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Energetic investigation of *Saccharomyces cerevisiae* during transitions. Part 2. Energy balance and thermodynamic efficiency ☆, 1

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Abstract

The technique for evaluating the material balance during transitions was presented in Part 1 of this series. In the present paper we investigate the energy balance in order to determine the consistency of the data. The same statistical test as used for the material balance can be applied to evaluate the error and, potentially, to reject the data consistency. Energy efficiency is calculated using cumulative quantities, and our results show that the efficiency based on the enthalpy and on the free energy remained constant, even for a changing metabolism. The results reported complete the data on themodynamic efficiency at steady state under various growth conditions.

Keywords: Energy balance; Enthalpy; *Saccharomyces cerevisiae*; Thermodynamic efficiency; Transition

List of symbols

A	number of substrates
a	corresponds to the ath substrate
B	number of products

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[1] Part 1 is the preceding paper in this issue.

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b	corresponds to the bth product
c_p	heat capacity in (kJ kg^{-1} K^{-1})
F	flow rate in (l s^{-1})
G	free enthalpy in (kJ mol^{-1})
H	enthalpy in (kJ mol^{-1})
h	test function
i	corresponds to the ith species
j	corresponds to the jth observation
m	mass in kg
n	number of moles in mol
P	heat production rate in kW
Q	heat, as defined in Eq. (3) in kJ
S	species concentration in (mol l^{-1})
s	number of species
T	temperature in K
t	time in s
V	volume in l
$x_{k/i}$	molar fraction of element k in species i
$\Delta_{c,aq}G^{\circ}_S$	standard free enthalpy of combustion of S in (kJ mol^{-1})
$\Delta_{c,aq}H^{\circ}_S$	standard enthalpy of combustion of S modified for the aqueous state in (kJ mol^{-1})
$\Delta_c H^*_X$	modified enthalpy of combustion of biomass, defined in Eq. (9) in (kJ mol^{-1})

Subscripts

aq	aqueous
g	gaseous
in	input
out	output

Greek symbols

$1-\theta$	significant level of χ^2 distribution
χ^2	chi-square probability distribution
ε	error on balance, defined in Eq. (5) in kJ
η	efficiency, defined in Eqs. (6)–(8)
η^*	modified efficiency, defined in Eq. (10)

1. Introduction

Material balances can be verified easily for different fermentation processes [2], whereas energy balances require the measurement of heat dissipated [3,4] and, therefore, can be achieved only using a calorimeter. Application of a χ^2 test is useful

to appreciate the deviation of the enthalpy balance from zero. Indeed, knowledge of the energy balance is important for identifying gross errors of measurement, undetected compounds in the stoichiometry, and erroneous assumptions concerning the thermodynamic properties of the biomass.

Cumulative quantities are used to calculate the thermodynamic efficiency [1,5]. The efficiency is constant for the yeast *Saccharomyces cerevisiae* grown under various conditions but at steady state [4,5]. One aim of this study was to determine whether the efficiency is also constant during transitions.

2. System boundary

The boundary of the system has to be specified very clearly, as do the flows across this boundary. In the case considered the system is open, not at steady state. The example concerns the growth of *S. cerevisiae* in continuous culture during transients resulting from shifts in the dilution rate. Fig. 1 indicates the boundary and the different flows across it.

3. Mass and energy flows for an open non-steady-state system

Enthalpy is the only form of energy for which it may be possible to achieve a balance. However, free enthalpy cannot be balanced because irreversible changes in entropy are not measurable. In addition, no work is performed because neither the pressure nor the volume changes. The various input and output flows and their characteristics are described below.

3.1. Material input

1. Liquid feed flow: the flow is thermostated at the same temperature as the reactor and the pH equals the pH set-point. The medium contains glucose and ammonium ions.

