The reference redox half reaction and calculation of γ and ΔG_e for chemical compounds

For methanol the following reference redox half reaction can be set up according to the preceding definition by converting methanol to the reference compounds HCO_3^-, H_2O, and H^+

$$-1CH_4O - 2H_2O + HCO_3^- + 7H^+ + 6e^-$$

In this reference redox half reaction, 1 C-mol methanol is converted and six electrons are produced, hence $\gamma = +6$ for methanol. Using the ΔG_{f-}^{01} values from Table 2, the ΔG_{ref} for the methanol-reference redox half reaction follows as (standard conditions)

$$\Delta G_{ref}^{01} = (7)(-39.87) + 1(-586.85) - (2)(-237.18) - (1)(-181.75) = -216.192 \text{ kJ}$$

This gives for the ΔG_e^{01} value of methanol by equation 4a

$$\Delta G_e^{01} = -\left(\frac{-216.192}{6}\right) = +36.032 \text{ kJ/e-mol}$$

Obviously ΔH_e can be calculated in a similar way by calculation of ΔH_{ref}.

For biomass the following redox half reaction can be set up, assuming that NH_4^+ is the N source:

$$-1CH_{1.8}O_{0.8}N_{0.2} - 2.5H_2O + HCO_3^- + 0.2NH_4^+ + 5H^+ + 4.2e^-$$

Obviously, the degree of reduction for biomass is 4.2. The ΔG_{ref}^{01} value is obtained similarly as earlier for methanol. ΔG_{ref}^{01} can be calculated to be -142.128 kJ, giving

$$\Delta G_e = -(-142.128)/(4.2) = +33.840 \text{ kJ/e-mol}$$

In a similar way as shown in Example 5 for each chemical compound, the values of γ, ΔG_e, and ΔH_e can be calculated for a large number of relevant compounds. Table 3 contains all relevant stoichiometric and energetic information for growth systems, clearly shown in the following. A point of attention is the finding (Table 3) that for biomass the degree of reduction depends on the N source used in the growth system. For example $\gamma = 4.2$ for NH_4^+ and 5.8 for NO_3^- as N source. This is a consequence of the reference definition. The advantage is that the N source disappears from the stoichiometric calculations using γ, ΔG_e, and ΔH_e (Examples 7a, 7b, and 8b). The defined reference system is closely related to the generalized degree of reduction as defined by Roels (1) and Erickson et al. (28). It can be seen that for reduced organic compounds γ is between 0 and 8 (per C-mol). For inorganic compounds, such an upper limit does not exist (because there is not a normalization per atom). For O_2, γ is negative (-4), which is logical for an acceptor. ΔG_e is related to the conventional redox potential of redox half reactions ($\Delta G_e^{01} = -FE_0^1$). ΔG_e is calculated using HCO_3^- (the most abundant form of carbon dioxide at pH = 7); ΔH_e has been calculated using CO_2 (gas) as reference, to take the large heat effect of $HCO_3^- \rightarrow CO_2$ (gas) into account.

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.

Balance of Degree of Reduction, Atomic Degrees of Reduction, and the COD Balance

In the previously defined "growth reference" system, γ_i was introduced as the degree of reduction of compound i. This parameter is important in stoichiometric calculations, because due to the principle of electron conservation, an electron balance can be defined. This is the so-called balance of degree of reduction. This is not an additional conservation principle (in addition to C, H, O, N, and charge conservation). The balance of degree of reduction can be obtained from the usual C, H, O, N, and charge balances by eliminating (by suitable substitutions) H^+, H_2O, HCO_3^-, and the N source. Hence, the balance of degree of reduction is a suitable linear combination of already available conservation equations. The importance of the balance of degree of reduction is that, by definition, in this balance, only biomass formation, consumption of electron donor, and consumption of electron acceptor are related. Based on the previous definition of the reference set of compounds in the growth reference system it is also possible to calculate the degree of reduction of atoms and of electric charge (Table 4).

It should be noted that the atomic degree of reduction for the N atom in biomass depends on the applied N source as a consequence of the defined growth reference system as explained earlier. Using the γ values of atoms and electric charge in Table 4, it is straightforward to calculate the γ values for any chemical compound for which the elemental composition is known (Example 6a). This is an equivalent alternative to writing the reference redox half reaction to obtain γ (Example 5).

EXAMPLE 6a

Direct calculation of γ from elemental composition

Using the atomic degrees of reduction (Table 4) it can easily be checked that indeed for the reference chemicals $\gamma = 0$:

H_2O	$\gamma = 2 \times 1 + 1(-2) = 0$
CO_2	$\gamma = 1 \times 4 + 2(-2) = 0$
HCO_3^-	$\gamma = 1 \times 1 + 1 \times 4 + 3(-2) + 1 = 0$
H^+	$\gamma = 1 \times 1 + 1(-1) = 0$

Table 4. Degree of Reduction of Atoms and Electric Charge According to the Definition of the Growth Reference System

Atom or charge	Degree of reduction of atoms
H	+1
O	−2
C	+4
Charge +1	−1
Charge −1	+1
S	+6
P	+5
N	+5
N in N index in biomass	+3 for NH_4^+ or NH_3 as N source 0 for N_2 as N source +5 for NO_3^- or HNO_3 as N source

For the degree of reduction of biomass (γ_X) it is easy to show that this is a function of the N source used. Using the standard elemental biomass composition $CH_{1.8}O_{0.5}N_{0.2}$ and using the N degree of reduction for the different N sources (Table 4) one obtains

$$-NH_4^+ \text{ as N-source } \gamma_X = 1 \times 4 + 1.8 \times 1 + 0.5(-2) + 0.2(-3) = 4.2$$

$$-NO_3^- \text{ as N-source } \gamma_X = 1 \times 4 + 1.8 \times 1 + 0.5 \times (-2) + 0.2(5) = 5.8$$

For the electron content of an organic substrate (e.g., acetate ion, $C_2H_3O_2^-$) the amount of electrons is $2 \times 4 + 3 \times 1 + 2(-2) + 1(+1) = 8$. Because for organic compounds γ is defined as the number of electrons per C atom, we obtain for acetate with 2 carbon atoms $\gamma = 8/2 = 4$.

The degree of reduction balance is also called chemical oxygen demand (COD) balance in wastewater engineering (26). The COD balance is equivalent to the balance of degree of reduction. COD is a number assigned to each chemical and represents the consumed O_2 on total oxidation in g O_2/g compound. There is a direct link with degree of reduction. Each mole of electrons represents 8 g COD. This is easily understood, because the consumption of 1 mol O_2 represents the acceptance of 4 mol electrons ($\gamma = -4$; see Table 3). One mol O_2 represents −32 g COD and therefore 1 mol electrons ≡ 8 g COD.

EXAMPLE 6b

Calculation of COD values

Consider glucose, in which 1 mol (≡ 180 gram) represents (according to Table 3) a total of 6×4 electrons ≡ $6 \times 4 \times 8 = 192$ g O_2. Clearly glucose has a COD value of 192/180 = 1.0667 g COD/g glucose.

The NO_3^-/N_2 acceptor couple has $\gamma_A = -5$ electrons. The COD value of NO_3^- − N is then $-5 \times 8/14 = -2.857$ g COD per gram nitrate-nitrogen.

The values of γ, ΔG_e, and ΔH_e from Table 3 can be used for very easy stoichiometric and energetic calculations as shown in Examples 7a and 7b.

EXAMPLE 7a

Calculation of the stoichiometry of example 1a using γ values

In Example 1a, five equations were solved to calculate the full macrochemical equation. Using the γ values of Table 3 we can first make the balance of degree of reduction. For the electron donor oxalate $\gamma_D = 1$ per carbon or 2 per mole oxalate; for biomass $\gamma_X = 4.2$ and for the electron acceptor $O_2 \gamma_A = -4$ (Table 3). For all the other chemicals (N source, H^+, H_2O, HCO_3^-) $\gamma = 0$ by definition. The γ balance is now

$$2f - 4c + 4.2 = 0$$

Because $f = -5.815$ we obtain $c = -1.857$ directly.

