

Editorial Manager(tm) for World Journal of Microbiology and Biotechnology
Manuscript Draft

Manuscript Number: WIBI2562R1

Title: Culture of *Penicillium ulaiense* in a batch-reactor. Stoichiometric studies.

Article Type: Full-length Paper

Section/Category:

Keywords: *Penicillium ulaiense*; culture; stoichiometry; black box description; energy balance.

Corresponding Author: Mrs. Veronica Beatriz Rajal, PhD

Corresponding Author's Institution: Universidad de California Davis

First Author: Veronica B Rajal, PhD

Order of Authors: Veronica B Rajal, PhD; Carlos M Cuevas, PhD

Manuscript Region of Origin:

Abstract: *Penicillium ulaiense* is a pathogenic, slow growing, fungus that affects citrus fruits post-harvest. This is the first study on the growth of this fungus with different carbon sources. The linear relations between the net conversion rates were used to make the first approach to simulate the system. To accomplish this, *Penicillium ulaiense* was cultured in a bioreactor using three carbon sources: glucose, sucrose, and lactose. The mass balance for C was closed. A black box model was adopted to describe the system, represented by two macrochemical reactions: 1) formation of biomass and 2) combustion of the C-source. The experimental yield coefficient and the elemental and charge balances were used to determine the linear relations between the net conversion rates of the relevant substances involved and the stoichiometric coefficients. The reaction rates were determined as a function of the independent conversion rates. The energy balance was based on the description of three redox half-reactions: production of biomass, utilization of substrate, and electron transfer. The application of the second law of thermodynamics allowed the calculation of the maximal theoretical yield coefficient. The dissipated energy, the theoretical yield coefficient, the maintenance energies and the thermodynamic efficiencies were also calculated.

[Response to the reviewer](#)

Comments for the Author:

ManuscriptWIBI2562

General Comments

The work of Rajal and Cuevas deals with the stoichiometry and energetics of the microbial growth, a very interesting and not much exploited topic. It is a good work and the manuscript has been carefully prepared, and as such no doubt deserves publication. I think a Nomenclature section would be useful. I only have a few specific suggestions detailed below.

[Nomenclature section was included, see p. 3-4](#)

Specific Comments

Summary. "The energy balance was...and electron acceptor". It would be better "electron transfer"

[Modification was done, see p.5](#)

p. 7. Explain the rationale of your medium. Why 30 g sugar/l and only 3 g nitrate/l, it seems disbalanced. Why the glycerophosphate, why the yeast extract, these two compounds are not considered in the stoichiometric equations. 30 g/l of glucose are not equivalent to 30 g/l of sucrose or lactose.

I agree that 30 g/l of glucose are not equivalent to 30 g/l of sucrose or lactose. In fact, 30 g/l of glucose provide 12 g/l of C while 30 g/l of sucrose or lactose provide 12.63 g/l of C (approximately 5% lower for glucose). The corresponding amounts of C were used in each case to close the C balance.

The information about the amount of C provided in each case was added in p. 9

In the stoichiometric equations only the main compounds were included, therefore only the main C and N source were considered. The contributions of C were 12 g from glucose, 12.63 g from sucrose or lactose, 0.46 g from yeast extract (assuming $\text{CH}_{1.78}\text{O}_{0.60}\text{N}_{0.19}$ as the composition for *Saccharomyces cerevisiae*) and 0.09 g from magnesium glycerophosphate ($\text{C}_3\text{H}_7\text{MgO}_6\text{P}$). The yeast extract was taken into account (assuming total consumption) for the final C balance. The glycerophosphate was not considered for the C balance but if it was the final difference between input and output would be decreased 0.7% approximately.

The N balance can also be closed, except for the culture on sucrose, if the contributions of N from yeast extract (0.10 g) and initial biomass from the inoculum (0.1 g, 0.02 g and 0.06 g for cultures with glucose, sucrose, and lactose, respectively) are included. The differences between the initial and final N contents were 6.4%, 19.8%, and 2.2%.

The culture media was selected according to the results of previous experiments in shaken flasks (data not shown) where different variables were studied, being the C and N sources and the addition of yeast extract the main ones. The starting point at that time was the Czapek-Dox medium, recommended for *Penicillium*, that includes 0.5 g/l of magnesium glycerophosphate, 2 g/l of NaNO_3 and 30 g/l of sucrose among other compounds. The addition of yeast extract gave yield coefficients more than three times higher than when it was absent; the three sugars (glucose, sucrose, and lactose) studied produced similar Y_{XS} when used as C source, and nitrate and ammonia nitrate gave the same yield coefficient when added providing equivalent amounts of N. (This complete paragraph was included in p. 9, old p.7)

Results and Discussion. No comments are included on the values the yields obtained. Y_{XS} of 0.20-0.26 seems very low as no extracellular metabolites are considered in the equations.

The low yield can be justified by the high maintenance energy required by *P. ulaiense* (see comments in p. 17 and Tables 2 and 3) due to its slowness in growing (it took many days to reach the stationary phase).

The quality of Figure that I received is below the limit of acceptance, I can hardly read the axis, but it seems you obtained 7 g cells/l. How come, with only 3 g/l nitrate? According to the biomass formula, the yield of nitrate should be around 1.6 that would support no more than 3.2 g/l growth

I apologize for the initial quality of the figure, it is improved now.

According to the biomass formula 2.24 g (0.16×14) are N per 24.2 g of biomass (one mole), so a final concentration of 7 g biomass/l corresponds to 0.65 g/l (0.16×14×7/24.2) of N when it the culture was on glucose. The total amount of N that was provided in this case was 0.69 g/l, discriminated as follows: 0.49 g/l from NaNO₃, 0.1 g/l from initial biomass (inoculum), and 0.1 g/l from the yeast extract.

Did you experimentally checked the biomass composition (you would need an Elemental Analyzer)?.

I agree with the reviewer that the best thing to do would have been to check the biomass composition experimentally. I did not do so since unfortunately I did not have any Elemental Analyzer available.

Running head: *Penicillium ulaiense*. Stoichiometry

Culture of *Penicillium ulaiense* in a batch-reactor.

Stoichiometric studies.

Verónica Beatriz Rajal* and Carlos Mario Cuevas

Instituto de Investigaciones para la Industria Química (INIQUI – CONICET), Universidad

Nacional de Salta

Buenos Aires 177, 4400 Salta, Argentina

Tel and Fax 0054-387-4251006

*E-mail: vrajal@unsa.edu.ar

Culture of *Penicillium ulaiense* in a batch-reactor. Stoichiometric studies.

