Department of Chemistry
Cumulative Examinations
March 2, 2013

You may choose to answer any exam from any area covered in the examination booklet. Each exam may contain multiple parts. You may answer more than one exam but each exam is scored separately and is treated as an individual examination result. Thus, answering parts of two exams with a score of 50% would not yield a 100% grade for this cumulative exam. Instead you would receive 50% on each examination attempted.

This booklet contains five examinations.

1) Analytical Cumulative Examination, Pages 1-3
2) Biochemistry Cumulative Examination, Pages 4-21
3) Inorganic Cumulative Examination, Page 22
4) Organic Cumulative Examination, Pages 23-25
5) Physical Cumulative Examination, Pages 26-27

On your examination booklet:

1) Print your student ID number.
2) Print the Exam Booklet number.
3) Print the question number you are answering.
4) Print the Exam Date.

Do not write your name anywhere on the examination booklet. Each exam will be scored anonymously. If you attempt more than one exam, you must use a separate examination booklet for each examination.

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Analytical Chemistry Cume  March 2 2013

Answer  Question 1 OR 2, and also Question 3 OR 4, as well as Question 5

1. [35 points] The statement has been made recently that “chemical analysis of molecules is passé”. [Definition passé: outmoded, beyond its prime]
   **Argue against this view** including examples of specific successes of analysis of particular molecules (the names and where reasonable, structures, of which should be given) using particular analytical methods. Try to select important examples and give the key characteristics of the methods discussed.
   [Answer should be approximately 3 blue book pages, with three separate examples based on different types of molecules and different analytical methods.]

2. [35 points] The statement has been made recently that “the analysis of molecules is passé”. [Definition passé: outmoded, beyond its prime]
   **Argue for this view** emphasizing that analytical chemistry is moving into a new phase where the task is to identify/characterize new types of aggregate materials (e.g. molecular machines; high temperature super-conductors; interacting microorganisms) and to do so on short distance and time scale in functioning environments.
   [Answer should be approximately 3 blue book pages, with three separate examples based on different types of molecules and different analytical methods.]

3. [30 points] Give approximate values and units for TEN of the following:
   (i) length of a single C-C bond
   (ii) strength of a single C-C bond
   (iii) thickness of graphene sheet
   (iii) number of molecules, molecular weight 100 Da, in a 1 fg sample
   (iv) Boltzmann constant
   (v) heat of formation of H₂ (gas)
   (vi) height equivalent of a theoretical plate in liquid chromatography
   (vii) binding constant of typical small molecule drug and receptor
   (viii) binding constant of avidin/streptavidin
   (ix) precision of standard FDA approved analytical methods
   (x) linear dynamic range in ESI mass spectrometry
   (xi) number of electrons/second in a 1 pA current
   (xii) sampling depth of SIMS, ESCA and Auger spectroscopy
4. [30 points] Briefly explain principal characteristics, information obtained and type of sample for SIX of the following [5 – 10 lines each]

(i) near-field optical microscopy – NSOM
(ii) laser induced breakdown spectroscopy – LIBS
(iii) x-ray photoelectron spectroscopy – XPS
(iv) second harmonic generation – SHG microscopy
(v) confocal microscopy
(vi) microcantilever sensor
(vii) microelectromechanical systems – MEMS
(viii) scanning electrochemical microscopy - SECM
(ix) Orbitrap mass analysis
(x) surface enhanced raman spectroscopy – SERS
(xi) hyperspectral imaging
(xii) reflectance absorption infrared spectroscopy - RAI RS
(xiii) electron energy loss spectroscopy – EELS
(xiv) partial least square linear discriminate analysis – PLS - LDA
5. [35 points] Explain how you would tackle **EIGHT** of these analytical problems, give method, instrument, equation, as appropriate. [5 – 10 lines each]

(i) spectroscopically characterization of gold nanoclusters (note: they self-aggregate and this must be avoided)

(ii) measure percent enantiomeric excess of synthetic limonene

(iii) determine the amino acid sequence of an octapeptide hormone

(iv) follow endocytosis of a drug candidate in a model cell culture system

(v) functionalize single walled nanotube using naphthylmethyl radicals

(vi) monitor immobilization of cisplatin on a metal surface

(vii) measure vincristine levels in blood of cancer patients undergoing therapy

![Vincristine molecule](image)

(viii) measure a set of trace metals in a meteorite sample recently recovered from a Siberian lake

(ix) measure traces of anabolic steroids in athlete’s urine and identify the steroid(s)

(x) quantify residual radioactivity in vegetables being consumed near the Fukushima nuclear plant
1. Identify at least 5 different ways metabolic pathways can be regulated in living organisms. Illustrate your answer with specific examples from metabolism.

2. Discuss the thermodynamic characteristics of metabolic reactions that are primarily controlled by a) changes in substrate concentration and, b) enzyme inhibition. Illustrate your answer with specific examples from metabolism.

3. Consider the supplied excerpts from two journal articles and address the following questions:

   - For the supplied 2006 paper:
     a) Interpret all the data and draw clear conclusions from the findings;
     b) Write a well-structured abstract for this paper;
     c) Select any ONE figure and illustrate an alternative way of visualizing/processing the data;
     d) Critically compare and contrast the way you suggest in "c" with the way it is done in the paper in terms of how well the data processing supports the interpretation reached from the findings;

   - For Table S1 below:
     e) Use the pH data in Table S1, as well as the findings in the 2006 paper, to explain why it is important to consider compartmentalization when studying metabolism and its thermodynamics.

