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A comparison of various Gibbs energy dissipation correlations for predicting microbial growth yields

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Abstract

Thermodynamic analysis may be applied in order to predict microbial growth yields roughly, based on an empirical correlation of the Gibbs energy of the overall growth reaction or Gibbs energy dissipation. Due to the well-known trade-off between high biomass yield and high Gibbs energy dissipation necessary for fast growth, an optimal range of Gibbs energy dissipation exists and it can be correlated to physical characteristics of the growth substrates. A database previously available in the literature has been extended significantly in order to test such correlations. An analysis of the relationship between biomass yield and Gibbs energy dissipation reveals that one does not need a very precise estimation of the latter to predict the former roughly. Approximating the Gibbs energy dissipation with a constant universal value of $-500\,\mathrm{kJ\,C\text{-}mol^{-1}}$ of dry biomass grown predicts many experimental growth yields nearly as well as a carefully designed, complex correlation available from the literature, even though a number of predictions are grossly out of range. A new correlation for Gibbs energy dissipation is proposed which is just as accurate as the complex literature correlation despite its dramatically simpler structure.

Keywords: Biomass yield; Metabolism; Thermodynamics of growth; Gibbs energy dissipation; Yield prediction; Growth yield database

1. Introduction

Whenever cells are grown in any kind of culture, it is of utmost importance to obtain as high a biomass density as possible. The achievable biomass concentration affects the ease with which scientific research projects can be carried out in that it determines the amount of biological material that can be derived from a given cellular strain. In industrial biotechnology the biomass concentration determines the amount, the synthesis rate and the concentration of the target product that can be expected and thus represents a prime factor influencing the economic viability of the project. The biomass concentration that can be obtained is in turn determined primarily by the growth yield characterizing the respective strain. It is therefore of practical significance to develop methods for roughly predicting the achievable biomass yields even before launching a project and/or carrying out in-depth experimental work.

Many different approaches for biomass yield prediction were formulated and reported in the literature. Early work was based on attempts to correlate measured biomass yields in terms of Y_{ATP} [1–3], or in terms of energetic efficiencies [4–9], and many others. In his analysis of thermodynamics of metabolism of *Saccharomyces cerevisiae* with impaired growth and that of normally growing cells, Battley correlated the biomass yield with the average free energy per C-mole of substrate using the efficiency of free energy conservation [5]. In 1972, Minkevic and Eroshin, used the enthalpic efficiency coefficient for biomass yield production. They stated that the energy stored per unit C atom is related to reducing power, *i.e.* the degree of reduction [4]. Roels showed that biomass yields for aerobic growth appear to depend on the degree of reduction of the carbon and energy substrate [10]. He explained this by pointing out that the bioenergetic growth efficiency has to be inferior to 1 and by showing that this imposes an energy limitation on the biomass yield when microorganisms grow on energy poor substrates, whereas the biomass yields in growth on energy rich substrates can be assumed to be determined by a C-limitation.

A more rigorous thermodynamic treatment was proposed by McCarty and later refined [11–14]. Although this method is

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Nomenclature

A	electron acceptor (see Table 1)
D	electron donor; energy source (see Table 1)
DOX	oxidized form of electron donor
ΔG°_{an}	standard Gibbs energy of anabolic reaction (kJ C-mol^{-1})
ΔG°_{cat}	standard Gibbs energy of catabolic reaction (kJ C-mol^{-1})
$\Delta_c G^{\circ}_i$	standard Gibbs energy of combustion of ith compound (kJ C-mol^{-1}) or (kJ mol^{-1}) ($i = A$, D, P or X)
$\Delta_c G^{*}_X$	modified standard Gibbs energy of combustion of dry biomass using as reference state CO_2, H_2O and nitrogen in the most oxidized form occurring in the respective growth systems (NH_4^+ or NO_3^-) (kJ C-mol^{-1})
$\Delta_r G^{\circ}_X$	standard Gibbs energy of overall growth reaction per C-mole of dry biomass grown (kJ C-mol^{-1})
NS	nitrogen source (see Table 1)
P	product resulting from reduction of electron acceptor A (see Table 1)
r_{an}	rate of anabolic reaction advancement (C-mol s^{-1})
r_{cat}	rate of catabolic reaction advancement (C-mol s^{-1})
$Y_{i/j}$	yield of i versus j, C-mol C-mol^{-1} or C-mol mol^{-1} if j is inorganic; i and $j = A$, D, NS, P, DOX

Greek letter

γ_i	degree of reduction of ith compound using as reference state CO_2, H_2O and nitrogen in its most oxidized form in which it occurs in the respective growth system (NH_4^+ or NO_3^-)

based on a more realistic formulation of the anabolic reaction than the treatment of Roels, it still relies on an energy transfer efficiency between catabolism and anabolism, which is correlated to reproduce experimental data. More recently, this method was further improved for substrate oxidations involving an oxygenase as an initial step [15,16] and for substrates with a low degree of reduction [17].

In 1992, Heijnen and van Dijken reviewed a number of methods and pointed out that those based on the concept of energetic growth efficiency are plagued with internal inconsistencies mainly due to the fact that the definition of an efficiency requires defining an energetic reference state and that changing the reference state modifies all efficiency values [18,19]. Instead of correlating the biomass yield in terms of bioenergetic efficiency, they proposed to correlate it in terms of Gibbs energy dissipation per amount of biomass grown, which is clearly independent of any arbitrary reference state. By using a large number of experimental data from the literature, they developed a rather complicated empirical equation for predicting this Gibbs energy dissipation and showed that the measured yields could be predicted with remarkable accuracy.

The aim of the present paper is to explore alternative, simpler correlations for the Gibbs energy dissipation, which can be used to predict biomass yields. As a basis for this, the accuracy required in estimating this thermodynamic property of microbial growth will be discussed. Finally, the ways to calculate the biomass yield from the Gibbs energy dissipation will be reviewed and simple methods will be formalized.

