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Combining yield coefficients and exit-gas analysis for monitoring of the baker's yeast fed-batch fermentation

J. E. Claes, J. F. Van Impe

Abstract Baker's yeast is one of the micro-organisms that is studied most in literature. Therefore, a lot of knowledge on the biochemical pathways and corresponding yield coefficients is available. This knowledge is combined with measurements of oxygen and carbon dioxide in the exit-gas to determine the coefficients appearing in the stoichiometric equations. In this manner, two measurements are sufficient to yield on-line estimates for biomass, glucose, ethanol and the specific growth rate, and information about the (ill-defined) nitrogen source NH_q. This is not possible if the yield coefficients are not included in the estimation procedure. A sensitivity analysis illustrates that this estimation scheme is rather insensitive to uncertainties on the yield coefficients.

List of symbols

$Y^o_{X/S}$	Yield for oxidative growth on glucose [gDW/g]
$Y^r_{X/S}$	Yield for reductive growth on glucose [gDW/g]
$Y^o_{X/E}$	Yield for oxidative growth on ethanol [gDW/g]
RQ	Respiratory quotient [–]

1 Introduction

Growth and ethanol formation of baker's yeast in aerobiosis is studied in a number of articles [1, 2, 3]. Basically three pathways are considered, depending on the glucose concentration [3]. The ammonium source is not specified a priori, since an ill-defined medium is used.

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1. Oxidative growth on glucose:

$$aC_6H_{12}O_6 + bO_2 + c \cdot NX\ NH_q \xrightarrow{r_A} cC_1H_{HX}O_{OX}N_{NX} + dCO_2 + eH_2O \quad . \tag{1}$$

2. Reductive growth on glucose:

$$fC_6H_{12}O_6 + g \cdot NX\ NH_q \xrightarrow{r_B} gC_1H_{HX}O_{OX}N_{NX} + hCO_2 + iH_2O + jC_2H_6O \quad . \tag{2}$$

3. Oxidative growth on ethanol:

$$kC_2H_6O + lO_2 + m \cdot NX\ NH_q \xrightarrow{r_C} mC_1H_{HX}O_{OX}N_{NX} + nCO_2 + pH_2O \quad . \tag{3}$$

Note that the equations presented here are not normalized with respect to biomass or substrate, as is the case in [3, 4]. With this normalization, it is assumed that the time interval used during the calculations is equivalent with the time necessary to produce or consume one mole of biomass or substrate, respectively. However, in the present work, the time interval is determined by the measurement discretization time, which does not allow to use the normalized version of these pathways.

In literature, the stoichiometric coefficients are determined using equations from the conservation of chemical elements and a set of measurements [5, 6, 7, 8]. The present paper validates a novel methodology, combining knowledge about yield coefficients with the measurements to solve for all coefficients. With this method, less measurements are needed. This approach is inspired on the work of Sonnleitner and Käppeli [3], who performed also a sensitivity analysis with respect to the yield coefficients. Experiments in continuous and in batch mode are described. In the present work, the approach is validated on a fed-batch experiment, without any assumptions for the specific rates for substrate uptake, ethanol uptake and oxygen consumption.

If the stoichiometric coefficients are known, other quantities such as biomass, glucose, and ethanol can be estimated. These variables are an important tool in process monitoring, optimization and control [6, 7, 8, 9].

2 Materials and methods

A YPD medium is used for the fed-batch baker's yeast fermentation. Oxygen (paramagnetic) and carbon dioxide (infrared) in the exit-gas are analyzed on-line by using the `exit-gas analyzer WA-376F` (The Analytical Development Co. Ltd., Herts, UK). Every 30 seconds, a measure-

ment of oxygen and carbon dioxide is logged with a user interface developed in LabVIEW (National Instruments Co., Austin, USA) [10]. The air flow to the fermentor is controlled with an El-Flow digital mass flow controller (Bronkhorst Hi-Tec, Ruurlo, The Netherlands). Off-line analyses are performed for biomass (dry weight), glucose (enzymatic kit, Granutest 100, Merck) and ethanol (gas chromatography). For all other experimental conditions, reference is made to Claes and Van Impe [10].