Summary

Penicillium ulaiense is a pathogenic, slow growing, fungus that affects citrus fruits post-harvest. This is the first study on the growth of this fungus with different carbon sources. The linear relations between the net conversion rates were used to make the first approach to simulate the system. To accomplish this, *Penicillium ulaiense* was cultured in a bioreactor using three carbon sources: glucose, sucrose, and lactose. The mass balance for C was closed. A black box model was adopted to describe the system, represented by two macrochemical reactions: 1) formation of biomass and 2) combustion of the C-source. The experimental yield coefficient and the elemental and charge balances were used to determine the linear relations between the net conversion rates of the relevant substances involved and the stoichiometric coefficients. The reaction rates were determined as a function of the independent conversion rates. The energy balance was based on the description of three redox half-reactions: production of biomass, utilization of substrate, and electron transfer. The application of the second law of thermodynamics allowed the calculation of the maximal theoretical yield coefficient. The dissipated energy, the theoretical yield coefficient, the maintenance energies and the thermodynamic efficiencies were also calculated.

Keywords: *Penicillium ulaiense*, culture, stoichiometry, black box description, energy balance.

Nomenclature

ΔG	Gibbs energy change (kJ mol^{-1})
ΔH	enthalpy change (kJmol^{-1})
ΔS	molar entropy change ($\text{kJ K}^{-1} \text{mol-g}^{-1}$)
a_i	stoichiometric coefficients in the macrochemical reactions
a_i-c_i	elemental composition coefficients
C_l	C-chain length
D_S	Gibbs energy dissipation in microbial growth systems ($\text{kJ l}^{-1} \text{d}$)
m_S	substrate maintenance coefficient ($\text{C-mol C-mol}^{-1} \text{d}^{-1}$ or $\text{g C-mol}^{-1} \text{d}^{-1}$)
m_E	energy maintenance coefficient ($\text{kJ C-mol}^{-1} \text{d}^{-1}$)
r	reaction rate ($\text{C-mol l}^{-1} \text{h}^{-1}$)
S	C-source concentration (g l^{-1})
T	absolute temperature (K)
t	time (d)
X	biomass concentration (g l^{-1})
Y_{XS}	yield coefficient of biomass formed from substrate consumed (g g^{-1} or C-mol C-mol^{-1})

Greek letters

γ	generalized degree of reduction
β_i	stoichiometric coefficients of the i substances in a redox half reaction
ϕ_i	stoichiometric coefficient of the element i in the formation reaction of a compound
η	efficiency
μ	specific growth rate (d^{-1})

Vectors and matrixes

- α** $p \times q$ metabolic stoichiometric matrix
- A** $p \times (q+p)$ metabolic reaction matrix
- I** $p \times p$ identity matrix
- r** $q \times 1$ reaction rate vector
- r_A** $p \times 1$ net conversion rates vector

Subscripts

- 1 macrochemical reaction for the production of biomass
- 2 macrochemical reaction for the combustion of the C-source
- A, a electron acceptor, oxygen in this study
- C, c carbon dioxide
- co combustion reaction
- D electron donor, also C-source substrate in this study
- e electron level in a redox half reaction
- f formation reaction
- h proton
- N, n nitrate
- o oxygen
- R reaction
- S, s C-source substrate
- w water

Introduction

Thermodynamic analysis plays a central role in chemistry, in chemical engineering, and in chemical process development for making judgments about the feasibility of processes occurring under certain conditions. In biotechnology, however, thermodynamic analysis is rarely applied (Stockar *et al.* 2005). The second law of thermodynamics establishes limitations for all chemical reactions. Energy balances are broadly used in chemical processes. However, their use is not so extensive in biological systems due to the large number of biochemical processes involved in growth and product formation (Merchuk & Asenjo 1995). The energy balance can be established by considering the standard free energy of the catabolic reaction, but sometimes can be a poor estimation of the energy available for anabolic reactions since they occur under conditions that are not standard and not all the energy released is used. Recent advances in computer modeling and simulations have created increased interest in the use of thermodynamic analysis to model the growth of microbial populations under various culture conditions. There is special interest in the application of these methods in biotechnology to fungal growth because of their potential applications to bioremediation (Boswell *et al.*, 2003) and to concerns about the role of fungal growth in humid enclosed environments such as “sick building syndrome” (Karunasena *et al.*, 2000).

Many elements and compounds are involved in biochemical processes catalyzed by microorganisms, but often just a few relevant substances are enough to properly describe the system, especially if the process is considered to be in a pseudo-steady state. Although the number of net conversion rates is still high, most of them will be a function of the independent ones. That stoichiometric relationship is determined by the metabolic restrictions and the mass balance equations.

The system can be macroscopically described by a “black box” or a metabolic model. In the “black box” description, the process is considered as a black box that exchanges compounds, substrates and products with the surroundings and dissipates energy. The linear relations between the conversion rates are obtained from the mass conservation principle and from the Gibbs free energy balance. Only the global conversion of the process is considered and the microorganisms are represented by their elemental composition.

In the metabolic description, the relevant compounds are involved in multiple metabolic reactions. Besides the compounds that are exchanged with the surrounding medium, there are others that are not exchanged but are active inside the cells, such as enzymes, metabolic intermediates, ATP, and others. The metabolic approach uses the available biochemical knowledge about the metabolic reactions (Gulik & Heijnen 1995). In this case, the linear relations between the conversion rates arise from the metabolic restrictions and from the enthalpy and Gibbs free energy balances.

Both of the descriptive models allow determination of (a) the non-measured conversion rates from the measured ones, (b) the maximum possible number of independent rates, and (c) the Gibbs dissipation energy (Noorman *et al.* 1991).

In our work herein *Penicillium ulaiense*, a citrus post-harvest pathogenic fungus, was cultured in a batch bioreactor using three different carbon sources: glucose, sucrose and lactose. The experimental yield coefficients were determined and utilized for the stoichiometric studies adopting the “black box” description for the growth of *P. ulaiense*. The aims of this study were: 1) to find the net reaction rates that would allow the simulation of exponential growth phase, and 2) to analyze the energetics involved in the process to evaluate the efficiency of this fungus during growth.

Materials & Methods

Theory and calculations

The “black box” model adopted to describe the growth of *P. ulaiense* does not require information about the intracellular biochemical properties of the microorganism, but rather the compounds that are exchanged with the medium: the C-source, electron donor and acceptor, N-source, and biomass composition (Heijnen & Dijken 1992). In most cases that use this approach, and in this particular work, the carbon source coincides with the electron donor and oxygen is the electron acceptor in the aerobic processes. All of the information about the substances involved in the system is given as inputs and outputs to a “black box” (Figure 1) and/or as several macrochemical equations describing the material balance for the production of 1 C-mol biomass and the combustion of the C source.

Figure 1

Application of mass balance

Penicillium ualiense was cultured using three different carbon sources: glucose, sucrose and lactose. The procedure followed for the stoichiometric studies was similar for all the cases; growth with glucose is explained in detail in the Appendix A. The relevant substances involved in the description of the growth with glucose as limiting reactant are presented in Table 1.

Table 1

Due to the lack of specific information for *P. ulaiense*, the empiric formula for *P. chrysogenum* was used for the biomass (Table 1). It is known that the biomass also contains phosphorous and sulfur, but the amounts are not significant for the purposes of this study; therefore, they were not considered in the balance equations (Nielsen & Villadsen 1994). The ash content was 7.9% (w/w).