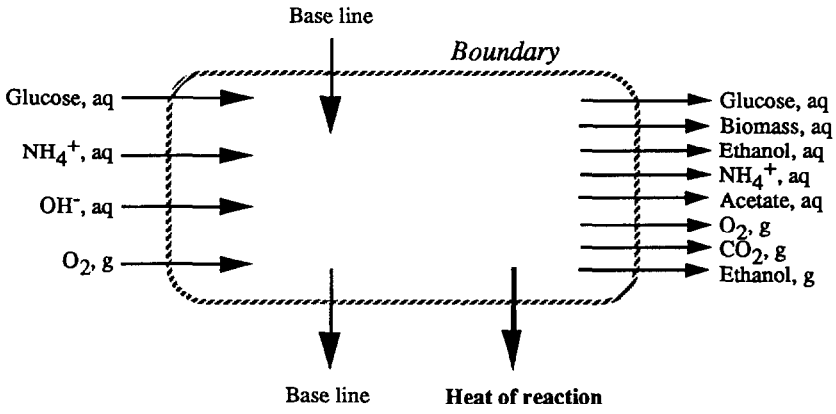

Fig. 1. Material and energy flows across the boundary of the system.

2. pH correction: the flow is intermittent and very small and, therefore, the feed is not thermostated.
3. Gaseous flow: air is preheated and saturated with water in a bubble column before entering the reactor. The temperature of the bubble column is 3°C higher than the temperature of the reactor and does not change.

3.2. Material output

1. Liquid overflow stream: the stream is at the reactor temperature and at the pH set-point. It contains the residual substrates, biomass and the products.
2. Gaseous output flow: the flow is at the reactor temperature and saturated with water at the reactor temperature. It contains nitrogen, O_2, CO_2 and ethanol, and its output rate is very close to the gas input rate. Small differences are due to CO_2 and ethanol production and we consider that the heat generation or consumption due to the thermal capacity of the air and varporization of water is constant during the experiment.

3.3. Energy input

1. Heat provided by the surroundings: heat is provided by stirring, aeration, feed rate and the thermostated head plate, but all these contributions are constant during one experiment.
2. Enthalpy provided by material input flows: it is necessary to define the reference state of enthalpy. For convenience we choose to work with enthalpy of combustion rather than with enthalpy of formation [4]. Thus, terms concerning water, O_2 and CO_2 disappear. The reference state of the substances in solution is aqueous, infinitely diluted at 30°C (Fig. 1). Heat is generated by the enthalpy change resulting from the biological reaction.

3.4. Energy output

We are interested in the rate of heat production during the reaction. Nevertheless, the calorimeter does not measure exactly this variable but rather measures the heat evolution rate. The difference is due to the dynamic of energy transfer through the inner wall of the calorimeter, the dynamics of oil circulation, the dynamic of temperature probes, etc. De Vallière [6] has shown that the time constants of these phenomena are much lower than the time constant of the biological processes. It is therefore not a problem to assimilate the measured heat production rate in the true one.

The calorimetrically measured power output is due to losses to the environment and to the heat of reaction, but only the contribution of the heat of reaction will change during the experiments. The sum of the constant heat exchange due to stirring and losses defines the baseline, and any additional heat release above this baseline is interpreted as the heat of reaction.

The flows involved in our example are shown in Fig. 1, as are the states.

4. Mass and energy balances for an open non-steady-state system

The elements and energy balances are presented below in a general formulation, and the technique for verifying the energy balance constraint on cumulated quantities is then explained.

4.1. Material balance

The mass balance across the boundary for any species i [1] gives

$$\frac{dn_i(t)}{dt} = \frac{d[S_i(t) \cdot V(t)]}{dt} - S_i^{in}(t) \cdot F^{in}(t) + S_i(t) \cdot F^{out}(t) \quad (1)$$

where n_i is the number of moles of i consumed or produced by the reactions, S_i is the concentration of the ith species, F is the flow rate, and V is the volume. n_i decreases if the species is consumed, and increases if the species is produced.