From the C balance we then obtain $e = +10.63$. From the N balance $a = -0.20$, from the charge balance $b = -0.8$, and from

the O or H balance we finally find d = −5.42. This is, as expected, the same result as before in Example 1a.

EXAMPLE 7b

Calculation of the Gibbs energy of reaction in example 1b using ΔG_e values

Using the now-available full macrochemical stoichiometry, it is possible to calculate the Gibbs energy of reaction using the Gibbs energy balance.

For each chemical compound the Gibbs energy contribution follows from the product of its number of electrons and its ΔG_e number. For example (using Table 3), the Gibbs energy contribution for oxalate, O_2, and biomass in the growth reference system follows as

$$\text{oxalate} = 2 \times 1 \times 52.522 = +105.04 \text{ kJ}$$

$$O_2 = -4 \times (-78.719) = 314.876 \text{ kJ}$$

$$\text{biomass} = 4.2 \times 33.840 = 142.128 \text{ kJ}$$

For all other reactants the Gibbs energy contribution in the growth reference system is zero. For the Gibbs energy of the macrochemical-reaction equation we obtain then from the available full stoichiometry:

$$-5.815(105.04) - 1.857(314.876) + 142.128 = -1053 \text{ kJ}$$

This is the same as obtained before, but now the calculation has only three terms.

Energetics of Redox Couples, Catabolic Redox Reactions, RET, and Energetic Regularities

It was pointed out earlier that for each microbial growth system a catabolic redox reaction is needed where an electron donor couple reacts with an electron acceptor couple. For the generation of maintenance energy the catabolic reaction is also required. For example, in aerobic growth on glucose the electron donor couple is glucose/HCO_3^- (glucose is oxidized to HCO_3^-) and the electron acceptor couple is O_2/H_2O (O_2 is reduced to H_2O). However, for anaerobic growth on glucose, where the catabolic reaction is the conversion of glucose into ethanol, the electron donor couple is glucose/HCO_3^- and the electron acceptor is the HCO_3^-/ethanol couple. To be able to quickly calculate the catabolic energy production, it is relevant to define the ΔG_e, ΔH_e, and γ values of redox couples i/j. These can be calculated from γ, ΔG_e, and ΔH_e values (Table 3) using equations 5a–5c.

$$\gamma_{couple} = \gamma_i - \gamma_j \tag{5a}$$

$$(\Delta G_e)_{couple} = \frac{(\gamma \Delta G_e)_i - (\gamma \Delta G_e)_j}{\gamma_i - \gamma_j} \tag{5b}$$

$$(\Delta H_e)_{couple} = \frac{(\gamma \Delta H_e)_i - (\gamma \Delta H_e)_j}{\gamma_i - \gamma_j} \tag{5c}$$

Equations 5a–5c have the following properties:

- If we invert the redox couple, for example, i/j into j/i, we are in fact inverting the redox half reaction of the redox couple. The value of γ changes sign, which is logical because produced electrons become consumed electrons. The value of ΔG_e and ΔH_e does not change, which is logical because the energetics of the reaction do not change.
- If the redox couple i/j contains a biological reference compound (e.g., j = HCO_3^-, H_2O, H^+, NO_3^-, SO_4^{2-}) then for compound j the value of γ, ΔG_e, and ΔH_e is zero. Equations 5a–5c then show that the value of γ, ΔG_e, and ΔH_e of the redox couple i/j becomes equal to the tabulated values of the i component in Table 3. For example, if the redox couple is an organic compound/HCO_3^-, then the γ, ΔG_e-, and ΔH_e value of the organic compound follow directly from Table 3.
- If the redox couple does not contain a reference chemical then equations 5a–5c must be used to calculate γ, ΔG_e, and ΔH_e. Table 5 shows some examples.

It can be seen from Table 5 that electron donor couples are characterized by positive γ values and electron-acceptor couples by negative γ values (which is logical). As stated earlier, in each microbial growth system there functions a catabolic reaction between an electron donor and an electron acceptor, which generates the required (for anabolism and maintenance) Gibbs energy. It is noted (equation 6c) that in a full catabolic redox reaction, the redox couple with the highest ΔG_e value must be the electron donor; the redox couple with the lowest ΔG_e value is the acceptor (Example 8a).

EXAMPLE 8a

Recognizing electron donor and acceptor

Consider the following catabolic reaction: $C_6H_{12}O_6 + 6O_2 \rightarrow 6HCO_3^- + 6H^+$. The two redox couples are $C_6H_{12}O_6/HCO_3^-$ and O_2/H_2O. According to Table 3, the glucose couple has $\Delta G_e = +39.744$ kJ/e-mol and the O_2 couple has $\Delta G_e = -78.719$ kJ/e-mol. Clearly glucose is the electron donor and O_2 is the acceptor. Consider now the catabolic reaction $C_6H_{12}O_6 + 2H_2O \rightarrow 2HCO_3^- + 2H^+ + 2C_2H_5OH$. This is the catabolic reaction in the ethanol fermentation. The redox couples are $C_6H_{12}O_6/HCO_3^-$ and

Table 5. ΔG_e, and ΔH_e Values of Redox Couples under Standard Conditions

Redox couple	γ (per (C-)mol)	ΔG_e (kJ/e-mol)	ΔH_e (kJ/e-mol)
$NH_4^+/\frac{1}{2}N_2$	3	+26.703	−44.3
NH_4^+/NO_2^-	6	−32.928	−99.665
NO_2^-/NO_3^-	2	−41.647	−108.5
Lactate/pyruvate	2	+18.283	−55.4
Fumarate/succinate	−2	−3.137	−64.5
$NO_3^-/\frac{1}{2}N_2$	−5	−72.194	−136.4
$NO_2^-/\frac{1}{2}N_2$	−3	−92.559	−155
$NO(g)/\frac{1}{2}N_2$	−2	−35.434	—
$N_2O(g)/N_2$	−2	−130.809	−183.8
Glucose/HCO_3^-	4	+39.744	−25.75

HCO_3^-/C_2H_5OH. From Table 3 we read for the glucose couple that $\Delta G_e = +39.744$ and for the ethanol couple $\Delta G_e = +30.353$. Now glucose is the donor and HCO_3^-/ethanol is the acceptor.

Because the catabolic reaction liberates the Gibbs energy required for the anabolism, it is important to calculate this amount of energy. Using the ΔG_e approach, we can then write directly equations 6a and 6b to calculate the Gibbs energy of reaction (ΔG_{CAT}) of the catabolic reaction. ΔG_{ED} and ΔG_{EA} are the electron Gibbs energy of the acceptor and donor couples:

$$-\Delta G_{CAT} = \gamma_D(\Delta G_{ED} - \Delta G_{EA}) \tag{6a}$$

The enthalpy of reaction ΔH_{CAT} of the catabolic reaction can be calculated similarly:

$$-\Delta H_{CAT} = \gamma_D(\Delta H_{ED} - \Delta H_{EA}) \tag{6b}$$

ΔG_{CAT} and ΔH_{CAT} represent the Gibbs energy and enthalpy of reaction of the catabolic reaction consuming 1 C-mol organic or 1 mol inorganic compound of electron donor. Dimensions are kJ/(C)-mol donor. γ_D is the degree of reduction of the donor couple in mol electrons/(C)-mol donor, which is always positive. According to the second law of thermodynamics ΔG_{CAT} must be negative. Therefore equation 6c, the second law of thermodynamics, holds:

$$\Delta G_{ED} > \Delta G_{EA} \tag{6c}$$

This shows that indeed the electron donor couple always has the highest ΔG_e value.

EXAMPLE 8b

Calculation of catabolic Gibbs energy production using ΔG_e

Consider the catabolic reactions in Example 8a. For aerobic glucose oxidation we can calculate, using equation 6a and Table 3

$$(-\Delta G_{CAT}) = 4 \times [39.644 - (-78.719)] = 473.852 \text{ kJ, so that } \Delta G_{CAT} = -473.852 \text{ kJ}$$

This is the Gibbs energy released for the aerobic combustion of 1 C-mol glucose. For 1 mol of glucose (6 C-atoms) the Gibbs energy of the catabolic reaction is $6 \times (-473.852) = -2{,}843$ kJ.