Table S1: pH in each of eight cellular compartments

<table>
<thead>
<tr>
<th>Compartment</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol and nucleus</td>
<td>7.20</td>
</tr>
<tr>
<td>Extracellular fluid</td>
<td>7.40</td>
</tr>
<tr>
<td>Golgi apparatus</td>
<td>6.35</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>5.50</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>8.00</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>7.20</td>
</tr>
<tr>
<td>Peroxisomes*</td>
<td>7.00 ± 1.2</td>
</tr>
</tbody>
</table>

Thermodynamics of microbial growth and metabolism: An analysis of the current situation

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Abstract

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Nomenclature

\[ E_a \quad \text{activation energy (kJ mol}^{-1}) \]
\[ G \quad \text{Gibbs energy content of system (cell)} \quad \text{(kJ)} \]
\[ \Delta G^\circ \quad \text{apparent standard molar Gibbs energy of a biochemical reaction (kJ mol}^{-1}) \]
\[ \Delta G^\circ_{\text{an}} \quad \text{standard Gibbs energy of anabolism (kJ C-mol}^{-1}) \]
\[ \Delta G^\circ_{\text{cat}} \quad \text{standard Gibbs energy of catabolism (kJ C-mol}^{-1}) \]
\[ \Delta_c G^\circ_i \quad \text{standard Gibbs energy of combustion of } i\text{th metabolite using CO}_2, \text{H}_2\text{O and N}_2 \text{as the reference state for total oxidation (kJ C-mol}^{-1}) \]
\[ \Delta_c G^\circ_{x} \quad \text{standard Gibbs energy of combustion of } x\text{th metabolite using CO}_2, \text{H}_2\text{O and NH}_4^+ \text{as reference state for complete oxidation (kJ C-mol}^{-1}) \]
\[ \Delta_r G^\circ_x \quad \text{standard Gibbs energy of growth reaction (kJ C-mol}^{-1}) \]
\[ \dot{h}_i \quad \text{partial molar enthalpy of imported } i\text{th metabolite (kJ mol}^{-1}) \]
\[ \Delta_r H^\circ_x \quad \text{standard enthalpy change of growth reaction (kJ C-mol}^{-1} \text{ of dry biomass)} \]
\[ \dot{n}_i \quad \text{molar rate of import of } i\text{th metabolite (mol s}^{-1}) \]
\[ \dot{n}_x \quad \text{rate of export of newly synthesized biomass (C mol s}^{-1}) \]
\[ \dot{Q} \quad \text{rate of heat import (W)} \]
\[ R \quad \text{ideal gas constant (kJ mol}^{-1} \text{ K}^{-1}) \]
\[ \dot{s}_i \quad \text{partial molar entropy of newly produced biomass (kJ K}^{-1} \text{ C-mol}^{-1}) \]
\[ S \quad \text{entropy of system (cell) (kJ K}^{-1}) \]
\[ S_{\text{prod}} \quad \text{rate of entropy production due to irreversible phenomena (kJ K}^{-1} \text{ s}^{-1}) \]
\[ \Delta_r S^\circ_x \quad \text{standard entropy of growth reaction (kJ K}^{-1} \text{ C-mol}^{-1} \text{ of dry biomass)} \]
\[ t \quad \text{time (s)} \]
\[ T \quad \text{absolute temperature (K)} \]
\[ W \quad \text{rate of energy import in the form of shaft work (W)} \]
\[ Y_{ij} \quad \text{yield of } j\text{th metabolite per } i\text{th metabolite, or stoichiometric coefficient} \]
\[ Y_{x/i} \quad \text{biomass yield (C mol of dry biomass per C mol or mol of } i\text{th metabolite)} \]

Greek symbols

\[ \gamma_i \quad \text{degree of reduction of } i\text{th metabolite, using CO}_2, \text{H}_2\text{O and NH}_4^+ \text{ as reference state} \]
\[ \mu \quad \text{specific growth rate (s}^{-1}) \]
\[ \mu_i \quad \text{chemical potential of } i\text{th metabolite (kJ C-mol}^{-1}) \]
\[ \mu_x \quad \text{chemical potential of dry biomass (kJ C-mol}^{-1}) \]
\[ \psi_i \quad \text{stoichiometric coefficient of } i\text{th metabolite in a reaction} \]
\[ \dot{\xi} \quad \text{rate of advancement of growth reaction (C-mol s}^{-1} \text{ per cell)} \]

Subscripts

\[ \text{an} \quad \text{anabolism} \]
\[ \text{A} \quad \text{electron acceptor} \]
\[ \text{cat} \quad \text{catabolism} \]
\[ \text{C} \quad \text{CO}_2 \]
\[ \text{D} \quad \text{electron donor (energy substrate)} \]
\[ \text{DOX} \quad \text{oxidized form of electron donor} \]
\[ i \quad \text{ith metabolite} \]
\[ \text{m} \quad \text{maintenance} \]
\[ \text{N} \quad \text{nitrogen source} \]
\[ \text{prod} \quad \text{produced internally, source term} \]
\[ \text{P} \quad \text{product, reduced form of A or of S} \]
\[ \text{r} \quad \text{growth reaction} \]
\[ \text{S} \quad \text{carbon substrate} \]
\[ \text{W} \quad \text{water} \]
\[ \text{X} \quad \text{biomass} \]

1. Introduction

Thermodynamics is instrumental in chemistry for judging whether reactions are feasible and for defining the physico-chemical conditions under which they can occur. Thermodynamic analysis thus plays a central role in chemistry, chemical engineering and in chemical process development.

In biotechnology, however, thermodynamic analysis is hardly ever applied (von Stockar and van der Wielen, 2003). Yet, one would expect that here again thermodynamic analysis should be able to predict whether a given microbial growth or metabolic reaction is feasible and under what conditions. Based on such analyses, it ought to be possible to roughly estimate key param-
eters of biotechnological cultures and thus to address the economic viability of the process before even performing experiments. Once the first measurements are carried out, the thermodynamic predictions could be used as benchmarks, in the sense that experimental results staying far behind the calculations might indicate that there is ample room for improvement. All of this would be of invaluable help in bioprocess development, which usually is carried out under intense time pressure.