4.2. Energy balance

Von Stockar et al. [4] give a comprehensive discussion of the energy balance in an open non-steady-state system. For s different species implied in the flows, the energy balance on input and output flows can be simplified to

$$m \cdot c_p \cdot \frac{dT}{dt} = P(t) + \sum_{i=1}^{i=s} \frac{dn_i(t)}{dt} \cdot \Delta_{c,aq} H^{\circ}_{S_i} \quad (2)$$

where m is the mass of the liquid, c_p its heat capacity, T is the temperature of the system, and $\Delta_{c,aq} H^{\circ}_{S_i}$ is the standard enthalpy of combustion of i at 30°C and infinitely diluted.

The temperature of the reactor is constant because the calorimeter runs in the isothermal mode. Therefore, the thermal-accumulation term is negligible.

5. Energetic linear constraint

The enthalpy of all the substrates consumed should be equal to the enthalpy of all the compounds produced plus the heat produced by the reaction (dissipation).

Balances cannot be calculated on flows during transitions, but are calculated using the cumulative quantities [1] involved between the beginning of the experiment and the observation at time t_j. In Part 1 [1] we showed how cumulative material quantities can be calculated. Here we focus on the calculation of the cumulative rate of heat production.

Let $P(t)$ represent the power output measured by a calorimeter. Thus, the heat produced between the first observation at time t_0 and the jth observation at t_j is

$$Q(j) = \int_{t_0}^{t_j} P(t) \cdot dt \quad (3)$$

and is approximated, by the method of rectangles, by

$$Q(j) \approx \sum_{t=t_0}^{t=t_j} P(t) \cdot \Delta t \tag{4}$$

where $\Delta t = t_j - t_{j-1} = 30$ s. The heat produced is evaluated recursively, so the energy balance $\varepsilon_{\Delta H}$ (in joules) calculated using the cumulative quantities for each observation j by integrating Eq. (2) with Eqs. (1) and (4) is

$$\varepsilon_{\Delta H}(j) = Q(j) + \sum_{i=1}^{i=s} n_i(j) \cdot \Delta_{c,aq} H^\circ_{S_i} \tag{5}$$

where s is the number of species. By definition, $Q(0) = 0$ and for any species $n_i(0) = 0$, because nothing has occurred before the first observation. This balance (Eq. (5)) should be equal to zero. However, due to the scatter of the measurements this will not be the case. The extent of the error is inspected by comparison with a χ^2 distribution, as described in Part 1 [1].

6. Thermodynamic efficiency

6.1. Definition of the thermodynamic efficiency

Several definitions of the thermodynamic efficiency of growth have been suggested [3,5,7]. Basically, we want to compare the energy actually stored in the biomass to what could have been stored. The reference state is combustion, and the substrates are glucose and ammonium ions.

A substrate is both an element and an energy source. Roels [3] has defined η as the ratio between the energy contained in the biomass and the energy of the substrate minus the energy of the product. This is applicable to enthalpy as well as to free energy and also to flows (at steady state) or to cumulative quantities (during transitions). The formulation proposed by Heijnen and van Dijken [7] is generalized to A substrates and B products.

Enthalpic efficiency

$$\eta_H(j) = -\frac{\Delta_{c,aq} H^\circ_X \cdot n_X(j)}{\sum_{a=1}^{a=A} \Delta_{c,aq} H^\circ_{S_a} \cdot n_{S_a}(j) + \sum_{b=1}^{b=B} \Delta_{c,aq} H^\circ_{P_b} \cdot n_{P_b}(j)} \tag{6}$$

Free-energy efficiency

$$\eta_G(j) = -\frac{\Delta_c G^\circ_X \cdot n_X(j)}{\sum_{a=1}^{a=A} \Delta_{c,aq} G^\circ_{S_a} \cdot n_{S_a}(j) + \sum_{b=1}^{b=B} \Delta_{c,aq} G^\circ_{P_b} \cdot n_{P_b}(j)} \tag{7}$$

where the subscript X refers to biomass, the subscript a to the ath substrate ($a = 1$ to A) and the subscript b to the bth product ($b = 1$ to B). The minus sign arises due to the convention that a consumed cumulated quantity is negative (as substrates