3 Determination of stoichiometric coefficients

The stoichiometric coefficients appearing in the stoichiometric equations are determined using equations from the conservation of chemical elements, knowledge about the yield coefficients and the measurements.

3.1 Conservation of chemical elements

The component vector $\mathbf{C}$[1] for the baker's yeast growth and fermentation can be selected as follows:

$$\mathbf{C} = [\mathrm{C_1H_{1.79}O_{0.57}N_{0.15}} \quad \mathrm{C_6H_{12}O_6} \quad \mathrm{C_2H_6O} \quad \mathrm{NH}_q \quad \mathrm{O_2} \quad \mathrm{CO_2} \quad \mathrm{H_2O}] \ .$$

Since chemical elements are conserved quantities, the product of the stoichiometry matrix $\boldsymbol{\alpha}$ and the vector with the elemental composition E (related with [C H O N]) must be equal to zero, resulting in the following Eq. (4):

$$\begin{bmatrix} c-6a+d & 1.79c-12a-0.15cq+2e & 0.57c-6a-2b+2d+e \\ g-6f+2j+h & 1.79g-12f+6j-0.15gq+2i & 0.57g-6f+j+2h+i \\ m-2k+n & 1.79m-6k-0.15mq+2p & 0.57m-k-2l+2n+p \end{bmatrix} = \bar{0} \ . \tag{4}$$

3.2 Yield coefficients

The yield coefficients for the three metabolic pathways are assumed to be known and established as [3]:

$$Y^o_{X/S} = 0.49\,\mathrm{gDW/g}, \quad Y^r_{X/S} = 0.08\,\mathrm{gDW/g},$$
$$Y^o_{X/E} = 0.72\,\mathrm{gDW/g} \ .$$

Combined with the molar mass of the different components, this yields three additional equations which can be used to solve for the stoichiometric coefficients:

$$-3.53a + c = 0, \quad -0.58f + g = 0,$$
$$-1.32k + m = 0 \ , \tag{5}$$

3.3 Respiratory quotient RQ

The occurrence of the three metabolic pathwyas depends on the respiratory quotient RQ. It can be calculated from the measured oxygen and carbon dioxide profiles according to:

$$RQ = \frac{\text{amount of carbon dioxide produced}}{\text{amount of oxygen consumed}} = \frac{\text{mol } CO_2}{\text{mol } O_2} \ . \tag{6}$$

In this equation, (mol O_2) is the amount of oxygen consumed during a specified time interval, while (mol CO_2) is the amount of carbon dioxide produced during that interval. In Fig. 1 measurements of oxygen, carbon dioxide, and the air flow rate are depicted. Fig. 2 represents the calculated respiratory quotient RQ according to Eq. (6).

The following three combinations of the metabolic pathways are distinguished, depending on the value for RQ [3, 7]. For each case, different sets of stoichiometric coefficients are to be determined combining the system of Eqs. (4), the yield Eqs. (5), and the available measurements.

1. RQ > 1.07: oxidative and reductive growth on glucose (Eqs. (1) and (2))

For the 10 coefficients a to j, six relations can be derived from the matrix (4), and the following four equations are available from the yield coefficients and the measurements. Therefore, all coefficients can be calculated as function of the two available measurements.

$$-3.53a + c = 0 \qquad \text{mol } O_2 = b$$
$$-0.58f + g = 0 \qquad \text{mol } CO_2 = d + h$$

2. $0.9 < RQ < 1.07$: oxidative growth on glucose (Eq. (1))

Three equations between the 5 coefficients a to e are available from Eqs. (4), together with the following equations from the yield coefficient and the measurements:

$$-3.53a + c = 0 \qquad \begin{matrix} \text{mol } O_2 = b \\ \text{mol } CO_2 = d \end{matrix}$$

The system is overdetermined and the additional equation is used to obtain information about the index q of the nitrogen source. From the measurements of oxygen and carbon dioxide in the exit-gas, q equal to 4 is shown to be the best value.