This contribution, which is based on a lecture given by the first author at Bioperspectives in Wiesbaden (von Stockar et al., 2004), attempts to review the thermodynamics of microbial growth and metabolism and to explore in how far it can be put to practical use in the sense just described. The prediction of the biomass yield is used as the main application example. Indeed, the biomass yield constitutes one of the key parameters in any biotechnological process or experiment involving microbial cultures, since it determines the final biomass or cell concentration, which must imperatively be optimized in order to obtain reasonable productivities. Yet, biomass yields vary by about two orders of magnitude from one microbial growth system to another (Heijnen and van Dijken, 1992).

2. Driving force for microbial growth

Biomass yields are intimately connected to the driving force for microbial growth, as will be shown later. We therefore describe the nature of this driving force first.

Microbial growth occurs spontaneously and is obviously a highly irreversible phenomenon. It must therefore be coupled with the production of entropy. In relation to growth reactions this seems contradictory, because growth reactions produce matter in a highly organized form from a set of very simple small molecules. One intuitively gets the impression that microbial growth decreases the entropy rather than producing it.

This contradiction may be resolved by contemplating an open-system entropy balance for the growing microbial cell (Fig. 1):

\[
\frac{dS}{dt} = \frac{\dot{Q}}{T} + \sum_i \tilde{s}_i \cdot \dot{n}_i - \tilde{s}_x \cdot \dot{n}_x + \dot{S}_{\text{prod}} \tag{1}
\]

According to this balance, the variation of entropy in the cell with time is given by the sum of all entropy fluxes exchanged with the environment plus the rate at which entropy is produced by irreversible processes (\(\dot{S}_{\text{prod}}\)). Entropy may be exchanged with the environment due to heat transfer to or from the cell denoted by \(\dot{Q}/T\) (\(dQ_{\text{rev}}/T\) represents the entropy increase in closed systems). In open systems, entropy is also imported or exported through metabolites entering or leaving the cell where \(\tilde{s}_i\) denotes the partial molar entropy carried by the \(i\)th metabolite and \(\dot{n}_i\) its molar rate of exchange, whereby positive values indicate assimilation rates. Newly formed biomass is treated as a product of the cell leaving it at a C-molar rate of \(\dot{n}_x\). Its partial molar entropy \(\tilde{s}_x\) is clearly low due to the high degree of organization of matter. The rate of entropy production by irreversible processes \(\dot{S}_{\text{prod}}\) can only be positive according to the Second Law of Thermodynamics and represents the real driving force for the process.

Due to constant entropy production at rate \(\dot{S}_{\text{prod}}\) and due to the fact that newly formed cells of low entropy content leave the cell but have been synthesized by importing high-entropy metabolites, entropy could in principle accumulate in the cell and lead to thermal cell death or to structural disorganization. In order to avoid this, the cell must constantly export the excess entropy, i.e. it must keep \(dS/dt\) at zero by making the
sum of the first three terms on the right-hand side of Eq. (1) negative. This is precisely the role of catabolism. It exports the excess entropy either by creating a large flux of small waste molecules from the substrate, thereby exporting it in the form of chemical entropy and making \( \sum_i \mu_i \delta_i \) strongly negative, or by releasing considerable amounts of heat, thereby making \( \dot{Q}/T \) strongly negative.

If Eq. (1) is multiplied by \( T \) and subtracted from an enthalpy balance (Eq. (2)) for a constant pressure process

\[
\frac{dH}{dt} = \dot{Q} + \dot{W} + \sum_i \bar{h}_i \cdot \dot{n}_i - \bar{h}_x \cdot \dot{n}_x
\]

a Gibbs energy balance results (Fig. 2):

\[
\frac{dG}{dt} = \dot{W} + \sum_i \mu_i \cdot \dot{n}_i - \mu_x \dot{n}_x - T \dot{S}_{\text{prod}}
\]

where \( \dot{W} \) stands for the power or work done on the cells, and \( \mu_i \) and \( \mu_x \) stand for the chemical potential of the \( i \)th metabolite and the newly grown cells, respectively. The latter is necessarily high due to the low entropy of biomass. In order to avoid death, the cell has to keep \( dG/dt \) at zero despite a constant loss of Gibbs’s energy through the newly formed biomass \( (\mu_x \dot{n}_x) \) and through dissipation or destruction of Gibbs energy \( (-T \dot{S}_{\text{prod}}) \), which can only be negative. In phototrophs, this loss is replenished by a positive \( W \) term in the form of photons. Chemotrophs, on the other hand, have a catabolism that feeds on high Gibbs energy substrates and release low energy waste products, thereby making \( \sum_i \mu_i \delta_i \) so positive that it overcompensates \( -\mu_x \dot{n}_x \) and \( -T \dot{S}_{\text{prod}} \). The result is a continuous decrease of Gibbs energy in the surrounding medium.

In order to express this change in Gibbs energy in terms of a Gibbs energy of reaction, \( \Delta G \), the whole process may be written in form of a macrochemical equation, of which an example for chemotrophic growth could have the following form:

\[
\frac{1}{Y_{XS}} S + Y_{A/X} A + Y_{N/X} NH_3
\]

\[
\rightarrow X + Y_{P/X} P + Y_{C/X} CO_2 + Y_{W/X} H_2O
\]

where \( S, A, X \) and \( P \) represent the carbon source, an electron acceptor or donor, the newly grown biomass, and a catabolic waste product, respectively. \( Y_{XS} \) denotes the biomass yield on the carbon source and \( Y_{UX} \) denotes the other yields. All \( Y \) coefficients may be regarded as stoichiometric coefficients of the growth reaction.

A molar balance over the cells for any of the metabolites in a macrochemical equation such as Eq. (4) yields:

\[
\frac{dn_i}{dt} = \dot{n}_i + v_i \cdot \dot{\xi}
\]

where \( v_i \) stands for either \( 1/Y_{XS} \) or any of the other \( Y_{UX} \) and \( \dot{\xi} \) denotes the rate of the growth reaction in \( \text{mol s}^{-1} \) per one cell. Substituting this into Eq. (3) and assuming the cells to be at steady state, i.e. setting both \( dG/dt \) and \( dn_i/dt \) to zero, yields (von Stockar and Liu, 1999):

\[
\Delta_r G_x \cdot \dot{\xi} = \dot{W} - T \dot{S}_{\text{prod}}
\]

with

\[
\Delta_r G_x = \sum_i v_i \mu_i = \Delta_r H_x - T \Delta_r S_x
\]

where \( \Delta_r G_x \) is the molar Gibbs reaction energy of the macrochemical reaction. The sum in Eq. (7) has to be performed over all constituents of the macrochemical Eq. (4) including biomass.