are) and a produced cumulated quantity is positive. For convenience, the efficiency is defined as a positive term, as usual. In addition, the enthalpy efficiency can be expressed as a function of the heat produced by the reaction if the energy balance (Eq. (5)) is satisfied, because in this case

$$Q(j) + \sum_{a=1}^{a=A} \Delta_{c,aq} H^{\circ}_{S_a} \cdot n_{S_a}(j) + \sum_{b=1}^{b=B} \Delta_{c,aq} H^{\circ}_{P_b} \cdot n_{P_b}(j) + \Delta_{c,aq} H^{\circ}_{X} \cdot n_X(j) = 0$$

then

$$\eta_H(j) = \frac{\Delta_{c,aq} H^{\circ}_{X} \cdot n_X(j)}{\Delta_{c,aq} H^{\circ}_{X} \cdot n_X(j) + Q(j)} \tag{8}$$

The advantage of this formulation is that it involves only two measures (biomass and heat), regardless of the number of the products formed. In the present case the consumption of ammonium is determined by the nitrogen content of the biomass. Thus, ammonium is a material source and its uptake cannot change independently of the biomass formation. In contrast, glucose is oxidized or reduced to ethanol as well as serving as a material for constructing biological material. It is basically an energy source. A second efficiency, which does not involve nitrogen, is defined as a modified enthalpy of combustion of biomass [5], describing the enthalpy change of a combustion leading to CO_2, H_2O and the nitrogen source (ammonium ions in the present case). Thus the only source of energy is the carbon source; it is the limiting substrate. So, this modified efficiency is basically related only to carbon metabolism. Glucose is metabolized to biomass, CO_2 and ethanol and the control of these three fluxes determines the efficiency of growth.

The modified definition of the enthalpy of combustion of the biomass is

$$\Delta_c H^{*}_{X} = \Delta_c H^{\circ}_{X} - x_{N/X} \cdot \Delta_c H^{\circ}_{NH^{+}_{4,aq}} \tag{9}$$

and the modified definition of the enthalpic efficiency is

$$\eta^{*}_{H} = -\frac{\Delta_c H^{*}_{X} \cdot n_X}{\sum_{\text{C substrates}} \Delta_c H^{\circ}_{S} \cdot n_S + \sum_{\text{products}} \Delta_c H^{\circ}_{P} \cdot n_P} \tag{10}$$

A schematic interpretation of the definition is shown in Fig. 2.

It is not necessary to measure the heat evolved because the calculation involves only the chemical properties of the species. In addition, the modified enthalpic efficiency can be expressed as a function of the heat produced (conversely to Eq. (8)), but the expression involves the enthalpy contribution of the nitrogen source consumed because, under the assumption that the enthalpy balance is correct, Eq. (10) is equivalent to the expression

$$\eta^{*}_{H}(j) = \frac{\Delta_{c,aq} H^{*}_{X} \cdot n_X(j)}{\Delta_{c,aq} H^{*}_{X} \cdot n_X(j) + x_{N/X} \cdot \Delta_c H^{\circ}_{NH^{+}_{4,aq}} + Q(j)} \tag{11}$$

The modified enthalpic efficiency cannot be calculated from the biomass and heat produced if the nitrogen content of the cells is not known and constant.