2. Theory

2.1. The relationship between Gibbs energy dissipation and the growth yield

In order to develop a relationship permitting calculation of the biomass yield from an estimation of the Gibbs energy dissipation, a stoichiometry of the growth reaction needs to be formulated. A rather general form of such a stoichiometry can be written as follows:

$$\frac{1}{Y_{X/D}} D + Y_{A/X} A + Y_{N/X}\,\mathrm{NS} \rightarrow X + Y_{P/X} P + Y_{\mathrm{DOX}/X}\,\mathrm{DOX} \quad (1)$$

where D, A, NS, X, P and DOX stand for the electron donor (energy source), the electron acceptor, the nitrogen donor, dry biomass, the reduced electron acceptor ("product") and the oxidized electron donor, respectively. Table 1 gives some examples for these compounds for various types of microbial growth.

In aerobic organothrophic growth, D can be any example of a large variety of organic compounds (Table 1). The electron donor acts simultaneously as carbon source and is oxidized into CO_2 (DOX) by the catabolic reaction. Oxygen functions as the electron acceptor and is reduced to water (P) in the process.

Anaerobic organotrophic growth may either be of a fermentative or respiratory type. In the first case, no electron acceptor A is present, but D is disproportionated into a (usually organic) highly reduced P and an oxidized DOX. This is normally CO_2, with the exception of homofermentative lactic acid production, where lactic acid is the sole product. In anaerobic organotrophic respiration, an inorganic compound other than oxygen acts as A. The electron donor (D) provides the carbon source in both cases.

A large variety of inorganic compounds may be used as oxidants by chemoautolithotrophic microorganisms. They will also consume CO_2 or HCO_3^- as a carbon source along with D and A.

In formulating a Gibbs energy balance for Eq. (1) it is most convenient to choose the completely oxidized state of matter (CO_2 and H_2O) as a reference. Wherever other elements come into play, the reference state will include them in the most oxidized state in which they occur. Thus, the reference state for nitrogen will either be NH_4^+ or NO_3^-, for sulfur SO_4^{2-} and for iron Fe^{3+}. Examining Table 1 reveals that with this choice the Gibbs energies of combustion of A, NS and DOX are zero in all cases. The same is true for the carbon source (CO_2) in autolithotrophic growth. The Gibbs energy balance for Eq. (1)

Table 1
Examples of electron donors, electron acceptors, and catabolic products for various types of microbial growth

	D	*A*	NS	*P*	DOX
Chemohetero-organo-trophic growth, aerobic	Any organic substrate: glucose, fructose, ethanol, methanol, methane, etc.	O_2	NH_4^+	H_2O	CO_2
Anaerobic, fermentation	As above	–	NH_4^+	Many organic compounds, as or more reduced than *D*: ethanol, lactic acid, butanol, glycerol, propionic acid, volatile fatty acids, methane, etc. Inorganic compound: H_2	Usually CO_2
Anaerobic, respiration e.g. denitrification	As above	NO_3^-	NO_3^-	N_2	CO_2
Chemoauto-lithotrophic growth, aerobic	H_2, NH_4^+, HNO_2, H_2SO_3, Fe^{2+}	O_2	Various	H_2O	H_2O, NO_3^-, SO_4^{2-}, Fe^{3+}
Anaerobic	H_2, S^{2-}, etc.	CO_2, Fe^{3+}, etc.	NH_4^+	CH_4, Fe^{2+}, etc.	H_2O, S^0, etc.

may therefore be written as:

$$\Delta_r G_X^\circ = \frac{\Delta_c G_D^\circ}{Y_{X/D}} - \Delta_c G_X^* - Y_{P/X}\,\Delta_c G_P^\circ \tag{2}$$

where $\Delta_r G_X^\circ$ denotes the overall standard Gibbs energy change or standard Gibbs energy dissipation generated by the growth reaction, counted per one C-mole of dry biomass grown. $\Delta_c G_D^\circ$ and $\Delta_c G_P^\circ$ stand for the standard Gibbs energy of combustion of one C-mole of electron donor and product, respectively, whereas $\Delta_c G_X^*$ represents the standard Gibbs energy of combustion going from one C-mole of dry biomass to CO_2, H_2O and either NH_4^+ or NO_3^-, depending on the most oxidized state of nitrogen in the respective growth process.

A degree of reduction balance based on Eq. (1) and using the same reference states as above yields:

$$Y_{P/X} = \frac{\gamma_D}{\gamma_P}\left(\frac{1}{Y_{X/D}}\right) - \frac{\gamma_X}{\gamma_P} \tag{3}$$

In all cases of aerobic growth, *P* is water and Eq. (3) cannot be used because $\gamma_P = 0$. In this case, however, $\Delta_c G_P^\circ$ disappears from Eq. (2), thus making $Y_{P/X}$ irrelevant.

Substitution of Eq. (3) into Eq. (2) yields:

$$\Delta_r G_X^\circ = \frac{1}{Y_{X/D}}\left(\Delta_c G_D^\circ - \frac{\gamma_D}{\gamma_P}\Delta_c G_P^\circ\right) + \frac{\gamma_X}{\gamma_P}\Delta_c G_P^\circ - \Delta_c G_X^* \tag{4}$$

Solving this for the growth yield:

$$Y_{X/D} = \frac{\Delta_c G_D^\circ - (\gamma_D/\gamma_P)\Delta_c G_P^\circ}{\Delta_r G_X^\circ + \Delta_c G_X^* - (\gamma_X/\gamma_P)\Delta_c G_P^\circ} \tag{5}$$

permits a computation in a straightforward manner using thermodynamic tables once the Gibbs energy dissipation $\Delta_r G_X^\circ$ is known. For aerobic growth, $\Delta_c G_P^\circ$ is zero and thus vanishes from Eq. (5).