3. RQ < 0.9: oxidative growth on glucose and ethanol (Eqs. (1) and (3))

Six relations between the 10 coefficients a to f, k to n and p are available from (4), and four equations are derived from the yield coefficients and the measurements.

$$-3.53a + c = 0 \qquad \text{mol } O_2 = b + l$$
$$-1.32k + m = 0 \qquad \text{mol } CO_2 = d + n$$

4 Estimation of biomass, substrate and ethanol

Since the three cases discussed in Sect. 3.3 are at least determined systems (and index q is known), the coeffi-

[1] Note that the biomass composition is fixed at $C_1H_{1.79}O_{0.57}N_{0.15}$ [3]

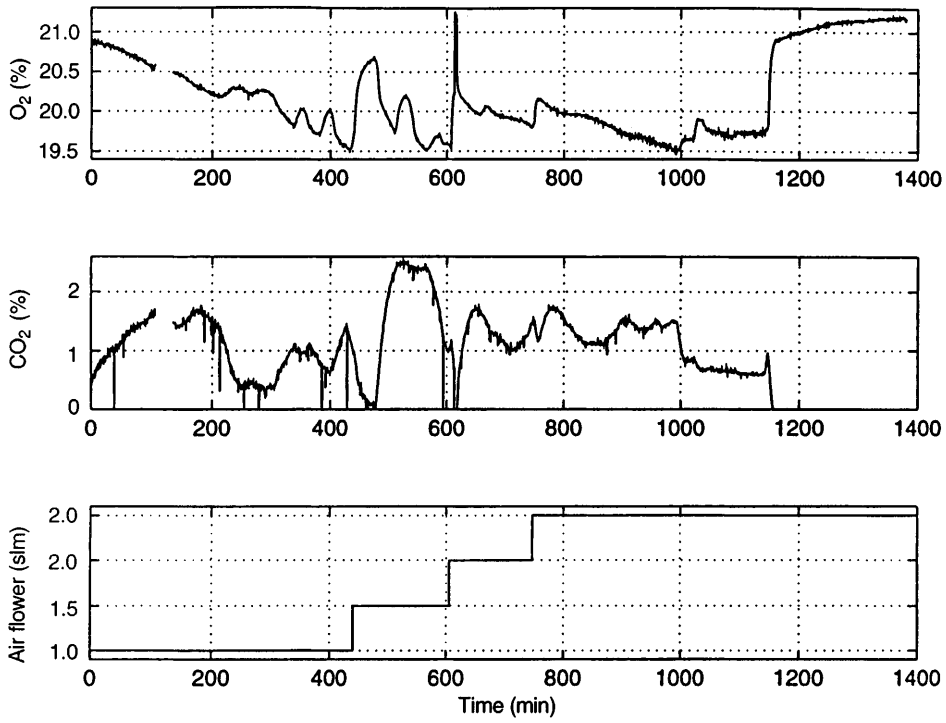

Fig. 1. Oxygen [%], carbon dioxide [%] and air flow rate [L air/min]

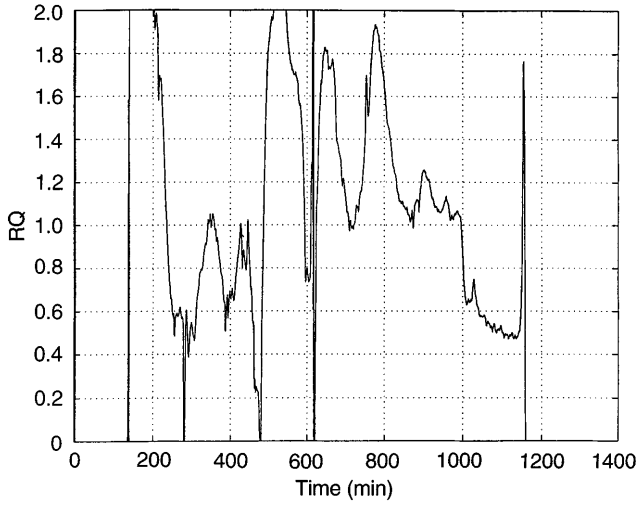

Fig. 2. Calculated respiratory quotient RQ

cients of the stoichiometric Eqs. (1) to (3) can be calculated as function of the available measurements.