For anaerobic ethanol fermentation of glucose we can calculate for the catabolic reaction $(-\Delta G_{CAT}) = 4 \times (39.744 - 30.353) = 37.564$ kJ per C-mol glucose. For 1 mol glucose the catabolic Gibbs energy of the catabolic reaction becomes $6 \times (-37.564) = -225.34$ kJ. This is the same as calculated in Example 3b.

The use of ΔG_e and ΔH_e now also reveals some interesting energetic regularities (4). Table 3 shows that for many organic donor compounds, the ΔH_e and ΔG_e values are rather close, with an average $\Delta G_e = +32 \pm 8$ kJ/e-mol and average $\Delta H_E = -28 \pm 5$ kJ/e-mol. These values are also close to the ΔG_E and ΔH_E value of biomass. Hence we can write for organic electron donors the important regularity

$$\Delta G_{ED} \approx \Delta G_{EX} \tag{7a}$$

$$\Delta H_{ED} \approx \Delta H_{EX} \tag{7b}$$

For autotrophic growth using inorganic electron donors, the ΔG_{ED} values are, in general, much lower than ΔG_{EX} (consider NH_4^+/NO_2^-, F_e^{2+}/F_e^{3+}, etc., in Table 4). For autotrophic growth (CO_2 as C source), this means that for these electron donors there is a need for Gibbs energy input in order to realize CO_2 reduction to biomass. This is achieved by RET. Knowing this we can write equation 7c to recognize RET.

$$\Delta G_{EX} > \Delta G_{ED} \tag{7c}$$

Finally, it is now easy to calculate the heat production and Gibbs energy dissipation in oxidative catabolism of organic compounds. As stated earlier, for organic compounds the average $\Delta G_{ED} = 32$ kJ/e-mol and for O_2 as acceptor $\Delta G_{EA} = -78.719$ kJ/e-mol. Hence, per mole of electron transferred between donor and acceptor, the available Gibbs energy is $32 - (-78.719) = 110.72$ kJ. Per mole of consumed O_2 (which accepts four electrons, $\gamma_A = -4$) in the combustion of any organic compound, the Gibbs energy made available by combustion of the organic compound is then $4 \times 110.72 = 443$ kJ per consumed mol O_2. Analogously, one can find for the produced heat per mole of O_2 in the combustion of organic compounds a value of 460 kJ per mole O_2. It is also obvious that the mentioned inaccuracy in the average ΔG_E or ΔH_E values for organic compounds only results in a minor error of 5 to 8% in the calculated Gibbs energy dissipation and heat production. These are very important rules of thumb for the fermentation industry (1).

MATHEMATICAL EQUATIONS TO CALCULATE THE GROWTH STOICHIOMETRY FROM KNOWN GIBBS ENERGY DISSIPATION

Deriving the Equations

In Example 1, it was shown that one suitably measured stoichiometric coefficient, for example, Y_{DX}, allows the calculation of all other stoichiometric coefficients, including the dissipated Gibbs energy $1/Y_{GX}$. This means that knowledge of $1/Y_{GX}$ should enable the calculation of all stoichiometric coefficients, as shown in Example 4. It has also been shown that Y_{GX} can be estimated for arbitrary growth systems under different growth rates and temperatures using the correlations (equations 2 and 3). Here we show that particularly simple equations to calculate all yields from Y_{GX} can be obtained by using the γ, ΔG_e, and ΔH_e parameters introduced in the previous section.

The general macrochemical reaction equation can be written as shown in Figure 2b. In this macrochemical equation, the electron donor and acceptor are written in C-mol (for organic compounds) or in mol (for inorganic compounds). The (. . .) stoichiometric coefficients are not given separate symbols. They follow easily from the charge balance (for H^+), N balance (for N source), from O or H balance (for H_2O), and the carbon balance (for HCO_3^-). For

autotrophic growth, the HCO_3^- stoichiometric coefficient is -1. Three balances can be written based on the conservation principles of electrons and enthalpy and the Gibbs energy balance.

Balance of degree of reduction

$$-\gamma_D/Y_{DX} - \gamma_A/Y_{AX} + \gamma_X = 0 \tag{8a}$$

Enthalpy balance

$$-\gamma_D \Delta H_{ED}/Y_{DX} - \gamma_A \Delta H_{EA}/Y_{AX} + \gamma_X \Delta H_{EX} + 1/Y_{QX} = 0 \tag{8b}$$

Gibbs energy balance

$$-\gamma_D \Delta G_{ED}/Y_{DX} - \gamma_A \Delta G_{EA}/Y_{AX} + \gamma_X \Delta G_{EX} + 1/Y_{GX} = 0 \tag{8c}$$

From equation 8a it follows that Y_{AX} is directly related to Y_{DX} (equation 9a)

$$\frac{1}{Y_{AX}} = \frac{\gamma_D}{(-\gamma_A)Y_{DX}} - \frac{\gamma_X}{(-\gamma_A)} \tag{9a}$$

The degree of reduction of the acceptor γ_A is (by definition) a negative number. Using equations 8a–8c, it is also possible to calculate directly Y_{DX}, Y_{AX}, and Y_{QX} as function of Y_{GX}. If we further use the found energetic regularity that $\Delta G_{EX} \approx \Delta G_{ED}$ (equation 7a) and replace $(\Delta G_{ED} - \Delta G_{EA})$ by $(-\Delta G_{CAT})/\gamma_D$ using equation 6a, it is possible to derive the simple equations 9b–9e. It is noted that ΔG_{CAT} is the Gibbs energy of the catabolic reaction of 1 C-mol organic or 1 mol inorganic compound, and that therefore $(-\Delta G_{CAT})$ is the Gibbs energy released in the catabolic reaction of 1 C-mol of organic or 1 mol of inorganic electron donor. $(-\Delta G_{CAT})$ is then by definition >0 and its units are kJ/(C)-mol donor. ΔG_{CAT} and ΔH_{CAT} follow from equations 6a and 6b using ΔG_{EA} and ΔG_{ED} values (Tables 3 and 5).

$$Y_{DX} = \frac{(-\Delta G_{CAT})}{1/Y_{GX} + \gamma_X/\gamma_D(-\Delta G_{CAT})} \tag{9b}$$

$$Y_{AX} = \frac{(-\gamma_A/\gamma_D)(-\Delta G_{CAT})}{1/Y_{GX}} \tag{9c}$$

$$1/Y_{QX} = \frac{(-\Delta H_{CAT})}{(-\Delta G_{CAT})}\, 1/Y_{GX} \tag{9d}$$

Furthermore it is often interesting to study Y_{DA}, which is the amount of electron acceptor couple consumed relative to the amount of electron donor consumed. For microorganisms growing aerobically on organic matter, this would be the mole of O_2 consumed per C-mole of organic compound consumed. For anaerobic growth this would be the amount of anaerobic products per amount of organic substrate in C-mole product per C-mole substrate. Because $Y_{DA} = Y_{DX}/Y_{AX}$ we obtain

$$Y_{DA} = [\gamma_D/(-\gamma_A)]\, \frac{1/Y_{GX}}{1/Y_{GX} + \gamma_X/\gamma_D(-\Delta G_{CAT})} \tag{9e}$$

Application of the Mathematical Stoichiometry Relations

The obtained stoichiometric relations (equations 9a–9e) can now easily be applied. In this section their use is demonstrated with the following subjects:

- Calculation of the complete growth stoichiometry
- Calculation of maintenance coefficients and maximal growth yields
- Calculation of the limit to growth yield posed by the second law
- Calculation of COD-based growth yields
- Calculation of the relation between heat production and Gibbs energy dissipation
- Calculation of maximal product yields in anaerobic metabolism

Calculation of the Complete Growth Stoichiometry. If for a given growth system the C source, the electron donor (ΔG_{ED} to decide on RET using equation 7c), the temperature, and the growth rate are known, then equations 2 and 3 allow a direct calculation of the required Gibbs energy $1/Y^m_{GX}$ to produce 1 C-mol of biomass at high growth rates μ. Knowing the electron donor couple and acceptor couple, and using Table 3 and equations 5a–5c, the values of ΔG_{ED}, ΔG_{EA}, ΔH_{ED}, ΔH_{EA}, γ_D, and γ_A can be calculated and from this $(-\Delta G_{CAT})$ and $(-\Delta H_{CAT})$ using equations 7a and b. Using equations 9b–9e subsequently allows the complete stoichiometric calculation where H^+, N source, H_2O, and HCO_3^- must be calculated using the conservation equations of electric charge, N, O or H, and carbon.