In non-photosynthetic growth, \( \dot{W} \) is usually zero. In this case, Eq. (6) shows that \( \Delta_r G_x \cdot \dot{\xi} \) reflects the rate at which cells export the entropy produced within them by irreversible processes into the fermentation medium. \( \Delta_r G_x \) must clearly be negative for growth to occur since \( \dot{S}_{\text{prod}} \) can only be positive. \( \Delta_r G_x \cdot \dot{\xi} \) therefore also reflects the rate at which Gibbs energy is dissipated by irreversible processes occurring in the cells. The Gibbs energy in the growth medium will thus decrease at a corresponding rate, as can be shown easily by a Gibbs energy balance over the whole bioreactor.

Due to its direct relationship with the rate of entropy generation in the case of \( \dot{W} = O \) (Eq. (6)), \( \Delta_r G_x \) is called the driving force for its conjugate flux \( \dot{\xi} \).
As shown by Eq. (7), the driving force has an enthalpic and an entropic part, which correspond to the export of entropy in the form of heat and in the form of high entropy molecules, respectively.

3. The relationship between the driving force and the biomass yield

3.1. Theory

The relationship between the driving force for microbial growth $\Delta r G_s$ and the biomass yield $Y_{X/S}$ is best understood by splitting the macrochemical reaction into a catabolic and an anabolic part (Fig. 3). Formation of biomass clearly produces matter with high Gibbs energy due to its low entropy content and therefore increases the Gibbs energy in the bioreactor. Anabolic reactions taken by themselves are thus subjected to a driving force in the opposite sense of growth and are endergonic. In order to pull the anabolic reactions against this driving force "up-hill", they are coupled to catabolism, which is characterized by a large negative $\Delta G$ and thus is highly exergonic. The net driving force $\Delta r G_s$ remaining for the whole process clearly depends on the stoichiometric "load" anabolism places on catabolism, i.e. on the biomass yield.

In inefficient growth systems, this load would be very small, producing only a small amount of biomass per carbon and energy substrate consumed. The biomass yield would be small, but the overall $\Delta r G_s$ would remain highly negative because the positive $\Delta G$ of anabolism would hardly make its weight felt on the downward pull of catabolism. Conversely, in efficient growth, the load would be much more important and would neutralize a large part of the negative $\Delta G$ of catabolism, thereby leaving only a small negative $\Delta G$ for the overall process. On the other hand, a lot of biomass would be formed per substrate consumed and $Y_{X/S}$ would be high. At the limit, the load could be so large that the two downward forces are in equilibrium and $\Delta r G_s$ is zero. This situation would define the highest $Y_{X/S}$ allowed by the Second Law of Thermodynamics. It would represent growth at the thermodynamic equilibrium and would thus proceed at an infinitely slow rate.

It is obvious that real microbial growth systems must strike a compromise between two unfavorable extremes: a very low "load" would provide a high overall driving force and the metabolism would proceed vigorously, but only little biomass would be formed and $Y_{X/S}$ would be small. If, on the other hand, growth occurred too close to thermodynamic equilibrium, the biomass yield would indeed be optimal, but growth would proceed so slowly that the organism would always be outgrown by competitors. Real organisms must therefore have a $\Delta r G_s$ and a $Y_{X/S}$ somewhere between these two extremes.

In order to formulate the relationship between $\Delta r G_s$ and $Y_{X/S}$ mathematically, the macrochemical Eq. (4) is best split up into catabolism and anabolism as follows:

Catabolism:

$$S + \gamma_A^p A \rightarrow \gamma_P^p P + \gamma_W^o H_2O + \gamma_C^c CO_2; \quad \Delta G_{\text{cat}}$$  \hspace{1cm} (8a)

Anabolism:

$$\gamma_P^p P + \gamma_W^o H_2O + \gamma_C^c CO_2 + \gamma_N^N NH_3 \rightarrow X + \gamma_A^p A; \quad \Delta G_{\text{an}}$$  \hspace{1cm} (8b)

This description suggests that the available carbon substrate $S$ is first completely catabolized and that biomass is then formed from the products of catabolism, which is of course unrealistic. Eqs. (8a) and (8b) have, on the other hand, the advantage of simple stoichiometry.
tries with clearly defined $\Delta G$ values, which are easy to evaluate as long as the catabolic reaction is known. To use correct formulations of anabolism is impractical because the real stoichiometry of anabolic processes is highly complicated (Heijnen and van Dijken, 1992) and therefore only rarely known. Despite the over-simplification in formulating anabolism, the results of calculations will be correct as long as Eqs. (8a) and (8b) are not used separately.

The quantitative relationship between $\Delta r G$ and $Y_{X/S}$ is obtained by observing that Eq. (4) results when Eq. (8a) is divided by $Y_{X/S}$ and added to Eq. (8b):

$$\Delta r G_x = \frac{1}{Y_{X/S}} \Delta G_{\text{cat}} + \Delta G_{\text{an}}$$

(9)

In this equation, only standard Gibbs energies have been used since life processes are so far from equilibrium that the concentration-dependent terms will not modify the calculations significantly.

3.2. Experimental results

The relationship between the driving force for growth and the biomass yield was investigated for several different growth processes in the laboratory of the first author (von Stockar and Liu, 1999).

The biomass yields were measured for batch and chemostat cultures under very many different growth conditions at varying specific growth rates and $\Delta r G_x$ values were calculated according to Eq. (9). In addition, the enthalpic part of the driving force of growth, $\Delta r H_x$, was measured directly by applying reaction calorimetry and compared with values computed according to the enthalpy analogue of Eq. (9). Experimental and computational details have been published elsewhere (von Stockar and Liu, 1999).