1. $RQ > 1.07$: oxidative and reductive growth on glucose:

$$\frac{\text{mol } O_2}{2.43}[C_6H_{12}O_6 + 2.43O_2 + 0.53NH_4 \xrightarrow{r_A} 3.53C_1H_{1.79}O_{0.57}N_{0.15} + 2.47CO_2 + 3.90H_2O] \ ,$$

$$(0.552\,\text{mol } CO_2 - 0.562\,\text{mol } O_2)[C_6H_{12}O_6 + 0.087\,NH_4 \xrightarrow{r_B} 0.58\,C_1H_{1.79}O_{0.57}N_{0.15} + 1.81CO_2 + 0.24\,H_2O + 1.80\,C_2H_6O] \ .$$

2. $0.9 < RQ < 1.07$: oxidative growth on glucose:

$$\frac{\text{mol } O_2}{2.43}[C_6H_{12}O_6 + 2.43O_2 + 0.53NH_4 \xrightarrow{r_A} 3.53C_1H_{1.79}O_{0.57}N_{0.15} + 2.47CO_2 + 3.90H_2O] \ .$$

3. $RQ < 0.9$: oxidative growth on glucose and ethanol:

$$(0.687\,\text{mol } CO_2 + 0.278\,\text{mol } O_2)[C_6H_{12}O_6 + 2.43O_2 + 0.53\,NH_4 \xrightarrow{r_A} 3.53C_1H_{1.79}O_{0.57}N_{0.15} + 2.47CO_2 + 3.90H_2O] \ ,$$

$$(1.009\,\text{mol } O_2 - 0.993\,\text{mol } CO_2)[C_2H_6O + 1.66O_2 + 0.198\,NH_4 \xrightarrow{r_C} 1.32C_1H_{1.79}O_{0.57}N_{0.15} + 0.68CO_2 + 2.21H_2O] \ .$$

From these equations, the amounts of biomass, substrate and ethanol, formed during the time interval (which is determined by the measurement discretization time), can be calculated. Using the volume of the liquid in the fermentor, concentrations are obtained. In Fig. 3 the results of these calculations are depicted. The correspondence between the off-line measured concentrations (indicated by the stars) and the calculated values (full lines) is good.

5 Sensitivity analysis

The sensitivity of the estimation procedure to uncertainties on the yield coefficients is analyzed. The upper and lower bounds reported by Sonnleitner and Käppeli [3] are enclosed in the boundaries studied here. In addition, the influence of an error in the boundaries defined for RQ is evaluated as well. Since the stoichiometric coefficients calculated in the previous section are function of the yield coefficients, these coefficients are recalculated if one or more yield coefficients change.

In Table 1 the results of the sensitivity analysis are summarized. For the calculation of the errors, the last data point (at 1375 minutes) is not considered. E_{abs} is an absolute error, defined as the sum of squared errors of bio-