EXAMPLE 9a

Calculation of stoichiometry using equations 9b–9e

Consider Example 4, where a microorganism is grown anaerobically on methanol, producing acetate. Assume first that the growth rate is high, such that maintenance can be neglected. Equation 3a then shows that $1/Y^m_{GX} = 698$ kJ Gibbs energy per C-mol biomass. Methanol is the C-source, methanol/HCO_3^- is the electron donor, and HCO_3^-/acetate is the electron acceptor. From Table 3 and using equations 5a–5c, we can then find that $\Delta G_{ED} = 36.032$ kJ/e-mol, $\Delta H_{ED} = -23$ kJ/e-mol; $\Delta G_{EA} = 26.801$ kJ/e-mol; $\Delta H_{EA} = -33.5$ kJ/e-mol. Also, $\gamma_D = 6$, $\gamma_A = -4$, and $\gamma_X = 4.2$.

This provides that $-\Delta G_{CAT} = 6(36.032 - 26.801) = 55.386$ and $-\Delta H_{CAT} = -23 - (-33.5) = 63$ kJ/C-mol methanol. Using equations 9b–9e, we obtain the maximal growth yields (maintenance neglected).

$$1/Y^m_{DX} = 13.3 \text{ mol methanol/C-mol biomass}$$

$$1/Y^m_{AX} = 18.9 \text{ C-mol acetate/C-mol biomass} = 9.45 \text{ mol acetate/C-mol biomass}$$

$$1/Y^m_{QX} = 794 \text{ kJ heat/C-mol biomass}$$

$$Y^m_{DA} = 1.42 \text{ C-mol acetate/C-mol methanol} = 0.71 \text{ mol acetate/mol methanol}$$

Using the C- balance $1/Y^m_{CX}$ is calculated as 6.6 mol HCO_3^- consumed/C-mol biomass produced. This overall stoichiometric result is very close to the exact solution obtained in Example 4.

The small deviation arises from the assumption that $\Delta G_{EX} \approx \Delta G_{ED}$ (as discussed before).

In general it can be shown that the simple set of equations 9b–9e seldom deviates more than 5% from the exact solution.

Maintenance Coefficients and Maximal Yield Coefficients. As indicated previously, relations between the maintenance coefficients follow from the catabolic reaction. The following relations can now be written to link the various maintenance coefficients to the maintenance Gibbs energy m_G using $-\Delta G_{CAT}$ (see also Example 9b). It is noted that m_G follows from the correlation (equation 2) and that ΔG_{CAT} is calculated for the catabolism of 1 (C)-mol of electron donor.

$$m_D = m_G/(-\Delta G_{CAT}) \tag{10a}$$

$$m_A = (\gamma_D/-\gamma_A)m_G/(-\Delta G_{CAT}) \tag{10b}$$

$$m_Q = m_G \frac{(-\Delta H_{CAT})}{(-\Delta G_{CAT})} \tag{10c}$$

$$m_C = m_G/(-\Delta G_{CAT}) \tag{10d}$$

Using equations 10a–10d, the value of the maintenance Gibbs energy requirement m_G can be calculated from either measured maintenance coefficients (for electron donor m_D, electron acceptor m_A, heat production m_Q, or carbon dioxide production m_C). Furthermore, it can easily be understood that the maximal biomass yields for electron donor, acceptor, and heat are found from equations 9b–9e by substitution of $1/Y^m_{GX}$ (instead of $1/Y_{GX}$) because the maintenance contribution is then neglected.

EXAMPLE 9b

Effect of maintenance on stoichiometry

In Example 9a the maintenance contribution was neglected. Assume that the microorganism is growing at 37 °C. Equation 2 then leads to $m_G = 13$ kJ/C-mol biomass h. Using equations 10a–10c and using ΔG_{CAT} and ΔH_{CAT} (Example 9) we obtain

$$m_D = 0.2347 \text{ mol methanol/C-mol biomass/h}$$

$$m_A = 0.3521 \text{ C-mol acetate/C-mol biomass/h}$$

$$m_Q = 14.787 \text{ kJ/C-mol biomass/h}$$

Further assume that the growth rate $\mu = 0.02\ h^{-1}$. Using equation 1b, the Y^m_{iX} values obtained in Example 9a, and the m_i values obtained here, one obtains for the stoichiometry:

$$\text{electron donor(D)}1/Y_{DX} = 13.3 + \frac{0.2347}{0.02} = 25.03 \text{ mol methanol/C-mol X}$$

$$\text{electron donor(A)}1/Y_{AX} = 18.9 + \frac{0.3521}{0.02} = 36.50 \text{ C-mol acetate/C-mol X}$$

$$\text{heat } 1/Y_{QX} = 794 + \frac{14.787}{0.02} = 1533 \text{ kJ heat/C-mol X}$$

$$Y_{DA} = 36.50/25.03 = 1.46 \text{ C-mol acetate/mol methanol}$$

These values can also be obtained directly from equations 9b–9e by substituting the complete Gibbs energy of growth and maintenance according to equation 1c:

$$1/Y_{GX} = 1/Y^m_{GX} + \frac{m_G}{\mu} = 698 + \frac{13}{0.02} = 1348 \text{ kJ Gibbs energy/C-mol X}$$

Clearly, comparing Examples 9a and 9b, one observes that the yield of biomass Y_{DX} drops from 0.077 to 0.04 due to maintenance, but the acetate/methanol yield Y_{DA} increases from 1.42 to 1.46 C-mol acetate/mol methanol.

Second Law Limit of Growth Yield. As for any chemical reaction, the microbial growth yield is also limited by the second law of thermodynamics. This limit is achieved if $1/Y_{GX} = 0$, because this defines equilibrium. Equations 9c–9f then show that for the thermodynamic limits we can write [see also Ref. 4] the following:

Thermodynamic limits for growth yields

$$Y_{DX} = \gamma_D/\gamma_X$$

$$1/Y_{AX} = 0$$

$$1/Y_{HX} = 0$$

Clearly, the more reduced electron donors (γ_D higher) have a higher Y_{DX} limit. This limit has already been determined (1).

COD-Based Yields. In wastewater treatment, the biomass yield is calculated on COD basis. Y_{COD} is the gram biomass COD over gram-substrate COD. Based on the COD definition we can write

$$Y_{COD} = \frac{\gamma_X}{\gamma_D} Y_{DX} \tag{11a}$$

This allows the following relation for Y_{COD} from equation 9b

$$Y_{COD} = \frac{(-\Delta G_{CAT})}{(-\Delta G_{CAT}) + (\gamma_D/\gamma_X)1/Y_{GX}} \tag{11b}$$

For aerobic growth on organic substrate $-\Delta G_{CAT} = \gamma_D[32-(-78.719)] \approx \gamma_D \times 110$ kJ/C-mol. Here the average value of $\Delta G_{ED} = +32$ kJ/e-mol for organic matter was used as shown before. Substitution of $-\Delta G_{CAT}$ gives Y_{COD} for aerobic growth on organic substrate

$$Y_{COD} = \frac{110}{110 + (1/Y_{GX})/\gamma_x} \tag{11c}$$

This equation shows that Y_{COD} for aerobic growth is not a constant, as often assumed (26) with $Y_{COD} \approx 0.50$–0.67, but it depends also on the type of C source, because this determines $1/Y_{GX}$ (equations 3a and 3b). Also, to decrease the Y_{COD} leading to lower surplus-sludge production, one must, according to equations 11b and 11c:

- Decrease ($-\Delta G_{CAT}$), by, for example, using anaerobic metabolism producing CH_4
- Increase $1/Y_{GX}$ by increasing temperature or decreasing the growth rate according to equation 1

These predicted phenomena are well known and applied in waste-treatment processes.