Fig. 4 summarizes the relationship between driving forces and biomass yields for a large number of aerobic growth processes on glucose. The thick line, obtained by Eq. (9), shows clearly the decrease of the driving force as a function of increasing assumed biomass yields (discussed earlier). The maximal value of $Y_{X/S}$ allowed by the Second Law is seen to amount to about 1.04 C-mol/(C-mol). The corresponding thin line for $\Delta r H_x$ is very close to the one for Gibbs energy, which indicates that $T \Delta r S_x$, which ought to separate the two lines, is close to zero. It may be concluded that aerobic growth on glucose is driven almost entirely by an enthalpy change, or that the entropy produced by irreversible processes is exported practically exclusively in the form of heat.

Despite the enormous differences in strains, growth conditions and rates, the measured biomass yields as well as the measured reaction enthalpies fall within quite a narrow range. The biomass yields are quite high, yet still far away from the theoretical maximum. This probably reflects the compromise between two unfavorable extremes discussed earlier. The driving forces $\Delta r G_x$ consequently do not vary widely and are comprised between $-250$ and $-500$ kJ C-mol$^{-1}$ of dry biomass grown. The measured $\Delta r H_x$ values fall into a similar range. If the energy balances had closed, the respective points should lie on the thin line, representing the predictions obtained from the enthalpy analogue of Eq. (9). (The open symbols fall on the thick line, because $\Delta r G_x$ values cannot be measured but were calculated from measured $Y_{X/S}$ values in the same way as the thick line (Eq. (9)).)

Fig. 5 represents a similar plot as Fig. 4, but for anaerobic growth of K. marxianus and S. cerevisiae. The catabolic reaction produces ethanol according to

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

(10)

The thin line representing $\Delta r H_x$ is now widely separated from the thick Gibbs energy of reaction line and indicates much lower negative values. The measured
reaction enthalpies were around $-100 \text{kJ mol}^{-1}$ only and confirm the calculated line quite well. Despite this dramatically lower heat generation, vigorous growth occurred and the calculated Gibbs energy change still reached between $-240$ and $-340 \text{kJ mol}^{-1}$ of dry biomass grown. The separation of $\Delta_r G^\circ_x$ and $\Delta_r H^\circ_x$ shows that a large $T \Delta_r S^\circ_x$ term contributed to the total driving force. In this case, about 2/3 of the driving force was entropic and the same fraction of entropy generated in the cells was exported as chemical entropy in the form of the small molecules generated by the catabolic reaction (see Eq. (10)).

It is noteworthy that the Gibbs energy change of this culture is still of the same order as the ones observed for aerobic growth, despite the fact that the thick line is located completely differently in the plot. Due to the much smaller Gibbs energy change of the catabolic reaction (Eq. (10)), this line now indicates much less negative $\Delta_r G^\circ_x$ for a given biomass yield. It could be hypothesized that the biomass yields in anaerobiosis are reduced to an extent permitting the culture to maintain the same overall driving force for growth.

Fig. 5. Standard Gibbs energies ($\Delta_r G^\circ_x$, calculated) and standard enthalpies ($\Delta_r H^\circ_x$, measured) of the growth reaction vs. biomass yields for ethanol fermentation on glucose. Thick line, $\Delta_r G^\circ_x$ calculated from Eq. (9); thin line, $\Delta_r H^\circ_x$ calculated from an enthalpy analogue of Eq. (9); open symbols, $\Delta_r G^\circ_x$ calculated for experimental biomass yields; full symbols, $\Delta_r H^\circ_x$ measured by calorimetry; dots/circles, S. cerevisiae; squares, K. marxianus. Reprinted with permission from von Stockar and Liu (1999).

Fig. 6. Standard Gibbs energies ($\Delta_r G^\circ_x$, calculated) and standard enthalpies ($\Delta_r H^\circ_x$, measured) of the growth reaction vs. biomass yields for homolactic fermentation on glucose by L. helveticus. Thick line, $\Delta_r G^\circ_x$ calculated from Eq. (9); thin line, $\Delta_r H^\circ_x$ calculated from an enthalpy analogue of Eq. (9); open symbols, $\Delta_r G^\circ_x$ calculated for experimental biomass yields; full symbols, $\Delta_r H^\circ_x$ measured by calorimetry. Reprinted with permission from Liu et al. (1999).

Fig. 6 represents the driving forces as a function of biomass yields for anaerobic growth of Lacticobacillus helveticus, which generates its energy from the following catabolic reaction (Liu et al., 1999):

$$C_6H_{12}O_6 \rightarrow 2\text{CH}_3\text{CHOHCOOH}$$

(11)

Although this catabolic reaction might be expected to export the excess entropy almost solely by producing small molecules from large ones, Fig. 6 shows a majority of the driving force to be enthalpic. The reason is the fact that at the growth pH lactic acid is immediately neutralized, thereby yielding a considerable amount of heat. This neutralization reaction has been taken into account when calculating the $-\Delta_r G^\circ_x$ curve in Fig. 6, and it may be seen that the Gibbs energy driving forces resulting from the measured biomass yields are again of the same order of magnitude.

The most extreme case of entropy-driven growth investigated as yet are anaerobic cultures of the bacterium Methanosarcina barkeri, which derives its energy from the following reaction (Liu et al., 2001):

$$\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$$

(12)

As may be seen in Fig. 7, the $-\Delta_r H^\circ_x$ curve is not only dramatically separated from the $-\Delta_r G^\circ_x$ curve, but it is even below the zero line. This means that this culture
Fig. 7. Standard Gibbs energies ($\Delta_r G_x$, calculated) and standard enthalpies ($\Delta_r H_x$, calculated or measured) of the growth reaction vs. biomass yields for acetotrophic methanogenesis by *M. barkeri*. Thick line, $\Delta_r G_x$ calculated from Eq. (9); thin line, $\Delta_r H_x$ calculated from an enthalpy analogue of Eq. (9); open symbols, $\Delta_r G_x$ and $\Delta_r H_x$, calculated for experimental biomass yields; full symbols, $\Delta_r H_x$ measured by calorimetry; squares, this work; circles/dots, literature values. Reprinted with permission from Liu et al. (2001).

not only does not produce any heat, but on the contrary absorbs heat and cools its environment down. As shown by the solid squares on the line, calorimetric measurements confirmed the endothermic nature of this culture and corresponded well with the calculated predictions. This is so far the only experimental demonstration of the existence of an endothermic life form. *M. barkeri* exports the excess entropy by turning one molecule in the aqueous state into two gaseous molecules, which increases the entropy in the bioreactor considerably.