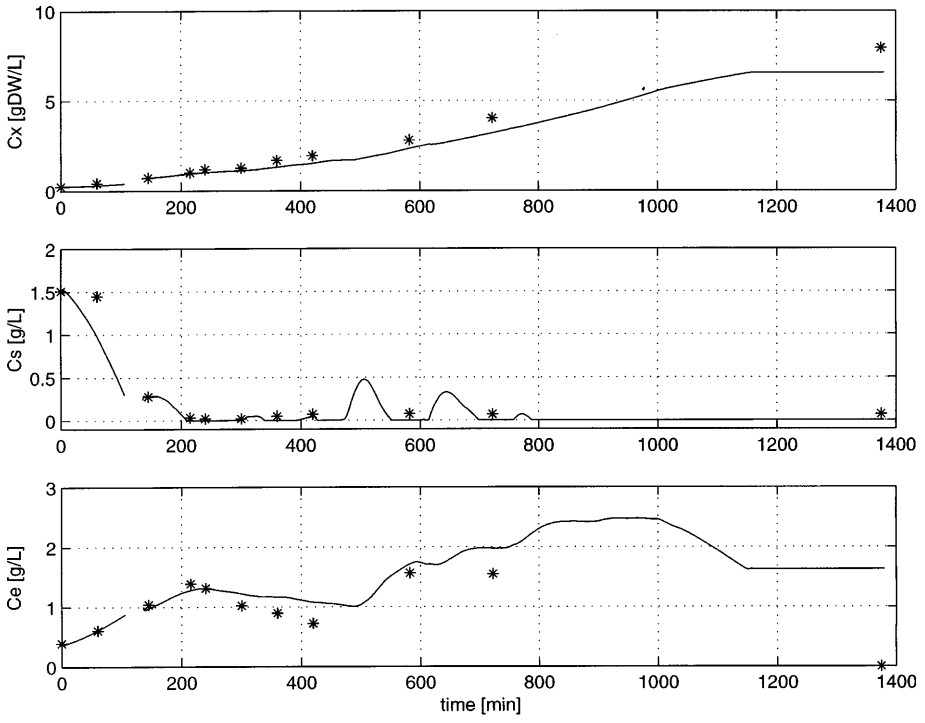

Fig. 3. Measured (✳) and estimated (full line) concentrations of biomass, substrate and ethanol

mass, substrate and ethanol, while E_{rel} represents the sum of squared errors, relative to the squared value of the mean biomass, substrate and ethanol measurement, respectively. It is worth noting that other combinations of upper and lower bounds on the yield coefficients are tested as well, and they result in an absolute and relative error between the values reported in Table 1.

In Fig. 4, the trajectories calculated with the lower and upper bounds on the yield coefficients of Table 1 are

Table 1. Sensitivity analysis with respect to the yield coefficients and the boundaries around RQ

$Y^o_{X/S}$	$Y^r_{X/S}$	$Y^o_{X/E}$	RQ_{low}	RQ_{upper}	E_{abs}	E_{rel}
0.49	0.08	0.72	0.9	1.07	2.00	2.80
0.45	0.04	0.60	0.9	1.07	4.31	3.53
0.55	0.12	0.80	0.9	1.07	0.81	2.58
0.49	0.08	0.72	0.8	1.20	1.98	2.82