Relation between Heat Production and Gibbs Energy Dissipation. According to equation 9d, the heat production ($1/Y_{QX}$) is related to Gibbs energy need ($1/Y_{GX}$) by the enthalpy and Gibbs energy of the catabolic reaction (ΔG_{CAT} and ΔH_{CAT}). Table 6 shows some examples of growth systems to illustrate the relation between heat production and dissipated Gibbs energy for growth (29). From Table 6 we can conclude the following rules of thumb:

- For aerobic (or denitrifying) growth systems on organic substrate, the Gibbs energy dissipation and heat production are nearly equal. The entropy contribution in the catabolic reaction is minimal (see glucose and acetate aerobic growth).
- For anaerobic growth, heat production and Gibbs energy dissipation can be substantially different, due to entropic effects.

Obviously, if in the catabolic reaction there is a net decrease of molecules or a consumption of gaseous molecules, then there is a strong negative entropy contribution (see H_2/CO_2 aerobic and anaerobic) and there is a much higher heat production than Gibbs energy dissipation. If, however, in the catabolic reaction there is a net production of the amount of molecules and/or production of gaseous molecules (e.g., the glucose/ethanol fermentation or the methane production from acetate), then there is a very large positive entropy contribution, leading to a much lower heat production than the Gibbs energy dissipation. The entropic effect can even be so large that there is a calculated heat uptake during growth (e.g., methanation of acetate). This is obviously endothermic growth. So, contrary to a common belief, growth of microorganisms is not necessarily related to heat production; there can be heat uptake as well. Experimental proof is, however, still lacking.

Maximal Product Yields in Anaerobic Metabolism. In many microbial processes the valuable product (e.g., ethanol or lactic acid) is related to catabolism. The relevant stoichiometric coefficient is then the yield of the electron acceptor couple to electron donor Y_{DA}.

Equation 9e shows how this coefficient is determined by various factors and it appears that Y_{DA} is maximized.

- For high Gibbs energy dissipation $1/Y_{GX}$. This means that high catabolic product yields are achieved for poor carbon sources, low growth rate, and high temperature, because $1/Y_{GX}$ is then maximized.
- For catabolic reactions with low ΔG_{CAT}. This is understandable, because then the growth yield is minimized, which leads directly to higher product yield.
- For highly reduced electron donors (γ_D high) and highly oxidized products (γ_A low). It is then even possible to achieve C yields larger than 1. An excellent example is the anaerobic production of acetate from methanol, where $Y_{DA} \approx 1.4$ C-mol acetate/C-mol methanol (Example 4).

KINETICS OF MICROBIAL GROWTH FROM A THERMODYNAMIC POINT OF VIEW

In the previous sections, the full stoichiometric description of growth has been given from a unifying thermodynamic point of view. However, for a complete description, growth kinetics are also required. In this section these kinetics are also presented from a thermodynamic point of view.

A Basic Kinetic Description of Microbial Growth

In Figure 1, the typically observed batch-growth curves of a microorganism growing on one substrate (electron donor) are shown. In this section we use the subscript S to denote substrate (electron donor, D). Usually these curves are described (for constant batch volume) with two mass balances for substrate (donor) and biomass, giving two differential equations (DE):

Table 6. Relation between Heat Production and Gibbs Energy Need

Microorganism	Growth condition	Y_{DX} (C-mol/(C)-mol)	$1/Y_{GX}$, Gibbs energy (kJ/C-mol biomass)	$1/Y_{QX}$, heat (kJ/C-mol biomass)	Entropy contribution (kJ/C-mol biomass)
Saccharomyces cerevisae	Glucose aerobic	0.57	332	+339	−7
Saccharomyces cerevisae	Glucose anaerobic	0.14	270	+95	+175
Hydrogenotroph	$H_2 + CO_2$ aerobic	0.13	1,265	+1686	−421
Methanobacterium arborophilus	$H_2 + CO_2$ anaerobic	0.015	1,035	+3,923	−2,888
Pseudomonas oxalaticus	Acetate aerobic	0.406	562	+593	−31
Methanobacterium soehngenii	Acetate anaerobic	0.024	597	−90	+687

Note: Heat production and Gibbs energy dissipation for (an)aerobic growth on glucose, H_2, and acetate; relative contribution of heat- and entropy-related dissipation.

$$\frac{dC_s}{dt} = q_s C_x \tag{12a}$$

$$\frac{dC_x}{dt} = \mu C_x \tag{12b}$$

where q_s and μ are the biomass-specific electron-donor (substrate) uptake and growth rate. To solve these DEs, we need kinetic expressions for q_s and μ. However, q_s and μ are stoichiometrically coupled to each other according to the Herbert–Pirt equation (equation 1a). This equation for substrate can be written as (realizing that $Y_{DX} \equiv Y_{SX} = \mu/-q_s$, and $m_S \equiv m_D$)

$$-q_s = \frac{1}{Y^m_{SX}}\mu + m_S \tag{13}$$

It is now only necessary to specify one kinetic equation, which can be for q_s of μ. In practice the Monod kinetic equation for μ ($\mu = \mu^m C_s/(C_s + K_s)$) is often chosen. This choice leads, however, to a very nasty inconsistency. According to Monod, $\mu = 0$ if the concentration of substrate C_s becomes 0. Substituting this result in the Herbert–Pirt equation shows, however, that (for $\mu = 0$) $q_s = m_s$. The inconsistency is then that in the absence of substrate ($C_s = 0$, $\mu = 0$) there is still consumption of substrate for maintenance ($v = -q_s = m_s$); this is very strange indeed. These problems can be avoided in a most simple way by introducing the kinetics of the substrate consumption rate in the most simplistic way (a Michaelis–Menten type of relation, see Figure 8):

$$-q_s = q_s^{max}\frac{C_s}{K_s + C_s} \tag{14a}$$

Combining equation 14a with equation 13 by eliminating q_s, leads to the kinetic relation of μ

$$\mu = Y^m_{SX}\, q_S^{max}\frac{C_s}{K_s + C_s} - Y^m_{SX}\, m_S \tag{14b}$$

The kinetic relations 14a and 14b contain four model parameters: Y^m_{SX}, K_s, m_s, and q_s^{max}. Figure 8 shows how μ and q_s depend on C_s.

Clearly, there is a maximal substrate uptake rate q_s^{max}, but also for μ a maximal value (μ^{max}) is seen at high C_s. The value of μ^{max} follows from equation 14b by taking the limit $C_s \gg K_s$, and the result is

$$\mu^{max} = Y^m_{SX}(q_s^{max} - m_s) \tag{15a}$$

In addition, it is clear from Figure 8 that there is a minimal substrate concentration ($C_{s,min}$) at which $\mu = 0$. For $C_s < C_{s,min}$, the growth rate μ becomes negative. An expression for $C_{s,min}$ can be found from equation 14b by putting $\mu = 0$, which results in

$$C_{s,min} = K_s\left(\frac{m_S}{q_s^{max} - m_s}\right) \tag{15b}$$

The occurrence of a minimal substrate concentration has indeed been observed (30,31). It can also be seen (Fig. 8) that at $C_s = 0$ the growth rate is negative.