The reactions for biomass synthesis and C-source combustion, occurring at rates r_1 and r_2 (C-mol Γ^{-1} h^{-1}), respectively, were based on the production of 1 C-mol of biomass. The stoichiometric coefficients of the relevant compounds in the macrochemical reactions were calculated by the application of the mass balance considering the measurements of two independent conversion rates, r_{Ax} and r_{As} , and their relation through the yield coefficient Y_{XS} (C-mol C-mol $^{-1}$). All of the linear relations between the net conversion rates were determined (see Appendix A) and later used to simulate the consumption of carbon and nitrogen sources and the production of carbon dioxide for the exponential growth phase (Guardia Alba & García Calvo 2000). A non-segregated non-structured first-order kinetic model for the growth was adopted to simulate the production of biomass at that phase.

Application of Gibbs energy balance

In order to estimate the theoretical yield coefficient, the Gibbs energy balance was applied. In principle, the relationship between Y_{XS} and the Gibbs energy dissipation per C-mol biomass (D_S^{01}/r_{Ax}) follows directly from the application of elemental conservation relations and the Gibbs energy balance (Heijnen & Dijken 1992). However, the equations obtained are complex and differ for various microbial systems. An alternative approach based on the description of four redox half-reactions (oxidation of biomass, C-source, and electron donor and acceptor; three half-reactions in these cases since the C-source coincides with the electron

donor) was presented by Heijnen *et al.* (1992) and was applied in this work. These redox half-reactions are mathematically equivalent to the “black box” description, using only the relevant substances of the system. The application of the second law of thermodynamics permitted the calculation of the maximum theoretical yield coefficient, the thermodynamic yield and the thermodynamic efficiency, all helpful to interpret the discrepancies between the experimental and the theoretical yield coefficients through the maintenance energy (see the detailed procedure in Appendix B).

Microorganism and medium

A strain of *Penicillium ulaiense* numbered as 0047, isolated in Salta, Argentina (Rajal *et al.* 2002) from a rotten lemon, was used for this study (deposited as FRR 4978, CSIRO, Australia). The strain was cultured in tubes with Pumpkin Agar and stored freeze-dried until use. A liquid medium was used for the preparation of the inoculum in a flask, and also for the fermentation culture. The composition per liter was: 3 g of NaNO₃, 0.5 g of KCl, 0.5 g of Mg glycerophosphate, 0.01 g of FeSO₄, 0.35 g of K₂SO₄, 1 g of yeast extract, and 30 g of the C source, glucose (12 g C/l), sucrose or lactose (both providing 12.63 g C/l), the pH was adjusted to 7.0.

The culture media was selected according to the results of previous experiments in shaken flasks (data not shown) where different variables were studied, being the C and N sources and the addition of yeast extract the main ones. The starting point at that time was the Czapek-Dox medium, recommended for *Penicillium*, that includes 0.5 g/l of magnesium glycerophosphate, 3 g/l of NaNO₃ and 30 g/l of sucrose among other compounds. The addition of yeast extract gave yield coefficients more than three times higher than when it was absent; the three sugars (glucose, sucrose, and lactose) studied produced similar Y_{XS} when

used as C source, and nitrate and ammonia nitrate gave the same yield coefficient when added providing equivalent amounts of N.

Culture conditions

Spores from *P. ulaiense* were suspended in sterilized water to a concentration of 5×10^5 spores ml^{-1} . Ten milliliters of the suspension (a total of 5×10^6 spores) was inoculated into a 1000-ml Erlenmeyer flask containing 200 ml of the medium. The spores were incubated at 28°C on an orbital shaker at 150 rpm, and after 4 days the resultant culture was used as the inoculum for the reactor. *P. ulaiense* was cultured in a 3 l Biostat B (B. Braun, Frankfurt, Germany) batch reactor containing 1.8 l of the sterile liquid medium and 200 ml of the inoculum previously described. The process was carried out at 28°C and 500 rpm, with 6 l min^{-1} of air, and the pH was controlled at 7.0. Variations of the volume due to evaporation were compensated for by the addition of sterile water. An emulsion of 0.03% antifoam DF7960 (B. Braun) in water was used. The culture was monitored for the presence of contamination by microscopy and also by plating in Petri dishes with Czapek-Dox agar.

Sampling and measurements

Samples were removed at various times and filtered to separate the mycelia from the cell-free extract (CFE). The biomass was determined by dry weight and the CFE was utilized to measure the concentrations of substrate by spectrophotometry at 620 nm after reaction with the anthrone-thiourea reagent (Southgate 1976). The experimental specific growth rate μ (d^{-1}) was determined for the exponential phase. The experimental net conversion rates of biomass

(r_{Ax}) and substrate (r_{As}) were used to calculate the experimental yield coefficient Y_{XS} (Eq. A-4, Appendix A).

The CO₂ production rate was determined by mass spectrometry (Spectramass PC2000). Calibration was performed periodically with gas mixtures of synthetic air with different CO₂ concentrations. The amount of CO₂ produced was calculated as the difference between the outlet and inlet of the bioreactor.

The biomass (X) obtained, the substrate (S) consumed, and the carbon dioxide (C) produced were used to calculate the carbon balance. All measurements were performed in duplicate and the averages were used for calculations.

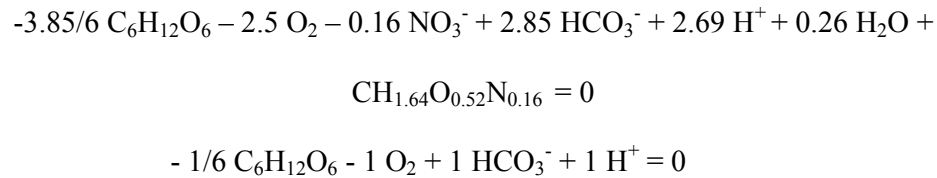
Results & Discussion

The experiments with *P. ulaiense* using three different carbon sources showed characteristics of color and viscosity that can be associated with typical phases of growth. During the lag phase, the medium was light brown; it turned to light yellow during the exponential phase, with an associated increase in viscosity. Regions of lower turbulence in the bioreactor favored the development of immobilized mycelia, which presented some inhomogeneities for sampling. As a consequence, the biomass concentration showed some variability, especially towards the end of the exponential growth phase and beginning of the stationary phase (highest concentrations); also, the value obtained from the last sample differed from the biomass determined from the volume left in the tank at the end of the process. This final value was used to perform the mass balance.

Growth of *P. ulaiense* in glucose

At the end of the process the C balance gave a final C content that was 7.5% higher than the initial amount. The difference can be attributed to the yeast extract, which was not counted as a C source at the beginning (in the medium), but was part of the mycelia at the end. If we assume that the composition of yeast extract is defined by the empirical formula of *Saccharomyces cerevisiae*, $\text{CH}_{1.78}\text{O}_{0.60}\text{N}_{0.19}$ (Nielsen & Villadsen 1994), the difference between the initial and final C content was decreased to 0.16%.