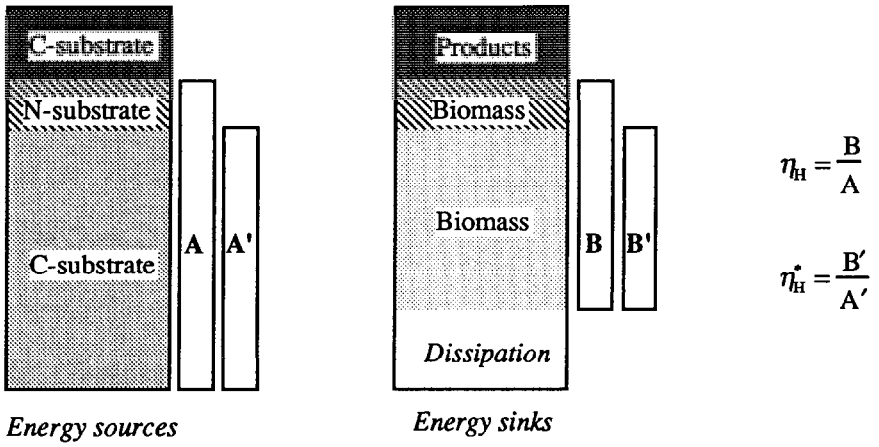

Fig. 2. Modified thermodynamic efficiency. The dark shaded area corresponds to the enthalpy of the carbon source converted to products; the light shaded area corresponds to the enthalpy of the carbon source converted to biomass and dissipated heat; the hatched area corresponds to the enthalpy content of the nitrogen source incorporated in the biomass.

The theoretical difference between the modified (Eq. (10)) and the first (Eq. (6)) definition of efficiency is not significant in the present case (see Appendix), but it was shown in Part 1 [1] that the nitrogen content of biomass changed significantly with the dilution rate. The heat of combustion also changes with dilution rate, whereas the modified heat of combustion is almost constant for different conditions [8]. The modified efficiency overcomes this problem in the calculations by eliminating all the contributions due to nitrogen.

The Gibbs free energy efficiency is almost constant at 60% in *S. cerevisiae* at steady state in aerobic or anaerobic cultures [5]. The free enthalpy of the ammonium ions is zero [7], and so it is not necessary to define a modified Gibbs free enthalpy efficiency.

7. Materials and methods

The methods were described in Part 1 [1]. The principle of the isothermal calorimeter has been described by Auberson-Huang [9].

The head plate is thermostated to avoid vapor condensation and the gas flow is preheated and saturated with water. The signal has been corrected for the baseline. In addition, the outlet gases are heated at 45°C to avoid water and ethanol condensation.

The elemental composition of the biomass was assumed to be constant. The composition [8] is (per C-mol) $CH_{1.65}O_{0.53}N_{0.15}$, and the ash content is 8.34% w/w. Thus, the molecular weight (ash free) is $MW_{ash\text{-}free} = 24.27$ g C-mol^{-1}, and the molecular weight of the measured biomass is $MW_X = 26.48$ g C-mol^{-1}. The enthalpy of combustion measured in a bomb calorimeter is $\Delta_c H^\circ_X = 516.2$ kJ C-mol^{-1}

[8] and $\Delta_c H^\circ_{NH_4^+} = 295.6$ kJ C-mol^{-1} [4]. The modified enthalpy of combustion is, according to Eq. (9)

$$516.2 - 0.15 \times 295.6 = 471.9 \text{ kJ C-mol}^{-1}$$

8. Results and discussion

We first briefly present the experiment [1], and then discuss the enthalpy balance. The continuous culture was grown aerobically at 0.1 h^{-1} in a bench-scale calorimeter and the dilution rate was then shifted to 0.40 h^{-1}, far above the critical dilution rate (around 0.16 h^{-1}) and ethanol and acetic acid productions immediately set in. The dilution rate was then decreased to 0.05 h^{-1} before the new steady state was reached. There was no nitrogen consumption for 2 h. Ethanol was no longer produced and consumption was evident 2 h after the shift down. Acetate accumulated in the broth and was rapidly consumed after ethanol exhaustion. This experiment involves the oxidation of glucose, ethanol and acetate and the reduction of glucose to ethanol.

8.1. Energy balance

The balance includes the following contributions: glucose, ammonium ions, NaOH consumed, biomass produced, ethanol in the liquid phase and in the gaseous phase, glycerol and acetate.

The enthalpies of combustion of the compounds were taken from von Stockar et al. [4], and the enthalpy of combustion of gaseous ethanol was corrected for the heat of vaporization.