$$(\mu)_{C_s=0} = -Y^m_{SX} m_S = -k_d \tag{15c}$$

Clearly, in the absence of substrate, the microorganism decreases its biomass; this phenomena is often called endogenous respiration, or biomass decay, where the kinetic parameter is k_d. Equation 14b can be rewritten by eliminating q_s^m and m_S using equations 15a and 15b leading to

$$\mu = \mu^{max}\frac{(C_s - C_{s,min})}{K_s + C_s} \tag{14c}$$

Alternatively equation 14b can be rewritten using the parameter k_d

$$\mu = \mu^{max}\frac{C_s}{K_s + C_s} - k_d\frac{K_s}{K_s + C_s} \tag{14d}$$

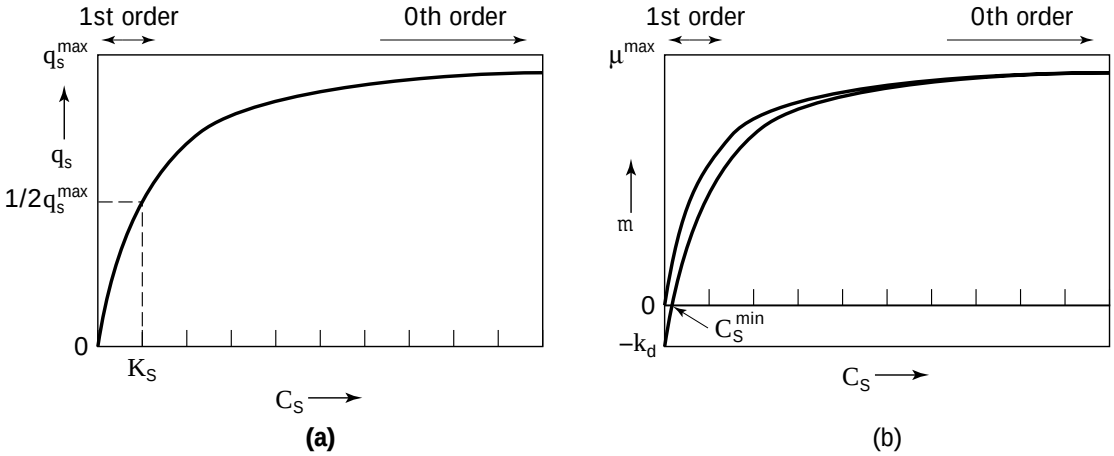


Figure 8. Dependence of q_s and μ as function of C_s for the Monod equation for μ (a) or the Michaelis–Menten equation for q_s (b).

It is clear that the obtained formulations for μ (equations 14b, 14c, and 14d) all become the familiar Monod equation if $m_S = 0$ (meaning $C_{s,min} = 0$ and $k_d = 0$; see equations 15b and 15c). The Monod equation and equations 14c and 14d are very close for $C_s \gg K_s$, but substantial differences arise for $C_s < K_s$ (see Fig. 8).

A final point to address is the implicit assumption (in equation 14a) that in the substrate-uptake process the substrate concentration can become zero. This is, in principle, not possible, because there always exists a substrate concentration where the catabolic reaction reaches a point where energy production in the form of ATP becomes impossible. This concentration is called the threshold concentration of substrate $C_{s,thresh}$.

From published data it is known that μ^{max} can vary in a wide range (0.001–1 h^{-1}) and that K_s is also covering an even wider range (10^{-6} to 10^{-3} mol/L). In the preceding sections m_S and Y^m_{SX} have been studied from a thermodynamic point of view. In the following, we present correlations and points of view that allow the estimation k_d, μ^{max}, K_s, and $C_{s,thresh}$, all based on a thermodynamic point of view. Such relations are very relevant in, for example, considerations of waste-treatment processes where the threshold concentration determines the effluent quality, where k_d determines the surplus sludge production, and where μ^{max} directly determines the reactor size or the type of reactor (suspended organisms or biomass retention systems). For batch-fermentation processes, μ^{max} is important because it determines the duration of a batch-growth process.

A Thermodynamic Relation for the Endogeneous/Decay Parameter k_d

According to equation 15c, k_d can be calculated from the relations for Y^m_{SX} and m_G found previously (equations 9c and 10a). Substitution gives

$$k_d = \frac{m_G}{1/Y^m_{GX} + (\gamma_X/\gamma_D)(-\Delta G_{CAT})} \qquad (16)$$

The values of m_G and Y^m_{GX} are obtained from the thermodynamic correlations equations 2 and 3. This equation shows that for aerobic heterotrophic growth (where $1/Y^m_{GX} \approx$ 300–600 kJ/C-mol biomass) the expected k_d will be a factor three times larger than for aerobic autotrophic growth (such as nitrification, for which $1/Y^m_{GX} \approx$ 3500 kJ/C-mol x). This has indeed shown to be the case (4), indicating that equation 16 is useful.

A Thermodynamic Correlation for μ^{max}

The value μ^{max} is the result of a limiting factor in metabolism. This metabolism can be represented very schematically as shown in Figure 9. Three possible bottlenecks are identified (Fig. 9):

1. The uptake rate of substrate
2. The rate of synthesis of biomass, as related to the ribosomal capacity to synthesize biomass protein

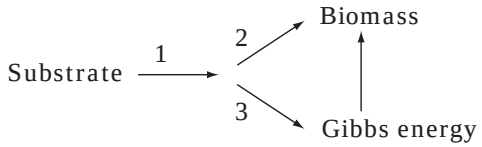


Figure 9. Possible bottlenecks in metabolism.

3. The rate of making Gibbs energy available to enable growth and maintenance

Here we assume as a simple working hypothesis that the rate of making Gibbs energy available for microorganisms is limited by a maximum rate of electron transport in the catabolic energy production. This hypothesis is supported by studies with *Escherichia coli*, where μ^{max} was measured aerobically (at the same temperature) for different growth substrates (32). It was observed that for all substrates the same maximal biomass specific O_2 uptake rate was achieved by *E. coli*. This supports the hypothesis of a constant maximal electron-transport capacity in microorganisms. A similar hypothesis was put forward by McCarthy (26). The following correlation is now proposed for the maximal electron-transport capacity in the microbial electron transport chain (in mol electrons/C-mol biomass/h) as a function of temperature:

$$\text{Maximal electron-transport capacity} = 3 \exp\left[\frac{-69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]$$

This correlation is an Arrhenius type of relation with an energy of activation of 69,000 J/mol. R is the gas constant (8.314 J/mol K). The value of 69,000 J/mol is an activation energy that follows from observations of the effect of temperature on μ^{max} of microorganisms (1).

For a given electron donor/acceptor combination, the maximal rate of Gibbs energy made available per unit biomass (q^{max}_G in kJ Gibbs energy per C-mol biomass per hour) follows then as

$$q^{max}_G = 3[(-\Delta G_{CAT})/\gamma_D] \exp\left[\frac{-69{,}000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]$$

It should be noted that $(-\Delta G_{CAT})/\gamma_D$ is the Gibbs energy made available per mole electron transported between donor and acceptor by the electron transport chain.

Now, by definition, the Gibbs energy is spent for growth and maintenance and we can write under maximum growth rate condition

$$q^{max}_G = \frac{1}{Y^m_{GX}}\mu^{max} + m_G$$

Eliminating q^{max}_G leads to the final equation (equation 17), which gives μ^{max} as a function of temperature (after replacing m_G using equation 2), Y^m_{GX}, γ_D, and ΔG_{CAT}.

Table 7. Estimated μ^{max} Values at 25 °C Using Equation 17

Microbial system	$-\Delta G_{cat}/\gamma_D$ (kJ/mol electron)	$1/Y^m_{GX}$ (kJ/C-mol biomass)	μ^{max} (h^{-1}, 25 °C)
Aerobic/glucose	118.5	236	1.5
Aerobic/acetate	105.5	432	0.7
Anaerobic/CH_4 from acetate	3.87	432	0.015
Anaerobic/ethanol from glucose	9.39	236	0.10
Aerobic/Fe^{2+} oxidation (pH = 1.5)	38.6	3,500	0.03
Aerobic/sulfide oxidation to SO_4^{2-}	99.6	3,500	0.08
Aerobic/nitrification	45.8	3,500	0.04

$$\mu^{max} = \frac{[3(-\Delta G_{CAT})/\gamma_D - 4.5]}{1/Y^m_{GX}} \exp\left[\frac{-69{,}000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right] \quad (17)$$

Table 7 shows the estimated μ^{max} values (25 °C = 298 K) for well-known growth systems. These estimated maximal growth rates are reasonable in the range of reported values. For the only prediction, which is rather wrong (system 4), it is indeed known that the Gibbs energy conversion is not due to electron transport, but due to substrate phosphorylation.