The experimental yield coefficient Y_{XS} was 0.20 g g^{-1} or $0.26 \text{ C-mol C-mol}^{-1}$. The stoichiometric coefficients for the production of biomass and for the combustion of glucose were determined. Thus, the reactions can be written as:



The linear relations between the net conversion rates (see Appendix A) are:

$$\begin{aligned}
 r_{An} &= -0.16 r_{Ax} & r_{Ac} &= -r_{Ax} - r_{As} & r_{Ah} &= -1.16 r_{Ax} - r_{As} \\
 r_{Aw} &= 0.26 r_{Ax} & r_{Ao} &= 1.35 r_{Ax} + r_{As}
 \end{aligned}$$

The reaction rates r_1 and r_2 are also a linear function of the independent net conversion rates:

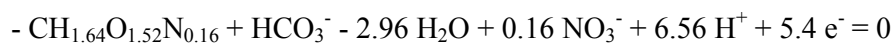
$$\begin{aligned}
 r_1 &= r_{Ax} & r_2 &= -3.85 r_{Ax} - r_{As}
 \end{aligned}$$

In most approaches to simulation, the reaction rates cannot be easily measured or modeled. The advantage of this procedure is that the reactions adopted for a process of growth in glucose can be quantified from the measured net conversion rates. The simulation of the system at the exponential growth phase was successful as shown in Figure 2, presented here as an example.

Figure 2

Prior to the application of the Gibbs energy and enthalpy balance to this system, the redox half-reactions were solved for the corresponding stoichiometry, and the generalized degrees of reduction were calculated (Appendix B):

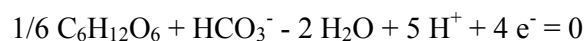
Reaction 1 Biomass:



$$\Delta G_{Rx}^{01} = -96.68 \text{ kJ C-mol}^{-1}$$

$$\Delta H_{Rx}^0 = 217.88 \text{ kJ C-mol}^{-1}$$

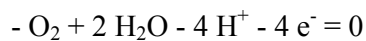
Reaction 2: electron donor/substrate



$$\Delta G_{Rs}^{01} = -159 \text{ kJ C-mol}^{-1}$$

$$\Delta H_{Rs}^0 = 90.67 \text{ kJ C-mol}^{-1}$$

Reaction 3: electron acceptor/oxygen



$$\Delta G_{Ra}^{01} = -316 \text{ kJ mol}^{-1}$$

$$\Delta H_{Ra}^0 = -572 \text{ kJ mol}^{-1}$$

From Eq. (B-2a) and (B-4a) $\Delta G_{eX}^{01} = 17.90 \text{ kJ e-mol}^{-1}$, $\Delta G_{eA}^{01} = -79 \text{ kJ e-mol}^{-1}$, and $\Delta G_{eS}^{01} = 39.75 \text{ kJ e-mol}^{-1}$.

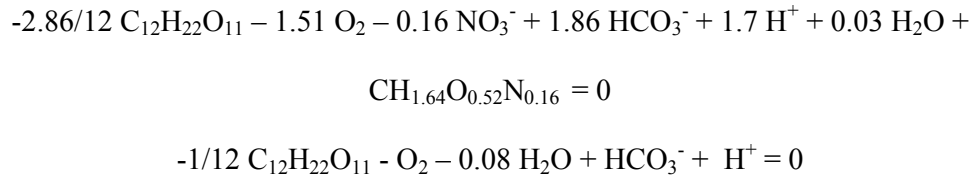
Growth of *P. ulaiense* in sucrose

The C balance showed a final content 10.8% higher than the initial amount, but when the C supplied from the yeast extract was considered the difference in the balance was reduced to 3.3%. The analysis was similar to that for glucose.

Experimentally $Y_{XS} = 0.30 \text{ g g}^{-1}$ or $Y_{XS} = 0.35 \text{ C-mol C-mol}^{-1}$ and the linear relations between the net conversion rates, used to simulate the system during the exponential growth phase (data not shown), were:

$$\begin{aligned} r_{An} &= -0.16 r_{Ax} & r_{Ac} &= -r_{Ax} - r_{As} & r_{Ah} &= -1.16 r_{Ax} - r_{As} \\ r_{Aw} &= 0.26 r_{Ax} + 0.08 r_{As} & r_{Ao} &= 1.35 r_{Ax} + r_{As} \end{aligned}$$

From the application of the elemental and charge balances the stoichiometric coefficients were calculated and the reaction equations for biomass synthesis and sucrose combustion are:



occurring at r_1 and r_2 , respectively, that linearly depend on the net conversion rates:

$$r_1 = r_{Ax} \quad r_2 = -2.86 r_{Ax} - r_{As}$$

The parameter D_S^{01} / r_{Ax} permits the estimation of the thermodynamic yield of the system. The correlation (B-3) proposed by Heijnen and Dijken (1992) for the calculation of the dissipated energy is valid for different C-chain lengths (C_l): C_1 to C_6 . It was not possible to apply it for sucrose since $C_l = 12$ and $\gamma_S = 4$ and no alternative method was available; therefore, a further analysis was performed. That correlation shows a minimum value for γ_S close to 4, and

different length of C-chain, and that the minimum decreases monotonically with the increment of C_l . The calculated values of D_S^{01} / r_{Ax} from Eq. (B-3) for $\gamma_S = 4$ and C_l between 1 and 6, were fitted to the potential curve: $D_S^{01} / r_{Ax} = 565.84 C_l - 0.4864$ ($R^2=0.98$), and by extrapolation for $C_l = 12$, it was determined that $D_S^{01} / r_{Ax} = 169 \text{ kJ C-mol}^{-1}$.

Following the method proposed by Heijnen *et al.* (1992), the system of redox half-reactions was formulated. The values ΔG_f^{01} and ΔH_f^0 for sucrose, not found in the literature, were calculated. The change in formation enthalpy, $\Delta H_f^0 = -2227.88 \text{ kJ C-mol}^{-1}$, was determined from the combustion enthalpy $\Delta H_{co}^0 = -1348 \text{ Kcal mol-g}^{-1}$ (Hougen *et al.* 1982). The Gibbs energy $\Delta G_f^{01} = -1002.81 \text{ kJ mol-g}^{-1}$ was obtained from

$$\Delta G_f^{01} = \Delta H_f^0 - T \Delta S_f^0 \quad (1)$$

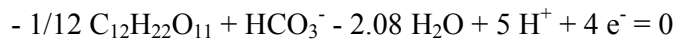
where T is the absolute temperature of the system in K (301 K in this case) and ΔS_f^0 ($-4.07 \text{ kJ K}^{-1} \text{ mol-g}^{-1}$) is the change of molar entropy for sucrose formation at standard conditions, according to

$$\Delta S_f^0 = \sum_{i=1}^q \phi_i S_i^0 \quad (2)$$

where ϕ_i is the stoichiometric coefficient of the substance i in the sucrose formation reaction from its elements, and S_i^0 the molar entropy for the substance i at standard conditions (Putnam & Boerio-Goates 1993, Hougen *et al.* 1978).