An alternative way to develop Eq. (5), which may be conceptually easier to understand, is to split up the overall growth reaction into a catabolic and into an anabolic reaction, as shown in Fig. 1 [20]:

$$\text{catabolism}: \quad D + Y_A^{\text{cat}} A \rightarrow Y_P^{\text{cat}} P + Y_{\text{DOX}}^{\text{cat}}\,\text{DOX} \quad (\Delta G_{\text{cat}}^\circ) \tag{6a}$$

$$\text{anabolism}: \quad Y_P^{\text{an}} P + Y_{\text{DOX}}^{\text{an}}\,\text{DOX} + Y_{\text{NS}}^{\text{an}}\,\text{NS} \rightarrow X + Y_A^{\text{an}} A \quad (\Delta G_{\text{an}}^\circ) \tag{6b}$$

As Fig. 1 shows, this split assumes arbitrarily that the electron donor is first completely catabolized and that a part of the products of catabolism is then used to synthesize the new biomass. Dividing Eq. (6a) by $Y_{X/D}$ and adding the result and Eq. (6b) yields again the overall reaction (1). Therefore, the overall Gibbs energy change is given by:

$$\Delta_r G_X^\circ = \frac{1}{Y_{X/D}}\,\Delta G_{\text{cat}}^\circ + \Delta G_{\text{an}}^\circ \tag{7}$$

This equation demonstrates in a very visual way the relationship between $\Delta_r G_X^\circ$ and $Y_{X/D}$. Large biomass yields are certainly in the interest of biology and one might assume that they were maximized during evolution. However, large biomass yields also place a large energetic burden on the catabolic reaction: since $\Delta G_{\text{an}}^\circ$ is positive (for anabolic reaction defined as in Eq. (6b)) (Fig. 1), $\Delta_r G_X^\circ$ will be less negative than $\Delta G_{\text{cat}}^\circ$. This effect will be the more pronounced the larger $Y_{X/D}$ becomes. This would reduce the chemical driving force for the overall growth reactions and therefore slow down growth. It is generally believed that an optimum range of Gibbs energy dissipation exists in microbial growth affording simultaneously reasonable biomass yields and a sufficient Gibbs energy dissipation as a driving force for vigorous microbial growth [20,21]. The correlation of these

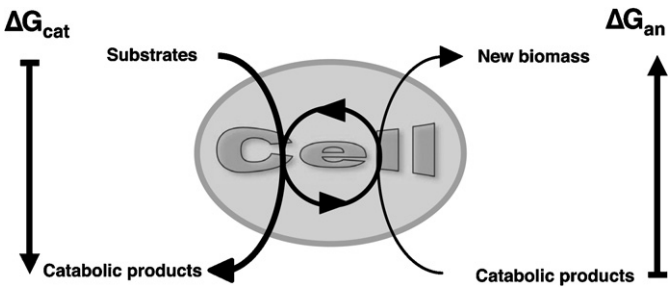

Fig. 1. Splitting the macrochemical reaction for the overall growth process up into a formal reaction for catabolism and anabolism. The ring in the middle of the cell represents the coupling of anabolism and catabolism by ATP and other biochemical mechanisms.

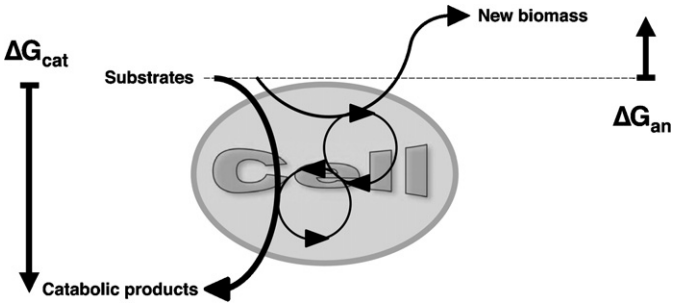

Fig. 2. A more realistic formulation of the anabolic reaction is to assume that some of the carbon- and energy-substrate is deviated from catabolism and that biomass is synthesized directly from there. This makes ΔG_{an} much smaller or even negative.

optimum $\Delta_r G^\circ_X$ values is the topic of the second part of this paper.

Once such a correlation is available, the biomass yield may be predicted by solving Eq. (7):

$$Y_{X/D} = \frac{\Delta G^\circ_{cat}}{\Delta_r G^\circ_X - \Delta G^\circ_{an}} \tag{8}$$

ΔG°_{cat} and ΔG°_{an} may be computed from thermodynamic tables. Applying a degree of reduction balance to Eq. (6) yields:

$$\Delta G^\circ_{cat} = \Delta_c G^\circ_D - \frac{\gamma_D}{\gamma_P}\Delta_c G^\circ_P \tag{9a}$$

and

$$\Delta G^\circ_{an} = \frac{\gamma_X}{\gamma_P}\Delta_c G^\circ_P - \Delta_c G^*_X \tag{9b}$$

where once again, the terms for P vanish in aerobic growth. Substituting this into Eq. (8) yields again Eq. (5).

One might argue that the formulation of the anabolic reaction, Eq. (6b), is quite unrealistic. In reality, biomass might be synthesized directly from the electron donor, which might make ΔG°_{an} much smaller as shown in Fig. 2 or even negative [22,23]. It is shown in Appendix A, however, that this does neither change the discussion following Eq. (7) nor the end result (Eq. (5)).

Eq. (5) or Eq. (8) combined with Eqs. (9a) and (9b), may be used for a very large part of microbial growth systems to predict the biomass yield. As already mentioned, the Gibbs energies of combustion for the electron donor and for the products of catabolism have been tabulated in thermodynamic tables (see Experimental).