The corresponding driving force for growth is sufficiently large to permit the culture to grow despite the fact that it must excrete waste products (CH$_4$ and CO$_2$) of higher chemical energy than the feedstock (acetate), thereby forcing it to absorb the difference in the form of heat. Nevertheless, with $-500$ kJ·mol$^{-1}$, the overall driving force $\Delta_r G_x$ is again of the same order of magnitude as in all the other cases.

Driving forces of between $-250$ and $-500$ kJ·mol$^{-1}$ of dry biomass grown are in line with a study by Heijnen and van Dijken (1992), who analyzed a large body of literature and found that growth on carbon substrates of a reduction degree of 4 is always driven by a driving force of that magnitude. This range may represent a good compromise between large driving forces and low biomass yields on the one hand and growth near thermodynamic equilibrium on the other hand, and it appears that biomass yields may have been adapted to this range by evolution.

In the cases of homofermentative lactic acid fermentation (Fig. 6) and acetotrophic methanogenesis (Fig. 7), these $\Delta_r G_x$ values are only obtained if the side reactions of lactic acid neutralization and gasification of CH$_4$ and CO$_2$ are included. Otherwise, Gibbs energy changes close to zero would result. It is thus concluded that these side reactions must be considered to be part of the energy metabolism, even though they occur outside the cell. They contribute considerably to the overall driving force by instantaneously removing the waste molecules lactic acid and CH$_4$ from the outer surface of the cells. (This is also the reason why growth of acetotrophic methanogenesis must be considered endothermic, even though the effect is mainly due to the vaporization of CH$_4$ and CO$_2$ outside of the cell. However, even without the evaporation of carbon dioxide growth of *M. barkeri* would still be endothermic, albeit very mildly so (Liu et al., 2001). On the other hand, it would be virtually impossible to grow *M. barkeri* at pressures which keep methane from evaporating.)

It may be concluded quite generally that the driving forces for growth $\Delta_r G_x$ appear to fall in a certain range, which may have been determined by evolution. However, the separation in an enthalpic and an entropic part differs widely from one case to another, as summarized in Fig. 8. Aerobic respiration is enthalpy driven practically to 100% with the $T \Delta_r S_x^\circ$ term (arrows) being insignificant. Fermentative metabolism, shown on the left of respiration, have lesser $-\Delta_r H_x^\circ$ values because no external electron acceptors are involved. However, fermentations invariably rip substrate molecules apart into smaller entities, thereby generating an increase of chemical entropy in the medium that can be harnessed as driving force. In the case of *M. barkeri*, shown just right of the overall driving force $\Delta_r G_x^\circ$, this entropic driving force has to overcome a positive enthalpy change, which in effect acts as a partial driving force in the wrong sense. It was thus proposed to call this enthalpy retarded growth. A case at the opposite extreme of the spectrum was also investigated calorimetrically: in the case of autotrophic methanogenesis, the catabolism leads to a reduction of chemical entropy in the medium, which has to be overcome by an overly negative enthalpic driving force. These growth processes are therefore very exothermic.
(Schill et al., 1999) and could be called entropy retarded.

3.3. Prediction of the biomass yield

Based on what has been presented in this review, thermodynamics allows estimating the biomass yield of an unknown microbial strain by solving Eq. (9) for it:

$$Y_{X/S} = \frac{\Delta G^\circ_{\text{cat}}}{\Delta_t G^\circ_x - \Delta G^\circ_{\text{an}}} \quad \text{(13)}$$

We will now describe how Eq. (13) may be used for predicting $Y_{X/S}$. The first step would be to estimate $\Delta G^\circ_{\text{cat}}$ and $\Delta G^\circ_{\text{an}}$ based on Eqs. (8a) and (8b). This is no problem as long as the compounds S, A and P have been identified and the stoichiometric coefficients appearing in Eqs. (8a) and (8b) are known from biochemistry, but in most cases it will also be possible to calculate these yields if they are unknown. The minimum information needed for this calculation is the nature of an eventual external electron acceptor A in Eq. (4) (e.g. oxygen), and the nature of an eventual waste product of the catabolism P (e.g. ethanol). In many cases, the metabolism will involve either A or P but not both, or either P or A will be CO2. (Occurrence of both A and P different from CO2 usually indicates the presence of more than one catabolic mechanisms and at least one additional piece of information, such as a $Y$ value in Eq. (4), must be known to treat the case (Heijnen et al., 1992; von Stockar and Liu, 1999).)

In the many cases in which only A or P is present, or either P or A is identical with CO2, the number of unknown $Y$ values in Eqs. (8a) and (8b) are reduced to 3 and 4, respectively. They may therefore straightforwardly be calculated by applying degrees of reduction, carbon, oxygen and nitrogen balances.