Affinity Constant of Electron Donor, K_s

The constant K_s is an affinity constant in the substrate-uptake kinetics. It is known that reported K_s values can vary over a wide range (10^{-3} to 10^{-6} mol/L). Even for the same organism, for example, *E. coli*, many different K_s values are found (30). Much of this wide spread is probably due to systematic errors in the determination of the K_s parameter. These errors can be due to ill-defined conditions of the microorganisms (adaptation), statistically unsound procedures to evaluate K_s from experimental data, neglect of mass-transfer limitation in the case of microorganisms growing as flocs, and analysis/sampling problems in determining the substrate concentration (see Ref. 30 for an extensive discussion). Moreover, K_s is a typical kinetic parameter, determined by the kinetic properties of the first step in substrate transport into the microorganism. For example, for the anaerobic acetate conversion into CH_4, the *Methanosarcina* type of organism has a high K_s value of 3 mM acetate and its substrate uptake is passive (non-energy linked). However, for the *Methanotrix* type of organism, where acetate is transported into the cell utilizing energy, $K_s \approx 0.5$mM (33).

Due to these factors, it is not possible to give, from a thermodynamic point of view, a generalization about the value of K_s for different microbial growth systems.

Threshold Concentration

In the previous section the substrate consumption kinetics q_s was assumed as an irreversible Michaelis–Menten kinetics equation (equation 14a). From this it follows that q_s becomes zero at substrate concentration $C_s = 0$. However, it is known that microbial metabolism stops at a certain concentration of the substrate. This is called the threshold concentration $C_{s,thresh}$.

This threshold concentration is thus the substrate concentration where substrate consumption q_s becomes zero. This should be distinguished from the minimal substrate concentration $C_{s,min}$, where the growth rate μ becomes zero.

This threshold situation is achieved when the coupled system of catabolic reaction and the energy generating system of the organism [most probably the proton motive force (pmf) process] are in equilibrium. Because the pmf requires at least 15–20 kJ for each proton (6), and assuming that a full catabolic reaction is minimally coupled to the extrusion of one proton, it appears that there must be a minimal catabolic Gibbs energy release of about 15–20 kJ. The following examples seem to support this idea where threshold behavior has been reported for a number for growth systems (Example 10):

- Anaerobic metabolism of CH_4 production from acetate (33)
- Aerobic metabolism of ferrous into ferric iron (12,13)
- Anaerobic production of H_2 converting 1 ethanol into 2 H_2 and 1 acetate and consumption of 4H_2 to produce acetate, CH_4, or H_2S from $2HCO_3^-$, HCO_3^- or SO_4^{2-} (34,35)

In all these situations of observed threshold concentration the actual ΔG_{CAT} could be calculated to be in the range of -10 to -30 kJ per catabolic reaction. Example 10 shows how the actual $(\Delta G)_{CAT}$ follows from textbook thermodynamic calculations.

EXAMPLE 10

Threshold concentrations and minimally required catabolic Gibbs energy for aerobic Fe^{2+}-oxidizing bacteria (12,13)

Fe^{2+} can be aerobically oxidized by specific bacteria. The catabolic reaction is $Fe^{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2H_2O$. The ΔG^0_{CAT} can be calculated to be -44.32 kJ (standard conditions). This aerobic iron oxidation can be performed by two different microorganisms, *Thiobacillus ferro oxidans* (T.f.) and *Leptospirillum ferro oxidans* (L.f.). Recently (12,13) the Fe^{2+} concentration was observed where the O_2 consumption stopped (threshold concentration). The following results were found (30 °C):

T.f.: $P_{02} = 0.12$ bar, pH = 1.85, $Fe^{3+} = 0.21$ M,
$Fe^{2+} = 3 \times 10^{-4}$ M (= threshold concentration)

L.f.: $P_{02} = 0.12$ bar, pH = 1.55, $Fe^{3+} = 0.21$ M,
$Fe^{2+} = 7 \times 10^{-6}$ M (= threshold concentration)

Using these concentrations the following ΔG_{CAT} can be calculated for *T. ferro oxidans*

$$\Delta G_{CAT} = -44.32 + 5.8 \log\left[\frac{0.21}{3 \times 10^{-4} \times 10^{-1.85}(0.12)^{0.25}}\right] = -15.75 \text{ kJ}$$

Similarly we can calculate for L. ferro oxidans $\Delta G_{CAT} = -8.03$ kJ.

Threshold concentrations and minimally required Gibbs energy for anaerobic acetate consuming methanogens

CH_4 can be produced anaerobically from acetate according to the catabolic reaction Acetate + $H_2O \rightarrow CH_4 + HCO_3^-$. For this reaction ΔG^0_{CAT} and ΔH^0_{CAT} at 298 K follow as

$$\Delta G^{01}_{CAT} = -31.0 \text{ kJ}$$
$$\Delta H^0_{CAT} = +5 \text{ kJ}$$

This provides, using the Van 't Hoff equation, for $\Delta S^{01}_{CAT} = \Delta H_r - \Delta G_r/T = 0.121$ kJ/mol K.

There are two organisms known to perform this reaction at 60 °C, both of which have threshold acetate concentrations. According to Ref. 32 Methano sarcina has a threshold concentration of 0.3×10^{-3} M and Methano trix of 16×10^{-5} M of acetate.

Using a CH_4 pressure of 0.6 bar, T = 333 K, and a bicarbonate concentration of 0.03 M, we can calculate the following ΔG_{CAT} of the catabolic reaction at the threshold acetate concentration (using the Van 't Hoff relation $\Delta G = \Delta H - T\Delta S$ to take the temperature effect into account):

Methanosarcina $\Delta G_{CAT} = -23$ kJ

Methanotrixe $\Delta G_{CAT} = -15.7$ kJ

It appears indeed that at the threshold concentration the coupled system of catabolic reaction and proton translocation may be in equilibrium. This leads to the statement that a threshold concentration can be estimated from the requirement that

$$(\Delta G_{CAT})_{thresh} \approx -20 \text{ kJ} \qquad (18)$$

This explanation is further supported by the observation that for anaerobic H_2-consuming systems (36), the observed effect of temperature on H_2 threshold values can be explained from correlation equation 18. Example 11 shows how the expected threshold concentration can be estimated for such systems.

EXAMPLE 11

Calculation of the threshold concentration as a function of temperature

Consider the anaerobic catabolic system $4H_2 + HCO_3^- + H^+ \Leftrightarrow CH_4 + 3H_2O$. One can calculate that $\Delta G^{01}_{CAT} = -135.57$ kJ, and $\Delta H^0_{CAT} = -241$ kJ. Using the Van 't Hoff relation ($\Delta G = \Delta H - T\Delta S$) one calculates $\Delta S^0_{CAT} = -0.354$ kJ/molK.

Assuming $P_{CH_4} = 1$ bar, $HCO_3^- = 0.01$ M, $H^+ = 10^{-8}$ M, T = 303 K, and ΔG_{CAT} equals the threshold minimum of −20 kJ, one can calculate at threshold conditions:

$$-20 = -241 - (-0.354) \times 303 + 0.008314 \times 303 \ln \frac{1}{(10^{-8}/10^{-7})(0.01)(P_{H_2})^4}$$

From this follows that the threshold hydrogen pressure is $P_{H_2} = 7 \times 10^{-5}$ bar at a temperature of 30 °C (303 K).

For a temperature of 75 °C (=348 K), one finds that $(P_{H_2})_{threshold} = 120 \times 10^{-5}$ bar. Such an increase of hydrogen threshold partial pressure with increasing temperature has indeed been observed (36). The calculated threshold H_2 pressures are also in the correct range.