2.2. The estimation of Gibbs energy dissipation

The amount of Gibbs energy dissipated, or "lost" ($\Delta_r G^\circ_X$) may be estimated based on an empirical correlation. Heijnen and van Dijken studied a large number of dissipation values based on literature results and concluded that $\Delta_r G^\circ_X$ does depend markedly on the degree of reduction of the carbon donor γ_s and to a lesser extent on the number of carbon atoms n_c of the latter. They fitted the following model to represent the data as well as possible:

$$-\Delta_r G^\circ_X = 200 + 18(6 - n_c)^{1.8} + \exp\{[(3.8 - \gamma_s)^2]^{0.16} \times (3.6 + 0.4 n_c)\}\ (\text{kJ C-mol}^{-1}) \tag{10a}$$

For chemotrophic growth involving reverse electron transport (RET), they proposed:

$$\Delta_r G^\circ_X = -3500\ \text{kJ C-mol}^{-1} \tag{10b}$$

In these equations, γ_s and n_c stand for the degree of reduction and the number of carbon atoms of the carbon substrate, respectively.

According to the authors, Eq. (10) correlates the Gibbs energies of the overall growth reaction based on literature data with an average error of ±30%. This may not be extremely accurate, but the biomass yields are not too sensitive with respect to $\Delta_r G^\circ_X$, so that the final estimation of $Y_{X/D}$ will nevertheless be reasonably good. This may be shown by differentiating Eq. (8) with respect to $\Delta_r G^\circ_X$. Dividing the result by the biomass yield to obtain a relative error yields:

$$\frac{1}{Y_{X/D}}\left(\frac{dY_{X/D}}{d\,\Delta_r G^\circ_X}\right) = -\frac{1}{\Delta_r G^\circ_X - \Delta G^\circ_{an}} \tag{11}$$

A plot of this equation appears in Fig. 3. It shows the approximate relative error on $Y_{X/D}$ in percent resulting from an overestimation of $\Delta_r G^\circ_X$ by 100 kJ C-mol^{-1}. In the way of examples, the figure also indicates the range of experimentally obtained values found in literature for some groups of microbial growth systems.

As may be seen, the prediction of growth yields for anaerobic growth without an electron acceptor is quite delicate. An error of ±100 kJ C-mol^{-1} in the estimation results in an uncertainty of ±20% to 40% in the biomass yield. In autotrophic methanogenesis, which often dissipates between −800 and

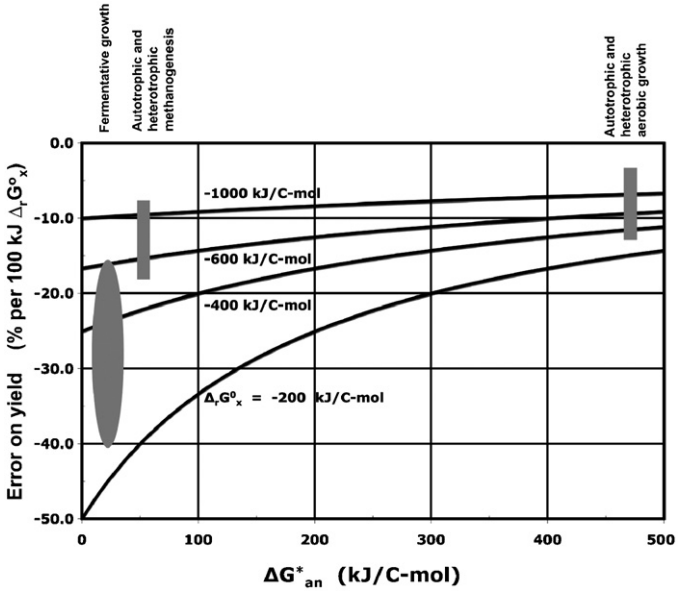

Fig. 3. The relative error resulting from an overestimation of the Gibbs energy dissipation $\Delta_r G^\circ_X$ by 100 kJ C-mol^{-1} as a function of the Gibbs energy change involved in anabolism and of the Gibbs energy dissipation per C-mole of dry biomass grown. The values for three typical growth systems are shown as lightly shaded areas in the way of examples.

$-1000\,\mathrm{kJ\,C\text{-}mol^{-1}}$, an error of $\pm 100\,\mathrm{kJ\,C\text{-}mol^{-1}}$ is less dramatic in that the yield could still be predicted within ±10%. Heterotrophic aerobic growth is equally favorable. An error of $\pm 100\,\mathrm{kJ\,C\text{-}mol^{-1}}$ in estimating the Gibbs energy dissipation, which often amounts to $-400\,\mathrm{kJ\,C\text{-}mol^{-1}}$, would still afford a biomass yield estimation within ±15%.

As will be shown in Section 4, correlation 10 may therefore be replaced by simpler equations or even by a constant value for $\Delta_r G^\circ_X$ without too much loss in accuracy.

3. Experimental

A database of experimentally obtained values for the biomass yields for a number of microorganisms growing on various substrates was used to test and to compare different correlations. It contains a large part of the data compiled by Heijnen and van Dijken [18] as well as additional literature data and represents a collection containing a total of 205 entries. The table in Appendix B contains the entire database together with values for free Gibbs energies of combustion for the nutrients and metabolites together with the respective degrees of reduction that were used in the calculations, and Gibbs energies of dissipation, when given in the original literature sources. If the literature source neither expresses biomass yields in units of C-mole of biomass per C-mole of the consumed electron donor nor the molar biomass composition for the particular microorganism, original values were converted to C-mole/C-mole units using the mean biomass composition values given in Table 2.

The average biomass degree of reduction used for prediction of biomass yields was 4.2. Gibbs energy of combustion values for different substrates and products of metabolism (to HCO_3^-(aq), N_2(g) and H_2O(l)) were taken from Roels [10], except for the reversed electron transfer cases that were from Thauer et al. [24].

The Gibbs energy of combustion of dry biomass may be estimated based on the low temperature calorimetric determination of the entropy of dry biomass performed by Battley et al. [25] as $\Delta_c G^\circ_X = -515\,\mathrm{kJ\,C\text{-}mol^{-1}}$ for all types of biomass [20]. Biomass is clearly synthesized in hydrated form; therefore this value should be corrected for the Gibbs energy of hydration of biomass. It has been shown experimentally, however, that the enthalpy of hydration would change the heat of combustion of dry biomass only by 1–2 kJ C-mol^{-1} [26]. This effect may thus safely be neglected both for $\Delta_c H^\circ_X$ and $\Delta_c G^\circ_X$. Since the reference state for the balances presented above has been chosen with respect to nitrogen in the most oxidized state occurring in the respective growth system, $\Delta_c G^\circ_X$ must be modified for the nitrogen contained in the dry biomass. For all growth systems using ammonium ions as a nitrogen source but not as an electron donor the modified Gibbs energy of combustion of dry biomass amounts to $\Delta_c G^*_X = -474\,\mathrm{kJ\,C\text{-}mol^{-1}}$.