For the growth of *P. ulaiense* in sucrose the redox half-reactions corresponding to biomass and electron acceptor/oxygen were similar to those obtained for the growth in glucose, while

Reaction 2 electron donor/substrate



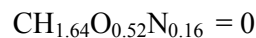
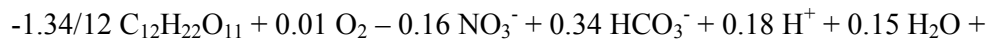
$$\Delta G_{Rs}^{01} = -209.39 \text{ kJ C-mol}^{-1}$$

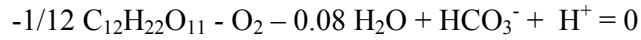
$$\Delta H_{Rs}^0 = 88.54 \text{ kJ C-mol}^{-1}$$

From Eq. (B-2a) and (B-4a), $\Delta G_{eX}^{01} = 17.90 \text{ kJ e-mol}^{-1}$, $\Delta G_{eS}^{01} = 52.35 \text{ kJ e-mol}^{-1}$, and $\Delta G_{eA}^{01} = -79 \text{ kJ e-mol}^{-1}$.

Growth of *P. ulaiense* in lactose

The consumption of carbon and nitrogen substrates during growth on lactose was different than for the other two sugars tested, and especially slow during the exponential phase. Although the concentration of carbon source at the end of the exponential phase was still high, growth did not increase. There was probably some inhibition in the production of biomass that prevented the complete utilization of the available lactose; i.e., some other nutrient in the medium became growth limiting. The stationary phase was also shorter when compared to the other sugars. The C balance showed a final content 13.1% higher than the initial amount, but when the C supplied from the yeast extract was considered the difference was reduced to 5.3%. The black box description for lactose is similar to that for sucrose by substitution of the lactose for the sucrose (with the same molecular formula $C_{12}H_{22}O_{11}$) as the electron donor/substrate. The generic chemical reactions that represent the system and the equations for the conservation of elements and charge are also the same. The experimental yield coefficient was $Y_{XS} = 0.63 \text{ g g}^{-1}$ or $Y_{XS} = 0.75 \text{ C-mol C-mol}^{-1}$. The reactions for the production of biomass and combustion of lactose and the respective reaction rates r_1 y r_2 were:





$$r_1 = r_{Ax}$$

$$r_2 = -1.34 r_{Ax} - r_{As}$$

The calculation of the parameter D_S^{01}/r_{Ax} for lactose presented the same problems as for sucrose; as $\gamma_S = 4$ and $C_l = 12$ the dissipated energy was the same $D_S^{01}/r_{Ax} = 169 \text{ kJ C-mol}^{-1}$.

The formation enthalpy $\Delta H_f^0 = -2219.5 \text{ kJ C-mol}^{-1}$ was calculated from the combustion enthalpy $\Delta H_{co}^0 = -1350 \text{ Kcal mol-g}^{-1}$ (Hougen *et al.* 1982). The Gibbs energy $\Delta G_f^{01} = -991.42 \text{ kJ mol-g}^{-1}$, was obtained from Eq. (1), for $T = 301 \text{ K}$ and $\Delta S_f^0 = -4.08 \text{ kJ K}^{-1} \text{ mol-g}^{-1}$, calculated by Eq. (2) (Hougen *et al.* 1978).

For the growth of *P. ulaiense* in lactose the redox half-reactions corresponding to biomass, electron donor/substrate, and electron acceptor/oxygen were similar to those obtained for sucrose. The values of ΔG_{Rs}^{01} , ΔH_{Rs}^{01} , ΔG_{eX}^{01} , ΔG_{eS}^{01} , and ΔG_{eA}^{01} were also the same.

The dissipated energy, the theoretical and the maximum yield coefficient, the experimental and theoretical efficiency, and the substrate and energy maintenance coefficients were calculated for the three cases (Table 2).

Table 2

Of the three carbon sources studied, sucrose proved to be the best for the growth of *P. ulaiense* since the process was faster (higher μ) and also gave higher concentrations of biomass at the end of the exponential phase. The C-balances were successfully verified when the carbon supplied by the yeast extract in the medium was taken into account. This fact confirmed that growth occurred without the production of significant amounts of other substances than those listed (Table 1) to describe the black box model.

The dissipated energy for the growth in sucrose and lactose was the same (Table 2) since the reduction degree γ_s , and the chain length C_l were the same. The corresponding value for glucose was higher due to the lower C_l . These results are consistent with the observations from Heijnen & Dijken (1992) when they analyzed data reported by other authors for the culture of different microorganisms. The theoretical yield coefficients were the same for sucrose and lactose, since they depend on the dissipated energy, and were higher than for glucose. The experimental yield coefficients were lower than the theoretical ones for the culture in glucose and sucrose, differences that can be explained by the maintenance energy, much higher for glucose and sucrose than for lactose. The maintenance coefficients for substrate and energy from this study can be compared to those compiled by Tjihuis *et al.* (1993) for different fungi growing in glucose (Table 3).

Table 3

In Tjihuis *et al.*'s (1993) work the maintenance coefficient m_S was quantified based on the comparison between the theoretical and the experimental yield coefficients for a discontinuous system. This procedure would be applicable to organisms with slow growth, like *Penicillium ulaiense*, such that a quasi-steady state can be assumed.

The high experimental yield coefficient for lactose in our study led to unusually high thermodynamic efficiencies and very low maintenance coefficients, differentiating *P. ulaiense* from other microorganisms.

The simulations for the exponential phase of growth using the linear relations between the net conversion rates were highly satisfactory for glucose and sucrose; however, some specific observations should be noted. For the concentration of nitrate, especially for the growth in glucose, the predicted values were zero at the end of the exponential phase while the actual

experimental values observed were very low. For the concentration of CO₂, the predicted values at the end of the exponential phase were higher than the experimental ones. The opposite situation was observed in the case of lactose, and the simulation failed since the concentrations predicted were much lower than the experimental ones. This phenomenon was due to the particularly high yield coefficient of substrate on biomass.

Summarizing, this is the first study on the growth of *Penicillium ulaiense* with different carbon sources. Since it is a slow-growing fungus, the process was treated as in a quasi-steady state and the mass and energy balances were applied to solve the stoichiometry and to find all the reaction rates involved as a function of the two independent net conversion rates. The linear relations between the net conversion rates were used to make the first approach to simulate the system at the exponential growth phase, which was successful for the cultures with glucose and sucrose.

References

- Boswell GP, Jacobs H, Gadd GM, Ritz K., FA Davidson (2003) A mathematical approach to studying fungal mycelia. *Mycologist* 17(4):165-171.
- Brown DE, Zaiduneen MA (1977) Growth kinetics and cellulose biosynthesis in the continuous culture of *Trichoderma viride*. *Biotechnol Bioeng* 19:941-958.
- Guardia Alba MJ, García Calvo E (2000) Characterization of bioreaction processes. Aerobic *Escherichia coli* cultures. *J Biotechnol*, 84:107-118.
- Gulik WM, Heijnen JJ (1995) A metabolic network stoichiometry analysis of microbial growth and product formation. *Biotechnol Bioeng* 48:681-698.
- Heijnen JJ, Dijken JP (1992) In search of a thermodynamic description of biomass yields for the chemotrophic growth of microorganisms. *Biotechnol Bioeng* 39:833-858.