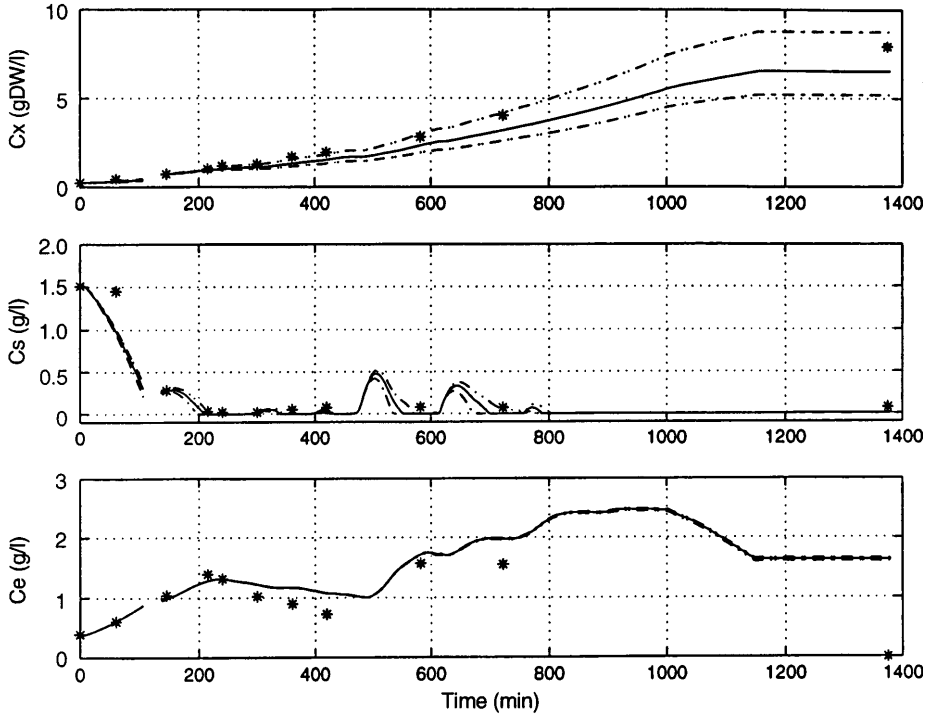

Fig. 4. Sensitivity analysis with respect to the yield coefficients. Full lines: trajectories with nominal values for the yields; dashed-dotted lines: trajectories with upper and lower bounds around the yields

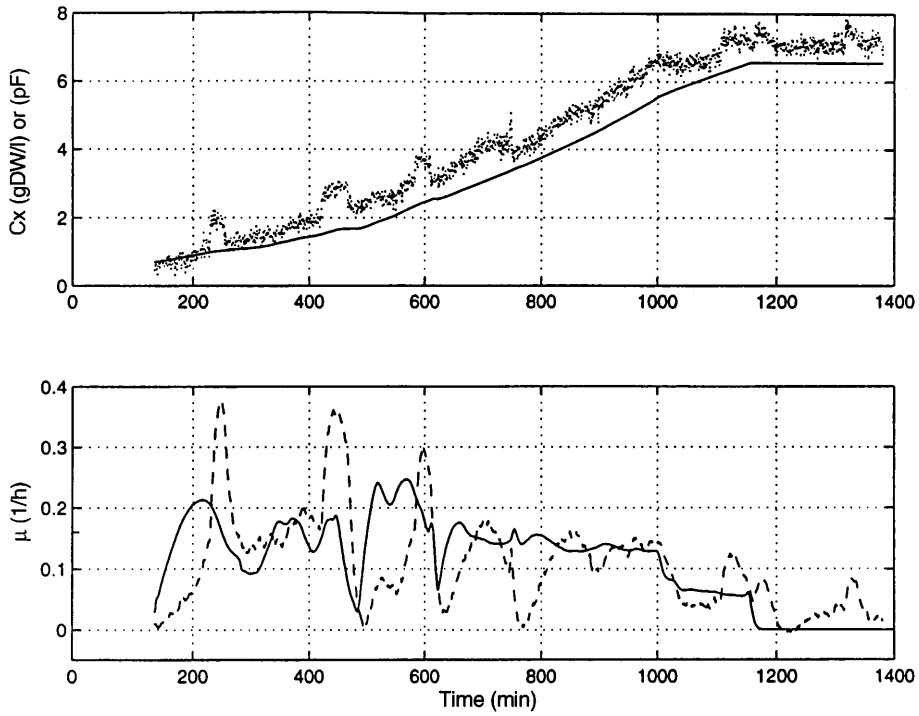

Fig. 5. Estimation of specific growth rate, using on-line measured ([pF], dashed line) and estimated ([gDW/L], full line) biomass concentration. Upper plot: biomass concentration, lower plot: estimated specific growth rate

compared with the nominal trajectory. It can be concluded from this sensitivity analysis that the estimation procedure presented here is rather insensitive to uncertainties on the yield coefficients and the respiratory quotient.