Growth yields were predicted using Eq. (8) based on $\Delta_r G^\circ_X$ values calculated according to Heijnen and van Dijken model (Eq. (10)), according to the simplified formula (Eqs. (15a) and (15b)), and using the constant value of $-500\,\mathrm{kJ\,C\text{-}mol^{-1}}$. A few cases in the database concern mixed catabolism. Instead of using Eq. (8), the respective yields were predicted by solving Eq. (2) for $Y_{X/D}$ and by substituting experimental values given in the references for $Y_{P/X}$.

The predicted yields were compared to the experimental values from the database. Relative error of each prediction was calculated according to the following equation:

$$\mathrm{Err}_i = \frac{Y^{\mathrm{p}}_{X/D_i} - Y^{\mathrm{exp}}_{X/D_i}}{Y^{\mathrm{exp}}_{X/D_i}} \tag{12}$$

The arithmetic mean of the relative errors of prediction (Err_i in the Eq. (13)) gives the statistical expectation of the prediction error, and is therefore a measure of the accuracy of the prediction. Hence it should, ideally, be close to zero:

$$\overline{\mathrm{Err}} = \frac{\sum_{i=1}^{n} \mathrm{Err}_i}{n} \tag{13}$$

As a measure of the overall precision of the model prediction, a standard error of correlation (SEC) was calculated as a square root of the average squared differences between the predicted and the experimental value in the following equation:

$$\mathrm{SEC} = \sqrt{\frac{\sum_i \left(Y^{\mathrm{p}}_{X/D_i} - Y^{\mathrm{exp}}_{X/D_i}\right)^2}{n}} \tag{14}$$

4. Results

When the carefully designed correlation of Heijnen and van Dijken [18] was tested against the database by using Eq. (10) in Eq. (5) or Eq. (8) to calculate $\Delta_r G^\circ_X$, a relatively good prediction was obtained as shown in the form of a parity plot (Fig. 4). The standard error of correlation was ±7.7 yield percentages. Average error shows a model overestimation of 17% (Table 3).

In view of the fact that the estimation of $\Delta_r G^\circ_X$ does not influence the yield prediction in a very pronounced way as shown in Fig. 3, the question arises whether simpler equations for estimating $\Delta_r G^\circ_X$ could be used without too much loss of prediction

Table 2
Mean composition, molecular weight per C-mole of biomass, degree of reduction of biomass carbon, enthalpy of combustion and the modified enthalpy of combustion of various microorganisms [50]

Organism	Elemental formula	M_X (g C-mol^{-1})	γ_X	$-\Delta_c H^\circ_X$ (kJ C-mol^{-1})	$-\Delta_c H^*_X$ (kJ C-mol^{-1})
Average bacteria	$CH_{1.66}O_{0.41}N_{0.21}$	27.76	4.21	521.35	460.29
Average algae	$CH_{1.63}O_{0.44}N_{0.09}$	23.35	4.48	530.08	504.19
	$CH_{1.71}O_{0.44}N_{0.10}$	24.52	4.55	535.01	506.26
Average yeast	$CH_{1.65}O_{0.54}N_{0.14}$	26.09	4.17	521.00	481.09
All microorganisms	$CH_{1.66}O_{0.46}N_{0.14}$	25.46	4.31	525.55	485.13

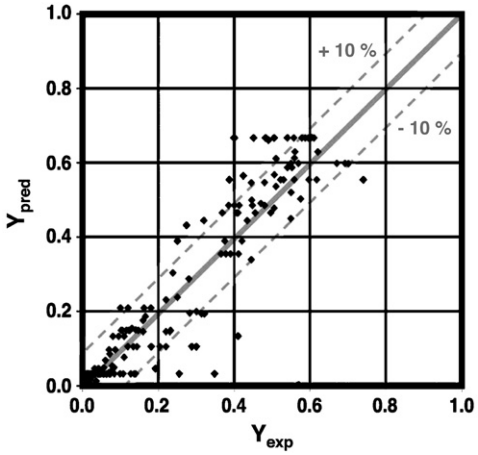

Fig. 4. Parity plot showing the predicted values for the biomass yield in C-mole/C-mole vs. the published experimental values based on correlation 10 for estimating the Gibbs energy change $\Delta_r G_X^\circ$ of the overall growth reaction.

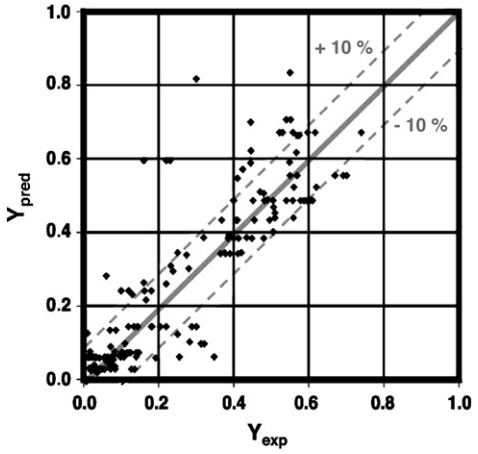

Fig. 5. Parity plot showing the predicted values for the biomass yield in C-mole/C-mole vs. the published experimental values assuming a constant value of $-500\,\mathrm{kJ\,C\text{-}mol^{-1}}$ for the Gibbs energy change $\Delta_r G_X^\circ$ of the overall growth reaction.