Fig. 3 shows the energy balance during the experiment. The enthalpy content of the glucose consumed was chosen as the basis of the balance to enable comparisons

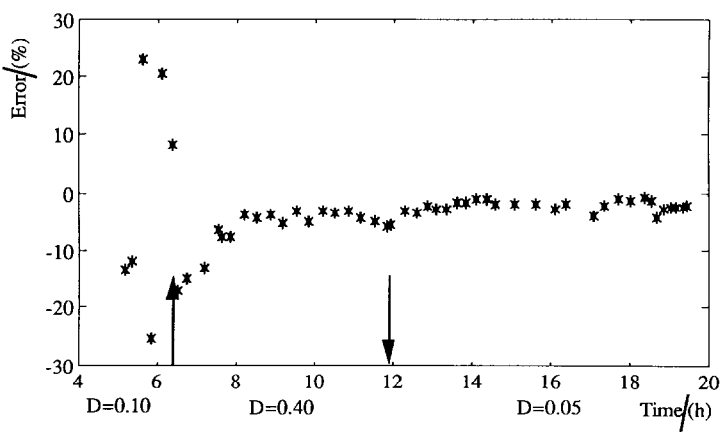

Fig. 3. Energy balance during the transitions as a percentage of glucose consumed (kJ kJ^{-1}). The arrows indicate the shift up from 0.1 to 0.4 h^{-1} and the shift down from 0.4 to 0.05 h^{-1}.

to be made. At the end of the experiment there is an excess as low as 0.7% (kJ/kJ) compared with the glucose consumed.

The contributions of the different species compared with the glucose input during the experiment are (kJ/kJ):

Glucose	100%	Ethanol in the broth	30–60%
Ammonium	3%	Ethanol in vapor	0–2.5%
OH^-	0.7%	Acetate	0–3%
Biomass	30–40%	Heat	10–20%

As expected, the main contributions are from glucose, biomass, ethanol and heat. Nevertheless, the energy input due to ammonium and hydroxide ions represents 3.7% of the glucose energy input. Conversely, the energy output of acetate and evaporated ethanol amount to 5.5% of the energy of glucose. Thus, these contributions are not negligible when calculating a precise energy balance.

A statistical analysis facilitates the interpretation of the errors shown in Fig. 3. The χ^2 test was performed assuming an error of 1% on the heat measurement and the quantity of added base. Errors on the other species involved were assumed to be 3%. These error levels are at the lower end of the range of possible real errors. Nevertheless, the hypothesis was not rejected (Fig. 4), except for some points at the beginning of the fermentation where small errors have a large influence on the result. The test function on energy balance was only 0.041 at the end of the experiment, compared with the cut-off value of 2.71 (χ^2 distribution with one degree of freedom and 10% significance level), so there is no reason to reject the energy balance.

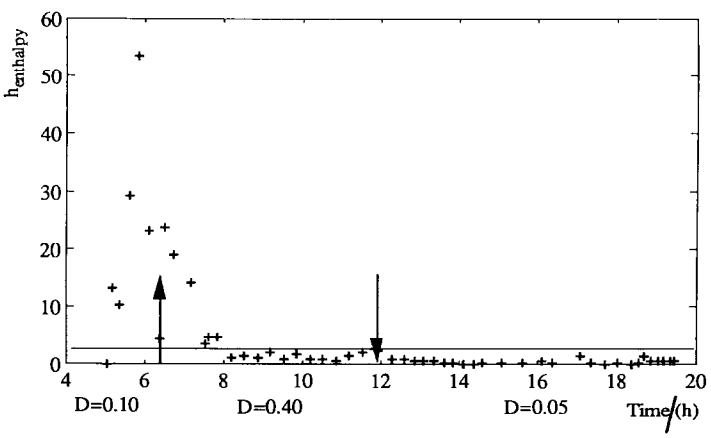

Fig. 4. Test function of the energy balance. The arrows indicate the shift up from 0.1 to 0.4 h^{-1} and the shift down from 0.4 to 0.05 h^{-1}. The horizontal line shows the upper control limit for a $\chi^2(1,\theta = 0.1)$ distribution.