6 Estimation of the specific growth rate

Since the estimates of biomass, glucose and ethanol are available on-line, they can be used in other estimation schemes as well. In [11], an observer based estimator for the specific growth rate is described, based on an on-line viable biomass measurement (expressed as a [pF] signal).

The same algorithm is applied to the estimated biomass concentration in the present work. In Fig. 5, the results of both estimations are compared.

It is clear that, although the specific growth rates are estimated from two completely different determinations of the biomass concentration, there is a correspondence in the order of magnitude and the main trend of both estimated growth rates. The oscillations in the viable biomass concentrations, which cause the oscillations in the estimated growth rate are partially due to the change in air flow rate. However, some of the variations in the [pF] signal can not be explained at this moment. Note that on-line knowledge of the specific growth rate is an important tool in model based control experiments [10].

7 Conclusions

In the present work, knowledge about the yield coefficients for the three main biochemical pathways of baker's yeast growth is combined with measurements of oxygen and carbon dioxide in the exit-gas. Depending on the respiratory quotient RQ, different combinations of the metabolic pathways will occur. Two measurements are sufficient to estimate the concentrations of biomass, substrate and ethanol during the fermentation. The correspondence between the estimated concentrations and off-line analyses of biomass, substrate and ethanol is good. Furthermore, a sensitivity analysis revealed that the presented estimation procedure is rather insensitive to uncertainties on the yield coefficients and the respiratory quotient. The specific growth rate is estimated from the on-line biomass estimate using an observer based estimator. The index q of the ill-defined nitrogen source NH_q is determined from the measurements as well, and set equal to 4.

Since this on-line information about the main process variables can be obtained from only two measurements, this is a very cost effective way for optimization and control of biotechnological processes.

References

1. **Alexander, M.A.; Jeffries, T.W.:** Respiratory efficiency and metabolite partitioning as regulatory phenomena in yeasts. Enz. Microb. Techn. 12 (1990) 2–19
2. **Barford, J.P.; Hall, R.J.:** An examination of the Crabtree effect in *Saccharomyces cerevisiae*: the role of respiratory adaptation. J. Gen. Microb. 114 (1979) 267–275
3. **Sonnleitner, B.; Käppeli, O.:** Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis. Biotechn. Bioeng. 28 (1986) 927–937
4. **Roels, J.A.:** Energetics and kinetics in biotechnology. Amsterdam: Elsevier Science Publishing Co. 1983
5. **Aiba, S.; Nagai, S.; Nishizawa, Y.:** Fed-batch culture of *Saccharomyces cerevisiae*: a perspective of computer control to enhance the productivity of Bakers' yeast cultivation. Biotechn. Bioeng. 18 (1976) 1001–1016
6. **Cooney, C.L.; Wang, H.Y.; Wang, D.I.:** Computer-aided material balancing for prediction of fermentation parameters. Biotechn. Bioeng. 19 (1977) 55–67
7. **Wang, H.Y.; Cooney, C.L.; Wang, D.I.:** Computer-aided bakers' yeast fermentations, Biotechn. Bioeng. 19 (1977) 69–86
8. **Wang, H.Y.; Cooney, C.L.; Wang, D.I.:** Computer control of Bakers' yeast production, Biotechn. Bioeng. 21 (1979) 975–995

9. **Levisauskas, D.; Simutis, R.; Borvitz, D.; Lübbert, A.:** Automatic control of the specific growth rate in fed-batch cultivation processes based on exhaust gas analysis. Bioprocess Eng. 15 (1996) 145–150
10. **Claes, J.E.; Van Impe, J.F.:** On-line monitoring and optimal adaptive control of the fed-batch baker's yeast fermentation. In: Yoshida, T.; Shioya, S.: (Eds.) Preprints of the 7th International Conference on Computer Applications in Biotechnology – CAB VII, pp. 405–410. Oxford: Elsevier Science Ltd. 1998
11. **Claes, J.E.; Van Impe, J.F.:** On-line estimation of the specific growth rate based on viable biomass measurements: experimental validation. Bioprocess Eng. (accepted for publication)