accuracy. One might even be tempted to adopt simply a fixed value in Eq. (5) or Eq. (8) for $\Delta_r G_X^\circ$. The literature values of the Gibbs energy dissipation from the database are plotted arbitrarily versus the degree of reduction of the electron donor in Fig. 7b. It is obvious that these values range from $-250\,\mathrm{kJ\,C\text{-}mol^{-1}}$ all the way up to $-3500\,\mathrm{kJ\,C\text{-}mol^{-1}}$ for growth systems with RET. But Fig. 7b also shows that a large number of cases cluster around $-500\,\mathrm{kJ\,C\text{-}mol^{-1}}$. By simply using this value in Eq. (5) or Eq. (8), biomass yields may indeed be roughly predicted as shown in the form of a parity plot (Fig. 5). It is obvious that the extreme values of the Gibbs energy dissipation give rise to some predictions that are grossly out of range. A large part of the predictions are however still reasonably close to the database and the overall standard error of prediction is ±10.5 yield percent. Calculation of the overall relative error shows an overestimation of the prediction of 27%. Analysis of the relative average error of prediction for the aerobic, anaerobic and autotrophic cases (Table 3) shows that this is a result of significant undervaluing of the dissipated Gibbs free energy of growth for the autotrophic microorganisms, which is estimated to be $-3500\,\mathrm{kJ\,C\text{-}mol^{-1}}$ in Heijnen and Dijken model. Using this value instead of the fixed $-500\,\mathrm{kJ\,C\text{-}mol^{-1}}$ for the autotrophic cases reduces the overall average error of prediction to 12%.

An intermediate solution may be to develop a simpler equation than correlation 10, but one that nevertheless retains the most important variations of the Gibbs energy dissipation. Roels showed [10] that aerobic biomass yields can roughly be predicted by correlating them in terms of the degree of reduction of the energy substrate as follows:

$$Y_{X/D} = 0.13\gamma_D \quad \text{for } \gamma_s \leq 4.67 \tag{15a}$$

$$Y_{X/D} = 0.6 \quad \text{for } \gamma_s > 4.67 \tag{15b}$$

The correlation is plotted graphically along with the points representing the experimental biomass yields for aerobic growth of the database in Fig. 6a.

As already mentioned in Section 1, the correlation for $\gamma_D \leq 4.67$ is assumed to reflect an energy limitation in the energy donor substrates. Eq. (15a) may be shown to be a result of a constant bioenergetic efficiency of 60%, the rest being dissipated for creating the necessary driving force for growth. Eq. (15b), however, was linked to the fact that the yield may not exceed unity even on energy rich substrates, and thus is thought to stem from a C-limitation. Roels could however not explain why the yield would not even exceed 0.6.

Table 3
Standard errors (SEC) and average relative errors of prediction of biomass yields for different models: 'H&D' model based on the thorough analysis of dissipated Gibbs energy (Eqs. (10a) and (10b)), '500' standing for the simple use of value of $500\,\mathrm{kJ\,C\text{-}mol^{-1}}$ for the $\Delta_r G_X^\circ$ in all cases, and a simplified model described in this paper ($\Delta_r G_X^{\mathrm{rational}}$ is the Gibbs energy dissipation from Eq. (18), and $\Delta_r G_X^{\mathrm{empirical}}$ the values for Gibbs energy dissipation calculated from polynomial fit of the literature $\Delta_r G_X$ values)

	H&D	500	This article		
			$\Delta_r G_X^{\mathrm{rational}}$	$\Delta_r G_X^{\mathrm{empirical}}$	
SEC (C-mol/C-mol)	0.077	0.105	0.063	0.071	
Relative error of prediction (%)	16.9	27.1	19.3	6.8	All
	13.3	9.4	9.9	−2.0	Aerobes
	22.2	20.6	35.9	18.5	Anaerobes
	5.3	251	22.0	2.3	Autotrophs

$\Delta_r G_X$ from Eq. (18); $\Delta_r G_X$ calculated from polynomial fit of literature values.

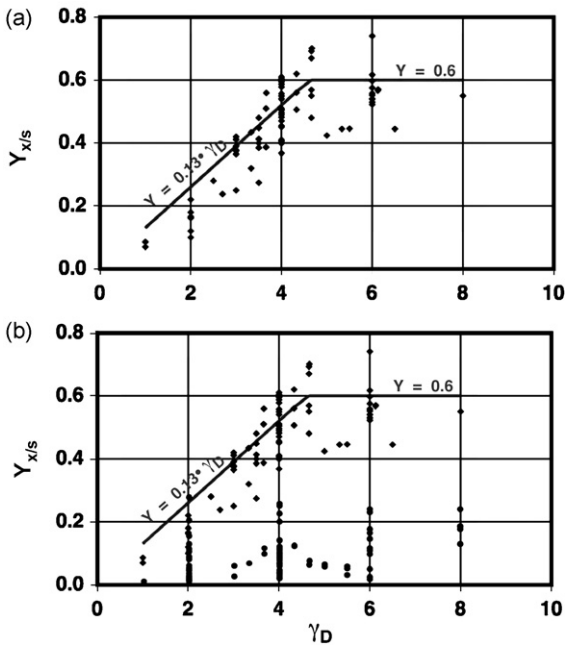

Fig. 6. Correlation of biomass yields for aerobic growth by Roels [10]. (a) Comparison with experimental aerobic growth yields and (b) comparison with all data. Keys: diamonds, aerobic growth yields; dots, anaerobic growth yields.

From a formal point of view, Eq. (8) reduces to the following for aerobic growth:

$$Y_{X/D} = \frac{\Delta_c G_D^\circ}{\Delta_r G_X^\circ - \Delta_c G_X^*} \tag{16}$$

It has been shown by several authors that both heats and Gibbs energies of combustion may be correlated quite accurately in terms of the degree of reduction of the respective substance. For $\Delta_c G^\circ$, Roels [10] gives the following correlation:

$$\Delta_c G^\circ = -86.6 - 94.4\gamma \tag{17}$$

Since the degree of reduction of dry biomass is similar for all types of cells, $\Delta_c G_X^*$ is constant in Eq. (16). If one assumes $\Delta_r G_X^\circ$ to depend as a first approximation only on the degree of reduction of the carbon substrate and since $\Delta_r G_D^\circ$ is given by Eq. (17), the biomass yield in Eq. (16) must, as a first approximation, depend primarily on γ_D (Fig. 6a).