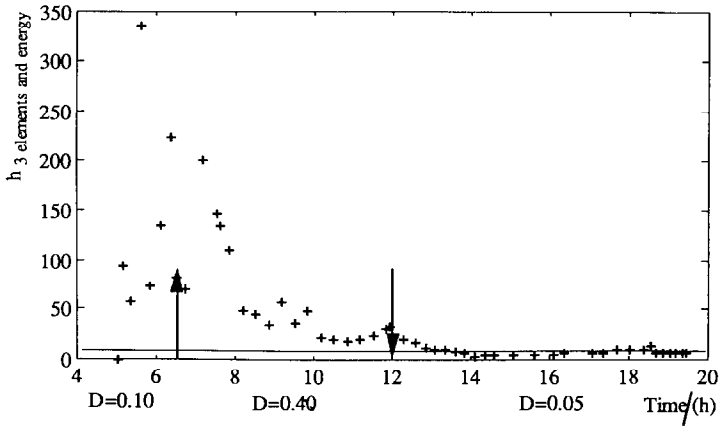

Fig. 5. Test function of elements (carbon, nitrogen and degrees of reduction) and energy balances. The arrows indicate the shift up from 0.1 to 0.4 h^{-1} and the shift down from 0.4 to 0.05 h^{-1}. The horizontal line shows the upper control limit for a $\chi^2(4,\theta = 0.1)$ distribution.

8.2. Combined elements and energy balances

The four balances (on carbon, nitrogen, degrees of reduction and enthalpy) were tested simultaneously. The test function was compared to a χ^2 distribution with four degrees of freedom and this test was investigated versus time to detect erroneous data, because the combined test is very sensitive to small errors (see Part 1 [1]). It is clear from Fig. 5 that the hypothesis is rejected as long as the dilution rate is higher than 0.05 h^{-1}. Indeed, analysis of individual balances indicates that the hypothesis about the nitrogen content of biomass is erroneous. Nevertheless, the errors in the first observations are smoothed by the new measurements, and at the end of the culture the test value is 6.16 compared with the cut-off value of 7.78. Thus, the hypothesis is not rejected after 13 h.

8.3. Enthalpic efficiency

The modified enthalpic efficiency (Fig. 6) was calculated using the modified enthalpy of combustion of biomass and Eq. (11). Investigation of the efficiency versus time shows that there are no significant changes during the experiment for the different metabolisms. The efficiency is randomly distributed between 60% and 65%, except for some points for the first observations, and the scatter on the result is due to measurement noise.

8.4. Gibbs free energy efficiency

The definition of "efficiency" does not involve ammonium: its free energy content is zero [7]. The profile of the efficiency versus time is very stable (Fig. 6); it is randomly distributed around 60% except for the first samples. The difference between enthalpy and free energy efficiencies is due to the entropy contribution.

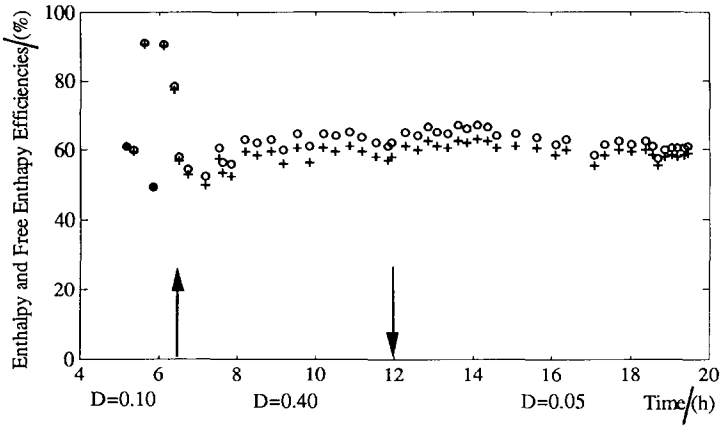

Fig. 6. (+) Gibbs free energy efficiency η_G and (○) modified enthalpic efficiency η_H^* during transients. The arrows indicate the shift up from 0.1 to 0.4 h^{-1} and the shift down from 0.4 to 0.05 h^{-1}.