Heijnen JJ, Loosdrecht MCM, Tijhuis L (1992) A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs Energy Dissipation. *Biotechnol Bioeng* 40:1139-1154.

Hougen OA, Watson KM, Ragatz RA (1978) *Principios de los procesos químicos. Parte II: Termodinámica*. Reverté, Barcelona.

Hougen OA, Watson KM, Ragatz RA (1982) *Principios de los procesos químicos. Parte I: Balances de materia y energía*. Reverté, Barcelona.

Karunasena E, Markham N, Brase T, Cooley JD, Straus DC (2000) Evaluation of fungal growth on cellulose-containing and inorganic ceiling tile. *Mycopathologia* 150:91–95.

Mason HR, Righelato RC (1976) Energetics of fungal growth: The effect of growth limiting substrate on respiration of *Penicillium chrysogenum*. *J Appl Chem Biotechnol* 26:145-152.

Merchuk JC, Asenjo JA (1995) Design of a bioreactor system. Overview. In: Asenjo JA, Merchuk JC (eds) *Bioreactor System Design*. Marcel Dekker Inc., New York.

Metwally M, El Sayed M, Osman M, Hanegraaf PPF, Stouthamer AH, Van Verseveld HW (1991) Bioenergetic consequences of glucoamylase production in carbon limited chemostat cultures of *Aspergillus niger*. *Ant V Leeuwenh* 59:35-43.

Nielsen J, Villadsen J (1994) *Bioreaction Engineering Principles*. Plenum Press, New York.

Noorman HJ, Heijnen JJ, Luyben Kch AM (1991) Linear relations in microbial reaction systems: a general overview of their origin, form and use. *Biotechnol Bioeng* 38:603-618.

Pirt SJ (1965) The maintenance energy of bacteria in growing cultures. *Proceeding of the Royal Society, B* 163, 224-231.

Putnam RL, Boerio-Goates J (1993) Heat capacity measurements and thermodynamic functions of crystalline sucrose at temperatures from 5 to 342K. Revised values for

ΔG_f^0 (sucrose, cr. 298.15 K), ΔG_f^0 (sucrose, aq, 298.15 K), S^0 (sucrose, aq, 298.15 K); and ΔG_r^0 (298.15 K) for the hydrolysis of aqueous sucrose. J Chem Thermodynam 25(5):607-613.

Rajal VB, Carrillo L, Cuevas CM (2002) Studies on exocellular hydrolases from a new *Penicillium ulaiense* strain. World J Microb Biot 18:713-714.

Rhigelato RC, Trinci APJ, Pirt SJ, Peat A (1968) The influence of maintenance energy and growth rate on the metabolic activity, morphology and conidiation of *Penicillium chrysogenum*. J Gen Microbiol 50:399-412.

Roels JA (1980) Application of macroscopic principles to microbial metabolism. Biotechnol Bioeng 22:2457-2514.

Southgate DAT (1976) Determination of food carbohydrates. In Applied Science Publishers 108. Applied Science Publishers Ltd., London.

Stockar U, Maskow T, Liu J, Marison IW, Patiño R (2005) Thermodynamics of microbial growth and metabolism: An analysis of the current situation. J Biotechnol 121:517-533.

Tijhuis L, Loosdrecht MCM, Heijnen JJ (1993) A thermodynamically based correlation for maintenance Gibbs energy requirements in aerobic and anaerobic chemotrophic growth. Biotechnol Bioeng 42:509-519.

Ying F (1990) Some linear characters in chemical reaction systems. Ind Eng Chem 29:34-39.

Figure legends

Figure 1. Growth of *P. ulaiense* according to the “black box” description.

Figure 2. Growth of *P. ulaiense* in a fermentor with glucose at 28°C and pH 7.0. Experimental values (symbols, subscript *exp*), and simulated results from the linear relations between the net conversion rates (lines, subscript *str*) are presented.

Table 1. Definition of the system for the growth of *P. ulaiense* in glucose.

Substance	Composition	Symbol	Net conversion rate (C-mol l⁻¹ h⁻¹)
Biomass	CH _{1.64} O _{0.52} N _{0.16}	<i>x</i>	<i>r_{Ax}</i>
Glucose	C ₆ H ₁₂ O ₆	<i>s</i>	<i>r_{As}</i>
Proton	H ⁺	<i>h</i>	<i>r_{Ah}</i>
Oxygen	O ₂	<i>o</i>	<i>r_{Ao}</i>
Bicarbonate	HCO ₃ ⁻	<i>c</i>	<i>r_{Ac}</i>
Water	H ₂ O	<i>w</i>	<i>r_{Aw}</i>
Nitrate	NO ₃ ⁻	<i>n</i>	<i>r_{An}</i>

Table 2. Parameters corresponding to the exponential growth phase of *P. ulaiense* in batch bioreactor, with different carbon sources.

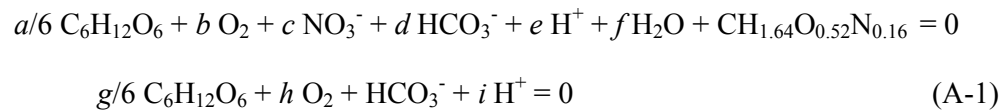
Parameters		Units	C-source/electron donor		
			Glucose	Sucrose	Lactose
μ	Experimental	d ⁻¹	0.3723	0.7391	0.3428
D_S^{01} / r_{Ax}	Growth		236	169	169
	Maintenance	kJ C-mol ⁻¹	1078.7	809.5	9.3
	Total		1314.7	978.5	178.3
Y_{XS}	Experimental		0.26	0.35	0.75
	Theoretical	C-mol C-mol ⁻¹	0.63	0.76	0.76
	Maximum		0.91	1.01	1.01
η^h	Experimental		0.29	0.35	0.74
	Theoretical	Dimensionless	0.69	0.75	0.75
m	For substrate (stationary phase)	C-mol C-mol ⁻¹ d ⁻¹	0.162	0.197	0.840
	For substrate		0.035	0.048	0.00025
	For energy	kJ C-mol ⁻¹ d ⁻¹	16.63	25	0.13

Table 3. Maintenance coefficients for substrate m_S and Gibbs energy m_E for the aerobic growth of fungi in glucose.

