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TOWARDS A GENERIC MODEL STRUCTURE OF BIOREACTORS

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Abstract: Model-based control of bioprocesses is a difficult task, mainly due to the associated modelling challenges. Therefore, robust controllers are a valuable solution, which efficiently allows for model uncertainties. Simplified generic models can thus be considered during controller design phases. In this direction, the paper proposes a methodological contribution towards a simplified batch or fed-batch bioreactor model structure. The first step defines a general straightforward framework of bioreactor model, based on its dynamical behaviour. The second characterises the specific growth rate of each process variable by a specified kinetics. This structure is validated in the particular yeast and penicillin production cases. *Copyright © 2007 IFAC*

Keywords: Modelling; Nonlinear systems; Biotechnology; Identification; Fermentation processes.

1. INTRODUCTION

System modelling is generally difficult and requires time to properly understand the system and identify a model. It becomes even more complicated when the system integrates living organisms. On the contrary to domains like physics where laws that are known since centuries (Ohm law, ideal gas relationship, fundamental principle in mechanics…) can apply, most biological models rely on empirical mathematical expressions (Hasar and Cumali, 2004; Stephan *et al*., 2003; Veglio *et al*., 1998). These laws result from a priori ideas on the behaviour of the system or, in some rare cases, have been estimated from some experiments. Since modelling can not be performed through commonly used and extensively validated laws, a general dynamical model of bioprocesses has further been proposed (Bastin and Dochain, 1990). However, the resulting structure remains complex, highly nonlinear, in particular due to the kinetics of the specific growth rates, and is consequently not appropriate for control purposes.

Therefore, robust controllers appear as a valuable solution, since they efficiently allow for model uncertainties (Renard *et al*., 2006). Simplified generic models can thus be considered during the controller design phase. This paper focuses on the elaboration of such a simplified model, characterizing the specific growth rate of each of the process variables by a dedicated kinetics. This general approach, only based on experimental identification techniques, is explained in the particular cases of the yeast (Saccharomyces cerevisiae) and penicillin production process.

The structure of the paper is as follows. Section 2 briefly reminds the dynamical model of bioreactors. Section 3 details the two-step methodology leading to the elaboration of a generic model, first the structure simplification of the dynamical model, then the identification of specific growth rates kinetics. Validation of this approach for yeast and penicillin production process is performed in Section 4. Section 5 finally presents some conclusions.

2. DYNAMICAL MODEL OF BIOREACTORS

The dynamical behaviour of a stirred tank bioreactor is often described by the following general macroscopic mass-balance model (Bastin and Dochain, 1990):

$$\frac{d\xi}{dt} = K\varphi(\xi, t) - D\xi - Q(\xi) + F \qquad (1)$$

In this model, the ξ vector is made-up with the concentrations of the various species inside the liquid

medium. The first term $K\varphi(\xi,t)$ describes the kinetics of the biochemical and microbiological reactions involved in the process. The remaining terms $-D\xi - Q(\xi) + F$ describe the transport dynamics of the components through the bioreactor. The K matrix is a constant pseudo-stoichiometric matrix. $\varphi(\xi,t)$ is the reaction rates vector, D the dilution rate, F the inlet feed rate. This model is detailed below in the yeast and penicillin production cases.

2.1 Yeast production example

During the aerobic growth, glucose and ethanol can be used as carbon sources according to the following reaction scheme (Sonnleitner and Käppeli, 1986; Renard *et al*., 2006):

Glucose oxidation:

$$S + k_5 O \xrightarrow{r_1 X} k_1 X + k_7 C \tag{2}$$

Glucose fermentation:

$$S \xrightarrow{r_2 X} k_2 X + k_4 P + k_8 C \tag{3}$$

Ethanol oxidation:

$$P + k_6 O \xrightarrow{r_3 X} k_3 X + k_9 C \tag{4}$$

where X, S, P, O and C are respectively the concentration in the culture medium of biomass, substrate (glucose), product (ethanol), dissolved oxygen and dissolved carbon dioxide and k_i are the pseudo-stoichiometric coefficients. For yeast production, two different operating regimes can appear: the respirative regime which is described by reactions 2 and 4 and the respiro-fermentative regime described by reactions 2 and 3. In the first regime $r_2 = 0$, and in the second one $r_3 = 0$. The reaction rates associated with these reactions are:

$$\begin{aligned} r_1 &= \min(r_S, \frac{r_{O\max}}{k_5});\ r_2 = \max(0, r_S - \frac{r_{O\max}}{k_5}) \\ r_3 &= \max(0, \min(r_P, \frac{r_{O\max} - k_5 r_S}{k_6})) \end{aligned} \tag{5}$$

The kinetic terms associated with the glucose consumption r_S, the respiratory capacity $r_{O\max}$ and the potential ethanol oxidative rate r_P are:

$$\begin{aligned} r_S &= \mu_S \frac{S}{S + K_S},\ r_{O\max} = \mu_O \frac{O}{O + K_O} \\ r_P &= \mu_P \frac{P}{P + K_P} \end{aligned} \tag{6}$$

where μ_S, μ_O and μ_P are the specific growth rates, K_S, K_O and K_P are the saturation constants of the corresponding substrate.