The same analysis fails completely for anaerobic growth, where the denominator and the numerator of Eq. (16) also depend on $\Delta_c G_P^\circ$, which may vary very widely. It is thus not astonishing that a plot of experimental biomass yields including also the anaerobic data cannot be described by a correlation based on the degree of reduction (Fig. 6b).

It may be hypothesized that the relation between $\Delta_r G_X^\circ$ and γ_D in Eq. (16) underlying the correlation by Roels [10] as illustrated by Fig. 6a captures the most important variations of the Gibbs energy of growth in aerobic and even in anaerobic growth. This variation may be back calculated by substituting Eq. (15) or Eq. (17) into Eq. (16):

$$\Delta_r G_X^\circ = \frac{-666.2}{\gamma_D} - 243.1 \quad (\gamma_D \le 4.67) \tag{18a}$$

$$\Delta_r G_X^\circ = -157\gamma_D + 339 \quad (\gamma_D > 4.67) \tag{18b}$$

For autotrophic growth with reverse electron transport (RET) a constant value of $-3500\,\mathrm{kJ\,C\text{-}mol^{-1}}$ is proposed, as in the correlation by Heijnen and van Dijken.

Although not fitted to the database but derived from the earlier correlation by Roels for aerobic growth, Eq. (18) reproduces at least the general trend of the data points for aerobic growth (Fig. 7a). While this correlation cannot predict extreme values of Gibbs energy changes, the general trend appears to be represented approximately correctly even if the anaerobic data points are also included (Fig. 7b).

Substituting Eq. (18) into Eq. (8) also yields quite a good biomass yield prediction despite the dramatically simpler form than Eq. (10). Surprisingly, the standard error of correlation is even somewhat lower and now amounts to 6.5 yield percentages (Fig. 8), with the overall average error of prediction of 19% (Table 3).

From Fig. 7b it appears that even more accurate predictions might be obtained if Eq. (18) was fitted directly to the data points instead of having been back calculated based on the classical correlation of aerobic yields published by Roels [10]. Using a simple third order polynomial fit resulted in more accurate predictions (overall average relative error of 7%) but somewhat bigger scatter of results resulting in a standard error of correlation of 7.1 yield percentages.

There is no doubt that the experimental data in the database must be affected by large scatter. Differences in strains used by various authors for the same type of growth system, differences in medium composition and culture condition, and a host of other uncontrolled factors may account for considerable uncertainty in the yield data. Even when measuring the yields repeatedly

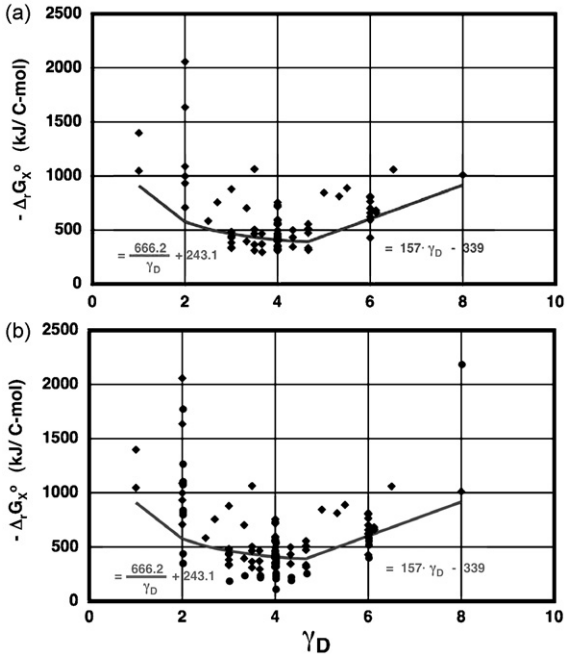

Fig. 7. Simple correlation of Gibbs energy dissipation. (a) Comparison with experimental data for aerobic growth and (b) comparison with all data. Keys: diamonds, aerobic growth; dots, anaerobic growth.

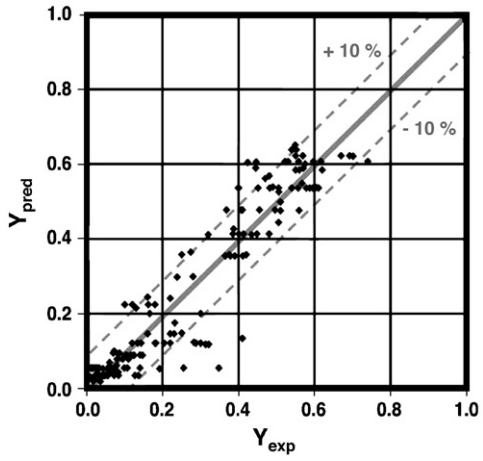

Fig. 8. Parity plot showing the predicted values for the biomass yield in C-mole/C-mole vs. the published experimental values based on correlation 18 for estimating the Gibbs energy change $\Delta_r G_X^\circ$ of the overall growth reaction.

during a single steady state in a chemostat culture of *Pichia pastoris* on glycerol gave rise to a relative standard deviation of 6% according to Jungo et al. [27]. A number of growth yields in the database is measured for cultures on complex media, and possible assimilation of compounds other than the designated main substrate was neglected in all analyses. This contributes to the model prediction error. Trying to design more accurate models may thus be limited by the inherent noise.