Fungi	T (°C)	m_S (C-mol C-mol ⁻¹ d ⁻¹)	m_E (kJ C-mol ⁻¹ d ⁻¹)	Reference
<i>P. ulaiense</i>	28	0.035	16.6	This study
<i>Penicillium chrysogenum</i>	25	0.019	9.5	(Mason & Righelato 1976)
	25	0.017	8.3	(Righelato <i>et al.</i> 1968)
<i>Aspergillus niger</i>	30	0.030	14.2	(Metwally <i>et al.</i> 1991)
<i>Trichoderma viride</i>	30	0.011	6.2	(Brown & Zaiduneen 1977)

Appendix A

Application of mass balance: The reactions for biomass synthesis from glucose and for glucose combustion, occurring at rates r_1 and r_2 , respectively, based on the production of 1 C-mol of biomass were:



where $a, b, c, d, e, f, g, h,$ and i are the stoichiometric coefficients of the relevant compounds in the macrochemical reactions. For the production of biomass they represent the conversion rates for each compound referred to the biomass formation rate:

$$\begin{aligned} a &= r_{As}/r_{Ax} & b &= r_{Ao}/r_{Ax} & c &= r_{An}/r_{Ax} \\ d &= r_{Ac}/r_{Ax} & e &= r_{Ah}/r_{Ax} & f &= r_{Aw}/r_{Ax} & i &= r_{Ax}/r_{Ax} \end{aligned} \quad (\text{A-2})$$

The stoichiometric coefficients can be determined by solving the equations for element and charge conservation in reactions (A-1):

$$\begin{aligned} \text{For C:} & \quad a + d + 1 = 0 \\ \text{For H:} & \quad 2 a + d + e + 2 f + 1.64 = 0 \\ \text{For O:} & \quad a + 2 b + 3 c + 3 d + f + 0.52 = 0 \\ \text{For N:} & \quad c + 0.16 = 0 \\ \text{For charge:} & \quad -c - d + e = 0 \end{aligned} \quad (\text{A-3})$$

Seven conversion rates were the unknowns of the system and were restricted through five conservation equations (A-3) so the system had two degrees of freedom. By the measurement of only two independent conversion rates, i.e. r_{Ax} and r_{As} , the system could be completely solved. These net conversion rates are also related by the yield coefficient Y_{XS} (C-mol C-mol⁻¹), which is the yield of biomass formed per unit mass of substrate consumed, as follows:

$$a = r_{As} / r_{Ax} = - 1 / Y_{XS} \quad (\text{A-4})$$

By combining equations (A-2), (A-3), and (A-4) the linear relations between the net conversion rates can be determined as:

$$\begin{aligned} r_{An} &= - 0.16 r_{Ax} & r_{Ac} &= - r_{Ax} - r_{As} & r_{Ah} &= - 1.16 r_{Ax} - r_{As} \\ r_{Aw} &= 0.26 r_{Ax} & r_{Ao} &= 1.35 r_{Ax} + r_{As} \end{aligned} \quad (\text{A-5})$$

A similar procedure was followed for the combustion of glucose at eq. (A-1).

The application of matrix algebra for this type of problem is very convenient (Roels 1980, Ying 1990, Noorman *et al.* 1991). The scheme that describes the global process of growth involves two reactions that must be linearly independent. Independence can be tested by determining the rank[α], with α ($p \times q$) the metabolic stoichiometric matrix, where $p=7$ is the number of relevant substances involved in this process, and $q=2$, the number of chemical reactions that are linearly independent:

$$\alpha = \begin{matrix} & r_1 & r_2 \\ \left[\begin{array}{ccc} x & 1 & 0 \\ s & a/6 & g/6 \\ c & d & 1 \\ o & b & h \\ n & c & 0 \\ h & e & i \\ w & f & 0 \end{array} \right] \end{matrix} \quad (\text{A-6})$$

As the $\text{rank}[\alpha] = q$, then the chemical reactions proposed to describe the system are linearly independent, and the reaction rate vector \mathbf{r} ($q \times 1$) can be determined as a linear function of the net conversion rates vector \mathbf{r}_A ($p \times 1$):

$$\alpha \cdot \mathbf{r} = \mathbf{r}_A \quad (\text{A-7})$$

or, the equivalent (Noorman *et al.* 1991):

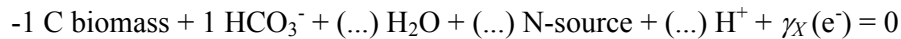
$$\mathbf{A} \cdot \mathbf{r}_r = \mathbf{0} \quad (\text{A-8})$$

where \mathbf{A} [$p \times (q+p)$] is the metabolic reaction matrix, $\mathbf{A} = [\alpha \mid -\mathbf{I}]$, with \mathbf{I} ($p \times p$) the identity matrix, and the $[(q+p) \times 1]$ vector $\mathbf{r}_r = [\mathbf{r} \mid \mathbf{r}_A]$.

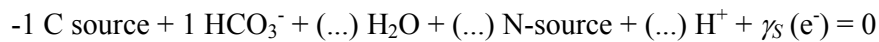
Appendix B

Redox half-reactions approach for the energy balance: This approach based on the description of four redox half-reactions (oxidation of biomass, C source, electron donor and electron acceptor), is mathematically equivalent to the “black box” description using only the relevant substances of the system (Heijnen *et al.* 1992):

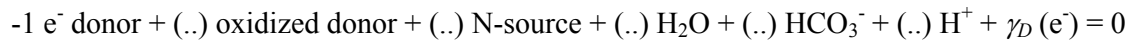
Reaction 1: Biomass



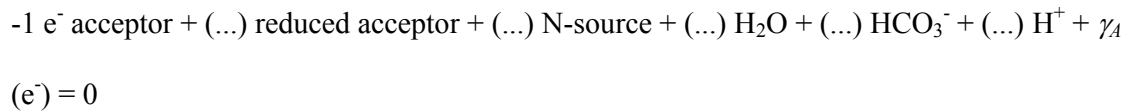
Reaction 2: C-source/substrate



Reaction 3: Electron donor



Reaction 4: Electron acceptor



These four half-reactions should contain all listed relevant compounds of the growth system. Products have positive and substrates have negative stoichiometric coefficients. In each reaction a number of electrons is produced. This number is called the generalized degree of reduction for biomass (γ_X), C source (γ_S), electron donor (γ_D), and electron acceptor (γ_A), and can be found in several tables for different C sources, biomass, and electron donors (Heijnen *et al.* 1992). It can also be calculated according to:

$$\gamma_i = 4 + a_i - 2 b_i - 3 c_i \quad (\text{B-1})$$

for a compound of elemental composition $\text{CH}_{a_i}\text{O}_{b_i}\text{N}_{c_i}$. In the present work, as the electron donor coincides with the substrate, only three half-reactions are necessary to describe the system.

Application of Gibbs energy balance: For each half-reaction the Gibbs energy $\Delta G_{R_i}^{01}$ (kJ mol⁻¹) and the enthalpy $\Delta H_{R_i}^{01}$ (kJ mol⁻¹) at biochemical standard conditions (298K, pH 7.0, and with ions, solutes and gases slightly soluble in aqueous solutions, indicated by superscript 01) can be calculated from the corresponding formation values $\Delta G_{f_i}^{01}$ and $\Delta H_{f_i}^{01}$ in kJ mol⁻¹:

$$\Delta G_R^{01} = \sum_i \beta_i \Delta G_{f_i}^{01} = \frac{-D_S^{01}}{r_{Ax}} \qquad \Delta H_R^{01} = \sum_i \beta_i \Delta H_{f_i}^{01} \qquad (\text{B-2 a \& b})$$

where β_i are the stoichiometric coefficients for the i substances participating in that redox half-reaction and D_S^{01} (kJ l⁻¹ d) is the dissipated energy in the formation of 1 C-mol of biomass.