9. Conclusions

The energy balance calculated during transitions is affected by an erroneous assumption regarding the biomass (nitrogen content). The balance is calculated as a simple balance across the boundary of the system and the contributions of species like ammonium, acetate and hydroxide ions are not negligible if an accurate balance is to be calculated. The soundness of the element and energy balances was inspected by a statistical χ^2 test, and the four simultaneous balances confirmed what independent tests have indicated.

The growth process runs at enthalpy and free-energy efficiencies that are both constant. The yeast controls its metabolism (oxidative and reductive) in a rapidly changing environment to a stable compromise between high efficiency (low dissipation) and high reaction rate (high dissipation). In addition, it is of prime importance to correct the enthalpy of combustion of biomass for its corresponding nitrogen content to have a specific value, regardless of the conditions.

The same comments apply to balances: the nitrogen content of biomass changes with the conditions and this affects its composition and enthalpy of combustion. It is necessary to subtract the corresponding contributions of the nitrogenous compounds to calculate the energy balance. It is not possible to calculate a precise nitrogen balance without further information on the evolution of the nitrogen content of biomass with the changing conditions. The statistical test χ^2 is accurate enough both to detect erroneous measurements and to find errors in the parameters concerning biomass.

The methods presented in Part 1 [1] and the present paper can be easily generalized to other elements (sulfur, phosphorus, etc.) and to more complex systems involving multiple substrates and nitrogenous products. Nevertheless, the only prerequisite to any investigation is an accurate determination of the biomass composition for the different conditions involved and a precise determination of all the major compounds consumed or produced during the experiment.

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Appendix

The relationship between the enthalpy efficiency η_H and the modified enthalpy efficiency η_H^* with glucose (S) and ammonium ions (N) as substrates, and biomass (X) and ethanol (P) as products is

$$\eta_H = -\frac{\Delta_{c,aq}H^\circ_X \cdot n_X}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_N \cdot n_N + \Delta_{c,aq}H^\circ_P \cdot n_P}$$

$$\eta_H = \frac{\Delta_{c,aq}H^\circ_X \cdot n_X}{(\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P) \cdot \left(1 + \dfrac{\Delta_{c,aq}H^\circ_N \cdot n_N}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P}\right)}$$

With a limited development of first order

$$\eta_H = -\frac{\Delta_{c,aq}H^\circ_X \cdot n_X}{(\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P)} \cdot \left(1 - \frac{\Delta_{c,aq}H^\circ_N \cdot n_N}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P}\right)$$

$$\eta_H = -\frac{\Delta_{c,aq}H^\circ_X \cdot n_X + \Delta_{c,aq}H^\circ_N \cdot n_N \cdot \left[\dfrac{\Delta_{c,aq}H^\circ_X \cdot n_X}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P}\right]}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P}$$

Let

$$\alpha = \frac{\Delta_{c,aq}H^\circ_X \cdot n_X}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P}$$

be the ratio of the enthalpic content of the biomass (containing nitrogen) to the enthalpic content of the carbon source and the products. Then

$$\eta_H = -\frac{\Delta_{c,aq}H^\circ_X \cdot n_X + \Delta_{c,aq}H^\circ_N \cdot n_N \cdot \alpha}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P}$$

where η_H^* is defined as

$$\eta_H^* = -\frac{\Delta_{c,aq}H^\circ_X \cdot n_X + \Delta_{c,aq}H^\circ_N \cdot n_N}{\Delta_{c,aq}H^\circ_S \cdot n_S + \Delta_{c,aq}H^\circ_P \cdot n_P}$$

which is Eq. (10). Note that n_N is negative and that $x_{N/X} = -(n_N/n_X)$.

Application: In the case of pure acrobic growth: $n_X/n_S = 0.6$, $n_P = 0$ and $\alpha = 0.66$. Therefore $\eta_H^* = \eta_H \times 0.969$.

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