This calculation and the evaluation of $\Delta G^\circ_{\text{cat}}$ and $\Delta G^\circ_{\text{an}}$ may be generalized and simplified for all cases treated in this review, including those in which the C-source and the energy source are not the same compound by rewriting Eqs. (8a) and (8b) as follows:

$$D + Y^a_A A \rightarrow Y^a_P P + Y^a_{\text{DOX}} \text{DOX}; \quad \Delta G^\circ_{\text{cat}} \quad \text{(14a)}$$

$$Y^b_P + Y^b_{\text{DOX}} \text{DOX} + Y^b_{N \text{NH}_3} \rightarrow X + Y^b_A A; \quad \Delta G^\circ_{\text{an}} \quad \text{(14b)}$$

where D is the electron donor or energy source in the growth reaction, A the electron acceptor, P the reduced form of A and DOX denotes the oxidized form of D. In heterotrophic growth, D is also the carbon source, yielding CO2 as DOX, whereas in autotrophic growth the carbon is provided by A, which then is CO2. In fermentative and reductive growth, A and D are the same compound.

In all cases treated in this review, as well as in a large majority of cases occurring in reality, only D, X and P
have a degree of reduction different from zero. As long as CO$_2$ (g), H$_2$O (l) and NH$_4^+$ (aq) are used as reference state for total combustion, all the other compounds have a degree of reduction and also a Gibb's energy of combustion of zero. The only relevant stoichiometric coefficients to compute in Eqs. (14a) and (14b) are thus $\gamma^P$ and $\gamma^B$, respectively. These may be found using a single degree of reduction balance for each of the two yields. Combining the results with Gibbs energy balances yields (von Stockar and Liu, 1999):

$$\Delta G_{\text{cat}}^\circ = \Delta G_D^\circ - \frac{\gamma^D}{\gamma^P} \Delta G_P^\circ$$  
(15a)

$$\Delta G_{\text{an}}^\circ = \frac{\gamma^x}{\gamma^P} \Delta G_P^\circ - \Delta G_x^\circ$$  
(15b)

where $\Delta G_x^\circ$ is the modified Gibbs energy of combustion for biomass, using CO$_2$ (g), H$_2$O (l) and NH$_4^+$ (aq) as reference state for total combustion. In oxidative heterotrophic growth, the second and the first terms on the right-hand side of Eqs. (15a) and (15b), respectively, vanish, because P is water which has a zero Gibbs energy of combustion.

Based on Eqs. (15a) and (15b), $\Delta G_{\text{cat}}$ and $\Delta G_{\text{an}}$ are then evaluated using tabulated molar Gibbs energies of formation or combustion for the various metabolites (Wagmann et al., 1968; Wilhoit, 1969; Battley, 1987; Roels, 1983; Heijnen and van Dijken, 1992; Sandler and Orbey, 1991; Heijnen, 1999a). In order to compute $\Delta G_{\text{an}}^\circ$, the C-molar Gibbs energy of formation or combustion of dry microbial biomass is also needed. There are many more or less speculative estimations in the literature for $\Delta_c G_\text{j}$ (von Stockar and Liu, 1999), but the only value with an experimental basis was determined by low temperature calorimetry by Battley et al. (1997) and amounts to $\Delta_c G_\text{j} = 515 \text{kJ C}^{-1} \text{mol}^{-1}$ for E. coli. Since the elemental composition of dry biomass is known to be fairly constant from one strain to another, this value may also be used for other types of biomass as a good estimate of $\Delta_c G_\text{x}^\circ$.

Once $\Delta G_{\text{an}}^\circ$ and $\Delta G_{\text{cat}}$ are known, an estimation of the overall driving force $\Delta_x G_\text{x}$ for the whole process is also needed, in order to use Eq. (13). While this driving force may have been optimized by evolution and does not seem to vary very much in the examples treated earlier, Heijnen and coworkers (Heijnen and van Dijken, 1992; Heijnen et al., 1992; Tijhuis et al., 1993) have shown, based on an investigation of data published in the literature, that $\Delta_x G_\text{x}$ is virtually independent of the nature of the electron acceptor used, but does depend in a statistically significant way on the number of carbon atoms C and the degree of reduction $\gamma$ of the carbon source on which the culture is growing. According to this study, cultures growing on "easy" substrates that have a degree of reduction $\gamma_x$ of 3.8, which is close to the one of dry biomass (4.2), and with six atoms of carbon (C = 6) need only to dissipate around $-200 \text{kJ C-mol}^{-1}$ of Gibbs energy. However, any deviation from this degree of reduction and/or growth on carbon sources with less carbon atoms increases the number of biochemical steps needed to incorporate the carbon source into the new biomass, such that a higher overall driving force is needed. The authors expressed this observation in terms of a quantitative, empirical correlation as follows:

$$-\Delta_x G_\text{x} = 200 + 18 \cdot (6-C)^{1.8} + 3.6 + 0.4C$$

(16a)

For chemotrophic growth with reverse electron transport:

$$-\Delta_x G_\text{x} = 3500 \text{(kJ C-mol)}$$

(16b)

The predictive power of correlations (16a) and (16b) together with Eqs. (13), (15a) and (15b) has been tested on an extended database consisting of almost all the data used and published by Heijnen and van Dijken (1992), all the experimental data accumulated over the years in the laboratory of the first author and additional literature data (Patiño et al., submitted for publication). By estimating $\Delta_x G_\text{x}$ by use of Eqs. (16a) and (16b) and substituting the result into Eq. (13), the biomass yields may be predicted with a standard error of prediction of about ±11% as shown in Fig. 9a. (Since Eqs. (14a), (14b), (15a) and (15b) were used to predict the biomass yields, these were obtained in C-mol of dry biomass per mole of electron donor ($Y_{\text{X/D}}$) and plotted in this form in Fig. 9.)

Liu (1999) developed a considerably simpler correlation for estimating the driving force for microbial growth. It was based on an old correlation by Roels (1983) who proposed to estimate aerobic biomass yields directly by assuming them to increase proportionally with the degree of reduction of the carbon
source ($Y_{X/S} = 0.13 \cdot \gamma_S$) up to a degree of reduction of 4.67 and then to remain constant at $Y_{X/S} = 0.6$. While this affords a very good prediction for aerobic growth, it fails completely for anaerobic processes. However, Liu (1999) calculated how $\Delta_r G_x^*$ must depend on $\gamma_S$ for this correlation to be valid in aerobic cases.