5. Conclusions

Microbial growth yields may roughly be predicted from a knowledge of the standard Gibbs energy change or dissipation of the overall growth reaction. The mathematical relationship between biomass yield and Gibbs energy dissipation was analyzed. A particularly intuitive way to represent this relationship is to split the overall growth reaction into a catabolic and an anabolic reaction, for which the Gibbs energies of reaction, ΔG_{cat}° and ΔG_{an}°, may be computed easily from thermodynamic tables. This way of representing the relationship shows clearly the trade-off that exists between high biomass yields and Gibbs energy dissipation. Growth yields are limited by the fact that too high a biomass yield will lower the Gibbs energy dissipation too much and thus reduce the thermodynamic driving force to insufficient values for fast metabolism and growth. The fact that there may be an optimal range of Gibbs energy dissipation in microbial growth is the implicit justification of Gibbs energy dissipation correlations. In splitting the growth process up into catabolism and anabolism, the latter can be formalized in many different ways, thus giving rise to very different values for ΔG_{an}°. The choice of a given formal description of anabolism does however neither affects these conclusions nor the quantitative end results of the analysis.

The Gibbs energy dissipation from a large number of literature data has been correlated using a carefully designed yet quite complex function published some time ago by Heijnen and van Dijken [18]. This correlation may be used as a basis for good biomass yield predictions. However, due to the nature of the relationship between biomass yield and Gibbs energy dissipation, one does not need a very precise estimation of the latter to predict the former roughly. Even assuming a universal constant value for the Gibbs energy dissipation predicts an extended collection of literature growth yields with a standard error of correlation that is not all that much larger than the complex correlation. A new dramatically simpler correlation is proposed which enables predictions that are just as accurate and in some ways even better than the complex one.

Acknowledgements

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Appendix A

Development of analogues of Eqs. (7) and (8) for more realistic formulations of the anabolic reaction.

Eq. (5) or Eq. (8) have been derived by splitting the overall growth process (Eq. (1)) up into a catabolic and a catabolic reaction as shown by Eq. (6) and Fig. 2. The underlying assumption has it that all the carbon and energy source is first completely catabolized and that biomass is resynthesized from the products of catabolism (Fig. 2).

It would be more realistic to assume that some of the carbon and energy source is deviated from catabolism and used for biosynthesis directly as shown in Fig. 3 (see, for example, Battley [23,28]). Catabolism and anabolism would thus formally be described by the following macrochemical equations:

$$D + Y_A^{cat} A \rightarrow Y_P^{cat} P + Y_{DOX}^{cat} \text{DOX}$$

$$Y_D^{an} D + Y_{NS}^{an} \text{NS} \rightarrow X + Y_{DOX}^{an} \text{DOX} \quad (\Delta G_{an1}^\circ) \tag{A1}$$

According to anabolic reaction (A1), the degree of reduction of the electron donor D in heterotrophic growth would be adjusted to the one of biomass (X) by decarboxylation reactions, thereby generating DOX (CO_2). In cases where the substrate is less reduced than biomass, Y_{DOX}^{an} becomes negative and the adjustment occurs by DOX (CO_2) fixation. In autotrophic growth, CO_2 or HCO_3^- would also be consumed as carbon source and would be reduced to X by D. In principle, the degree of reduction could also be adjusted by A:

$$Y_D^{an} D + Y_A^{an} A + Y_{NS}^{an} \text{NS} \rightarrow X + Y_P^{an} P \quad (\Delta G_{an2}^\circ) \tag{A2}$$

In many cases, however, Eq. (A1) is closer to reality.

No matter how the anabolism is formulated, the remarks made after Eqs. (7) and (5) will always remain valid. Assuming that anabolism is best described by Eq. (A1), Eq. (7) would have to be reformulated as:

$$\Delta_r G_X^\circ = \frac{r_{cat}}{r_{an}} \Delta G_{cat}^\circ + \Delta G_{an1}^\circ \tag{A3}$$

While r_{cat} and r_{an} denote the rate at which catabolism and anabolism proceed, their ratio is not equal to $1/Y_{X/D}$, because

both reactions consume D. Rather, the biomass yield must be formulated based on Eq. (6a) and Eq. (A1) as:

$$Y_{X/D} = \frac{r_{\text{an}}}{Y_D^{\text{an}} r_{\text{an}} + r_{\text{cat}}} \tag{A4}$$

or

$$\frac{r_{\text{an}}}{r_{\text{cat}}} = \frac{Y_{X/D}}{1 - Y_D^{\text{an}} Y_{X/D}} \tag{A5}$$

Substituting γ_X/γ_D for Y_D^{an} based on a degree of reduction balance and introducing the result into Eq. (A3) yields:

$$\Delta_{\text{r}} G_X^\circ = \frac{\Delta G_{\text{cat}}^\circ}{Y_{X/D}} - \frac{\gamma_X}{\gamma_D} \Delta G_{\text{cat}}^\circ + \Delta G_{\text{an1}}^\circ \tag{A6}$$

This equation would thus replace Eq. (7). The remarks made after Eq. (7) in the main text obviously also hold for Eq. (A6). Solving it for the biomass yield results in an analogue of Eq. (8):

$$Y_{X/D} = \frac{\Delta G_{\text{cat}}^\circ}{\Delta_{\text{r}} G_X^\circ - \Delta G_{\text{an1}}^\circ + (\gamma_X/\gamma_D)\Delta G_{\text{cat}}^\circ} \tag{A7}$$

Based on Eq. (6a) and Eq. (A1), $\Delta G_{\text{cat}}^\circ$ and $\Delta G_{\text{an1}}^\circ$ are given by:

$$\Delta G_{\text{cat}}^\circ = \Delta_{\text{c}} G_D^\circ - \frac{\gamma_D}{\gamma_P} \Delta_{\text{c}} G_P^\circ \tag{A8}$$

$$\Delta G_{\text{an1}}^\circ = \frac{\gamma_X}{\gamma_D} \Delta_{\text{c}} G_X^\circ - \Delta_{\text{c}} G_X^* \tag{A9}$$

If Eqs. (A8) and (A9) are substituted into Eq. (A7), one obtains again Eq. (5).

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tca.2007.01.016.

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