From the previous findings it is clear that, especially for systems with a low ΔG_{CAT} (e.g., anaerobic, inorganic electron donors), threshold values can be found. For aerobic growth, ΔG_{CAT} is so large that a measurable threshold value is not expected because the thermodynamically calculated $C_{s,thresh}$ would be extremely low.

The existence of threshold concentrations makes it desirable to change the kinetics of substrate uptake (q_s, equation 14a) from the irreversible to reversible form

$$q_s = \frac{q_s^m(C_s - C_{s,thresh})}{K_m + (C_s - C_{s,thresh})} \qquad (19)$$

In conclusion it appears that threshold concentrations of electron donor (or substrate) do exist and their value can be estimated from the catabolic reaction using the value of $(\Delta G_{cat})_{thresh}$ in equation 18. In addition a more proper kinetic expression for q_s is then given by equation 19.

NOMENCLATURE

Symbols

C_i	Concentration of compound i, mol/m^3
Y_{ij}	Yield of compound j on compound i, mol$_j$/mol$_i$
μ^{max}	maximal specific growth rate of biomass, h^{-1}
k_d	Decay coefficient of biomass, h^{-1}
m_i	Biomass specific maintenance requirement of compound i, mol$_i$/C-mol biomass h
K_s	Affinity constant for substrate uptake, mol/m^3
r_i	Rate of conversion of compound i per reactor volume, (C)mol/m^{3}h
q_i	Biomass specific rate of uptake or secretion of compound i, (C) mol i/C-mol biomass h
H	Enthalpy, kJ
G	Gibbs energy, kJ
Q	Heat, kJ
γ	Degree of reduction: for organic compounds it is defined per C-mol, electron/C-mol; for inorganic compounds it is defined per mol, electron/mol

Subscripts

D	Electron donor
A	Electron acceptor
X	Biomass
e	Calculated per mol electron
E	Calculated per mol electron
O	Oxygen
S	Substrate
C	Carbon dioxide
N	N-source
Q	Heat
H	Proton
W	Water
CAT	Catabolic reaction

Superscripts

m	Value at infinite substrate concentration
max	Value under maximal conditions
o	Reference conditions (1 mol/L, 1 atm, 298 K)
01	Reference conditions, but with pH = 7 ($H^+ = 10^{-7}$ mol/L)

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BIBLIOGRAPHY

1. J.A. Roels, Energetics and Kinetics in Biotechnology, Elsevier, New York, 1983.
2. J.J. Heijnen and J.P. van Dijken, Biotechnol. Bioeng. 39, 833–858 (1992).
3. J.J. Heijnen, M.C.M. van Loosdrecht, and L. Tijhuis, Biotechnol. Bioeng. 40, 1139–1154 (1992).
4. L. Tijhuis, M.C.M. van Loosdrecht, and J.J. Heijnen, Biotechnol. Bioeng. 42, 509–519 (1993).
5. E.H. Battley, Energetics of Microbial Growth, Wiley New York, 1987.
6. H.V. Westerhoff and K. van Dam, Mosaic Non-Equilibrium Thermodynamics and the Control of Biological Free Energy Transduction, Elsevier, Amsterdam, 1987.
7. J.J. Heijnen, Antonie van Leeuwenhoek 60, 235–256 (1991).
8. J.J. Heijnen and J.P. van Dijken, Biotechnol. Bioeng. 42, 1127–1130 (1993).
9. E.H. Battley, Appl. Environ. Microbiol. 61, 1655–1657 (1995).
10. A.H. Stouthamer, Antonie van Leeuwenhoek 29, 545–565 (1973).
11. R.K. Thauer, K. Jungermann, and K. Decker, Bacterial. Rev. 41, 100–180 (1977).
12. M. Boon, J.J. Heijnen, and G.S. Hansford, in D.S. Holmes and R.W. Smith eds., Minerals Bioprocessing II. Proceedings of the Engineering Foundations Conference: Minerals Processing II, Snowbird, Utah, July 10–15, 1995; TMS, Warrendale, Pa., 1995, pp. 41–62.
13. M. Boon, J.J. Heijnen, and G.S. Hansford, in D.S. Holmes and R.W. Smith eds., Minerals Bioprocessing II. Proceedings of the Engineering Foundations Conference: Minerals Processing II, Snowbird, Utah, July 10–15 1995, TMS, Warrendale, Pa., 1995, pp. 63–82.
14. N. Schill, W.M. van Gulik, D. Voisard, and U. von Stockar, Biotechnol. Bioeng. 51, 645–658 (1996).
15. B.H.A. van Kleeff, J.G. Kuenen, and J.J. Heijnen, Biotechnol. Bioeng. 12, 510–518 (1996).
16. U. von Stockar, L. Gustafsson, C. Larsson, J. Marison, P. Tissot, and E. Gnaiger, Biochim. Biophys. Acta 1183, 221–240 (1993).
17. U. von Stockar and I.W. Marison, Adv. Biochem. Eng. Biotechnol. 40, 93–136 (1989).
18. H.J. Noorman, B. Romein, K.Ch.A.M. Luyben, and J.J. Heijnen, Biotechnol. Bioeng. 49, 364–376 (1996).
19. R.T.J.M. van der Heijden, J.J. Heijnen, C. Hellinga, B. Romein, and K.Ch.A.M. Luyben, Biotechnol. Bioeng. 43, 3–10 (1994).
20. R.T.J.M. van der Heijden, B. Romein, J.J. Heijnen, C. Hellinga, and K.Ch.A.M. Luyben, Biotechnol. Bioeng. 43, 11–20 (1994).
21. R.T.J.M. van der Heijden, B. Romein, J.J. Heijnen, C. Hellinga, and K.Ch.A.M. Luyben, Biotechnol. Bioeng. 44, 781–791 (1994).
22. C. Hellinga and B. Romein, in M.N. Karim and G. Stephanopoulos eds., Modeling and Control of Biotechnical Processes 1992, IFAC Symposia Series 10, 1992.
23. J. Monod, Ann. Rev. Microbiology 3, 371–394 (1949).
24. S.J. Pirt, Proc. R. Soc. London, Ser. B 163, 224–231 (1965).
25. O.M. Neijssel and D.W. Tempest, Arch. Microbiol. 107, 215–230 (1976).
26. P.L. McCarty, in S.D. Faust and J.V. Hunter eds., Organic Compounds in Aquatic Environments, Marcel Dekker, New York, 1971, pp. 495–531.
27. J.J. Heijnen, J.A. Roels, and A.H. Stouthamer, Biotechnol. Bioeng. 21, 2175–2201 (1979).
28. L.E. Erickson, I.G. Minkevich, and V.K. Eroshin, Biotechnol. Bioeng. 20, 1595–1605 (1978).
29. J.J. Heijnen, TIBTECH 12, 483–492 (1994).
30. K. Kovárová, A.J.B. Zehnder, and T. Egli, J. Bacteriol. 178, 4530–4539 (1996).
31. M.E. Tros, G. Schraa, and A.J.B. Zehnder, Appl. Environ. Microbiol. 62, 437–442 (1996).
32. K.B. Andersen and K. von Meyenburg, J. Bacteriol. 144, 114–123 (1980).
33. S.H. Zinder, FEMS Microbiol. Rev. 75, 125–138 (1990).
34. H.J. Seitz, B. Schink, N. Pfennig, and R. Conrad, Arch. Microbiol. 155, 82–88 (1990).
35. H.J. Seitz, B. Schink, N. Pfennig, and R. Conrad, Arch. Microbiol. 155, 89–93 (1990).
36. R. Conrad and B. Wetter, Arch. Microbiol. 155, 94–98 (1990).

See also ANAEROBES; BIOREMEDIATION; CLOSTRIDIA, SOLVENT FORMATION; ELECTRON TRANSPORT; ENERGY METABOLISM, MICROBIAL AND ANIMAL CELLS; YEAST, BAKER'S.