The result was:

$$\Delta_r G_x^* = \frac{666.2}{\gamma_S} + 243.1 \text{ (kJ/C-mol)} \quad \text{for } \gamma_S \leq 4.67$$

(17a)

$$\Delta_r G_x^* = 157 \gamma_S - 339 \text{ (kJ/C-mol)} \quad \text{for } \gamma_S > 4.67$$

(17b)

It was then assumed that $\Delta_r G_x^*$ can be estimated from Eqs. (17a) and (17b) for both aerobic and anaerobic growth processes. Correlations (17a) and (17b) were tested together with Eq. (13) using the same database as before. The result (Fig. 9b) shows that the biomass yields may be predicted with a slightly smaller standard error of ±8%, despite a dramatically simpler estimation for $\Delta_r G_x^*$. A rough prediction to within ±9% is even possible by simply substituting an average value of −500 kJ C-mol⁻¹ for $\Delta_r G_x^*$ into Eq. (13). However, this method may result in very large relative prediction errors for anaerobic growth.

3.4. Discussion

In summary, it may be concluded that the very wide variations occurring in biomass yields can indeed be understood in the framework of a thermodynamic analysis. According to the nature of the catabolic mechanisms employed by given strains, the Gibbs energy driving forces for both the driving catabolic ($\Delta G_{cal}$) and the "payload" ($\Delta G_{pm}$) reactions may differ very considerably from one microbial growth system to another. A given biomass yield would thus give rise to completely different overall Gibbs energy driving force $\Delta_r G_x^*$ from one system to another as shown by Eq. (9) and Fig. 10. It appears, however, that microorganisms may have adapted in the course of evolution the yield of their "payload" reaction, i.e. the biomass yield, in such a way that the overall driving force $\Delta_r G_x^*$ represents a reasonable compromise between the extremes of ultrafast, wasteful metabolism on the one hand and very efficient but slow metabolism on the other hand. Since correlations for such optimal $\Delta_r G_x^*$ values have been developed, the actual biomass yields for given growth systems may very roughly be predicted.

Other developments than the one presented here have also been published as a basis for predicting biomass yield. Early work was based on attempts to cor-
Fig. 10. Relationship between biomass yield and the overall driving force for the growth reaction $\Delta_r G^\circ$ for: (a) oxidative growth on glucose, (b) ethanol fermentation of glucose, (c) lactic acid fermentation on glucose and (d) acetotrophic methanogenesis. The shaded Gibbs energy dissipation range is believed to represent a favorable compromise between high growth efficiency and high growth rate. Also shown are the ranges into which biomass yields of oxidative growth on glucose and on acetotrophic methanogenesis have to fall in order to grow at this optimal Gibbs energy dissipation (pairs of horizontal broken lines).

relate the biomass yield in terms of $Y_{\text{ATP}}$ (e.g. Bauchope and Elsdon, 1960) or in terms of energetic efficiencies (Minkevich and Eroshin, 1973; Roels, 1983; Battley, 1960, and many others). In developing their correlation for Gibbs energy dissipation, Heijnen and van Dijken (1992) reviewed these methods and showed that they were all plagued with serious internal inconsistencies. An alternative method first proposed by McCarty in 1965 and later refined (McCarty, 1969, 1975; Lawrence and McCarty, 1970; Christensen and McCarty, 1975; Rittmann and McCarty, 2001) is based on a combination of a Gibbs energy and an electron balance. It essentially sets a sum that is analogous to the two right-hand side terms of Eq. (9) equal to zero but multiplies the term referring to the catabolic reaction with an efficiency factor $K$, thereby implicitly reintroducing the Gibbs energy dissipation. This parameter $K$ has no physical meaning and is usually assumed constant when using the McCarty method. Nevertheless, it has been shown that the yield predictions for oxidative organo-heterotrophic growth are comparable with the ones based on correlations of $\Delta_r G^\circ$ (Van Briesen, 2002). The yield predictions obtained by this method seem not to have been tested broadly for reductive growth or for litho-autotrophic metabolism. On the other hand, its usefulness has been demonstrated for predicting the stoichiometry of biodegradation reactions. Also, it has been extended by Van Briesen and coworkers to cope with accumulation of slowly biodegradable intermediates (Van Briesen and Rittmann, 2000) and biodegradations involving monoxygenases (Van Briesen, 2001; Yuan and Van Briesen, 2002).

All of these prediction methods may be particularly useful to assess biomass yields where exotic forms of catabolisms occur, such as in applications in environmental sciences, e.g. for producing biohydrogen or bioelectricity (Angenent et al., 2004), but more work is needed to ascertain this. So far, the predictions are restricted to chemotrophic growth, but work on the energy budget and the thermodynamics of phototrophic growth may yield analogous results for phototrophic growth (Janssen et al., 2005).

4. Thermodynamic analysis of key culture growth parameters other than growth yield

During the 1990s, Heijnen and coworkers undertook thermodynamic analyses of a number of further essential parameters characterizing cellular growth. For instance, the correlations for the biomass yield discussed so far do not allow for the decrease of this parameter observed at low growth rates due to maintenance reactions. A complete prediction of the growth stoichiometry thus requires a correlation for estimating the maintenance coefficient, which then enables one to compute $Y_{X/S}$ or $Y_{X/D}$ at low specific growth rates using the well-known Herbert–Pirt relation. Tijhuis et al. (1993) placed this argument on a thermodynamic foundation by observing that maintenance reactions will increase the Gibbs energy dissipation rate as shown by Eq. (18):

$$\Delta_r G^\circ \cdot \mu = (\Delta_r G^\circ)_{\text{min}} \cdot \mu - (T \cdot \dot{S}_p)_{m}$$  \hspace{1cm} (18)$$

The left-hand side and the first term on the right-hand side of Eq. (18) represent the total and the minimal Gibbs energy dissipation, respectively, in a form similar to Eq. (6), except for $\dot{\xi}$ which was replaced by $\mu$. $(\Delta_r G^\circ)_{\text{min}}$ may be calculated as discussed in Section 3.