Heijnen and Dijken (1992) analyzed several cases reported by different authors for cultures with synthetic medium in both continuous and discontinuous systems, without the formation of product, and involving a variety of conditions in terms of microorganisms, chemotropic and autotrophic growth, C sources, length of C chain (C₁ to C₆) and electron donor and acceptor for a certain microorganism. They proposed a correlation valid for different electron donors when there is no reverse electron transport:

$$\frac{D_S^{01}}{r_{Ax}} = 200 + 18 (6 - C_i)^{1.8} + \text{Exp} \left[\left\{ (3.8 - \gamma_s)^2 \right\}^{0.16} (3.6 + 0.4 C_i) \right] \qquad (\text{B-3})$$

where C_l is the number of C-atoms (chain length) and γ_S the reduction degree of the carbon source. The relations between Gibbs energy and enthalpy with the generalized degree of reduction are, according to Heijnen *et al.* (1992):

$$\Delta G_{ei}^{01} = -\frac{\Delta G_{Ri}^{01}}{\gamma_i} \quad \Delta H_{ei}^{01} = -\frac{\Delta H_{Ri}^{01}}{\gamma_i} \quad (\text{B-4 a \& b})$$

where $-\Delta G_{Ri}^{01}$ is the liberated Gibbs energy in an electron donating redox half-reaction relative to the hydrogen reference (where the Gibbs energy of the electron is zero by definition), ΔG_{ei}^{01} represents the measure of the Gibbs energy level of an electron in a redox half-reaction, relative to the H₂ reference. The same holds for ΔH_{ei}^{01} , which provides the enthalpy level of the electrons in the redox half-reaction. The subscript i corresponds to X (biomass), S (substrate and electron donor: glucose, sucrose and lactose in this work), and A (electron acceptor, oxygen in this case).

Assuming that the i half-reactions occur at the rate r_i , the energy dissipated for growth in biochemical standard conditions D_S^{01} can be calculated as:

$$D_S^{01} = \sum_{i=1}^p r_i (-\Delta G_{Ri}^{01}) = r_1 (\gamma_X \Delta G_{eX}^{01}) + r_2 (\gamma_S \Delta G_{eS}^{01}) + r_3 (\gamma_A \Delta G_{eA}^{01}) \quad (\text{B-5})$$

The rates of reaction r_i are related to the net conversion rates r_{Ai} (C-mol l⁻¹ h⁻¹) from the elemental balances based on the stoichiometry:

$$r_1 = -r_{AX} \quad r_2 = -r_{AS} \quad r_3 = -r_{AA} \quad (\text{B-6})$$

The electrons generated in the three redox half-reactions should also be conserved:

$$\gamma_X r_{AX} + \gamma_S r_{AS} + \gamma_A r_{AA} = 0 \quad (\text{B-7})$$

Combining Eqs. (B-4a)-(B-7), the theoretical relation between Y_{XS}^{theo} and D_S^{01} / r_{AX} is obtained:

$$Y_{XS}^{theo} = \frac{\gamma_S (\Delta G_{eS}^{01} - \Delta G_{eA}^{01})}{\frac{D_S^{01}}{r_{AX}} + \gamma_X (\Delta G_{eX}^{01} - \Delta G_{eA}^{01})} \quad (\text{B-8})$$

The maximum limit imposed by thermodynamics for Y_{XS} is given by the application of the second law: $D_S^{01} = 0$. When this term is replaced in Eq. (B-8), we obtain the maximum theoretical yield coefficient or the thermodynamic yield Y_{XS}^{th} :

$$Y_{XS}^{th} = \frac{\gamma_S (\Delta G_{eS}^{01} - \Delta G_{eA}^{01})}{\gamma_X (\Delta G_{eX}^{01} - \Delta G_{eA}^{01})} \quad (\text{B-9})$$

Heijnen and Dijnen (1992) showed that the thermodynamic efficiency definitions (based on the black box and the Gibbs energy convertor concept) were all subject to serious intrinsic problems. To avoid them, they defined the thermodynamic efficiency as the ratio of the experimental to the thermodynamic maximal yield coefficient:

$$\eta^{th} = \frac{Y_{XS}}{Y_{XS}^{th}} \quad (\text{B-10})$$

and the maximum theoretical efficiency:

$$\eta^{th,theo} = \frac{Y_{XS}^{theo}}{Y_{XS}^{th}} = \frac{\gamma_X (\Delta G_{eX}^{01} - \Delta G_{eA}^{01})}{\frac{D_S^{01}}{r_{AX}} + \gamma_X (\Delta G_{eX}^{01} - \Delta G_{eA}^{01})} \quad (B-11)$$

with $0 \leq \eta^{th,theo} \leq 1$, the resulting $\eta^{th,theo} = 1$ when $D_S^{01} = 0$.

Effect of the maintenance energy: The experimental (Y_{XS}) and the theoretical (Y_{XS}^{theo}) yield coefficients usually show some discrepancy. The correlation used for the calculations of the theoretical yield coefficient took into account the C source, the N source, and the electron acceptor, but did not consider the consumption of substrate for functions not related to growth. Pirt (1965) suggested that the substrate consumption rate r_{As} be expressed as:

$$r_{As} = \frac{1}{Y_{XS}^{theo}} r_{Ax} + m_S X \quad (B-12)$$

where m_S (C-mol C-mol⁻¹ d⁻¹) is the substrate specific consumption rate for purposes related to maintenance, or is simply called the maintenance coefficient, and Y_{XS}^{theo} is the theoretical yield coefficient, the yield in growth that would be observed if m_S was null. Eq. (B-12) can also be written as:

$$\frac{1}{Y_{XS}} = \frac{1}{Y_{XS}^{theo}} + \frac{m_S}{\mu} \quad (B-13)$$

where μ , in d⁻¹ or h⁻¹, is the specific growth rate of the microorganism.

Tijhuis *et al.* (1993) proposed calculation of the free energy maintenance coefficient m_E (kJ C-mol⁻¹ d⁻¹) according to:

$$m_E = m_S \gamma_S (\Delta G_{eS}^{01} - \Delta G_{eA}^{01}) \quad (\text{B-14})$$

The total Gibbs energy dissipation D_S^{01} / r_{Ax} necessary for growth and maintenance is:

$$\left. \frac{D_S^{01}}{r_{Ax}} \right|_{total} = \left. \frac{D_S^{01}}{r_{Ax}} \right|_{growth} + \left. \frac{D_S^{01}}{r_{Ax}} \right|_{maintenance} = \left. \frac{D_S^{01}}{r_{Ax}} \right|_{growth} + \frac{m_E}{\mu} \quad (\text{B-15})$$

Figure 1