Based on the reaction scheme 2, 3, and 4, the following macroscopic mass balances can be derived:

$$\begin{aligned} \frac{dX}{dt} &= (k_1 \cdot r_1 + k_2 \cdot r_2 + k_3 \cdot r_3) \cdot X - \frac{F}{V} X \\ \frac{dS}{dt} &= -(r_1 + r_2) \cdot X - (S - S_{in}) \frac{F}{V} \\ \frac{dP}{dt} &= (k_4 \cdot r_2 - r_3) \cdot X - \frac{F}{V} P \\ \frac{dO}{dt} &= -(k_5 \cdot r_1 + k_6 \cdot r_3) \cdot X - O \frac{F}{V} + OTR \\ \frac{dC}{dt} &= (k_7 \cdot r_1 + k_8 \cdot r_2 + k_9 \cdot r_3) \cdot X - \frac{F}{V} C - CTR \\ \frac{dV}{dt} &= F \end{aligned} \tag{7}$$

where F is the inlet feed rate, V the culture medium volume, OTR the oxygen transfer rate, CTR the carbon dioxide transfer rate, S_{in} the feed substrate concentration. Parameters and initial values considered for simulation are reported in Tables 2 and 3 in Appendix.

2.2 *Penicillin production example*

The penicillin fermentation process considers input and output variables as described in Figure 1.

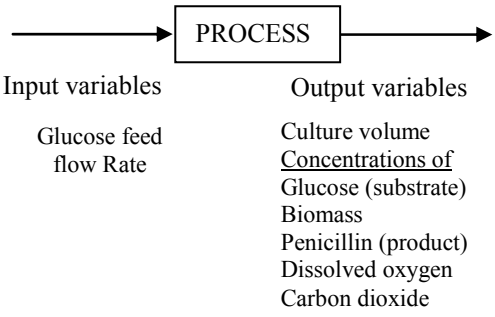

Fig. 1. Process input/output structure

Experimental findings suggest a high degree of dependence of biomass growth on the carbon source (glucose) and oxygen as substrate (Bajpai and Reuss, 1980), assuming there is no oxygen limitation. The specific growth rate μ in this case is:

$$\mu = \mu_x \frac{S}{K_x X + S} \tag{8}$$

The production of penicillin is described by non-growth associated product formation kinetics. The hydrolysis of penicillin is also included in the rate expression (Bajpai and Reuss, 1980). The specific penicillin production rate μ_{PP} is given assuming again no oxygen limitation by:

$$\mu_{pp} = \mu_p \frac{S}{\left(K_p + S + \frac{S^2}{K_I}\right)} \tag{9}$$

The mathematical model of penicillin fermentation is as follows:

$$
\begin{aligned}
\frac{dX}{dt} &= \mu X - \frac{F}{V}X \\
\frac{dS}{dt} &= -(\frac{\mu}{Y_{x/s}} + \frac{\mu_{pp}}{Y_{p/s}} + m_x)X + \frac{F}{V}(S_{in} - S) \\
\frac{dP}{dt} &= \mu_{pp}X - KP - \frac{F}{V}P \\
\frac{dO}{dt} &= -(\frac{\mu}{Y_{x/o}} + \frac{\mu_{pp}}{Y_{p/o}} + m_o)X + OTR - \frac{F}{V}O \\
\frac{dC}{dt} &= \alpha_1 \frac{dX}{dt} + \alpha_2 X + \alpha_3 \\
\frac{dV}{dt} &= F
\end{aligned}
\qquad (10)
$$

where X, S, P, O and C are respectively the concentration in the culture medium of biomass, substrate (glucose in this case), product (penicillin), dissolved oxygen and dissolved carbon dioxide, V is the culture volume, K is the penicillin hydrolysis rate constant, m_x is the maintenance coefficient on substrate, S_{in} the feed substrate concentration, $Y_{\gamma/\delta}$ are the yield constants related species (X, S, P and O), OTR is the oxygen transfer rate, F is the flow rate and m_o is the maintenance coefficient on oxygen. The values of α_1, α_2 and α_3 are chosen to give CO_2 profiles similar to the predictions of (Montague *et al*., 1986). Parameters and initial values considered for simulation are reported in Tables 4 and 5 in Appendix.

3. TOWARDS A GENERIC MODEL

Taking as a starting point the previous dynamical model of bioreactors, the aim is now the elaboration of a generic structure, which can be applied to a majority of bioreactors. This model should be as simple as possible since the final objective may be the design of a robust controller taking into account model uncertainties. Two directions are explored: selection of useful differential equations, and identification of widespread kinetics for the specific growth rates.

3.1 Simplified model structure

In order to simplify the previous bioreactor dynamical structure, the yeast and penicillin models are compared. Based on the mathematical model of these cultures (7, 10), the structure of the two models appears to be quite similar, except for the equation describing the evolution of the dissolved carbon dioxide. In fact, this equation does not affect the other relations, since the concentration of the dissolved carbon dioxide does not appear in any other expression (e.g. specific growth rates). Generalizing to many kinds of bioreactors allows cancelling this differential equation from the model structure useful for controller design. This yields to the following 5 ODE model:

$$
\begin{aligned}
\frac{dX}{dt} &= \mu_X \cdot X - \frac{F}{V}X \\
\frac{dS}{dt} &= -\mu_S \cdot X - \frac{F}{V} \cdot (S - S_{in}) \\
\frac{dP}{dt} &= \mu_{pp} \cdot X - \frac{F}{V}P - \varepsilon KP \\
\frac{dO}{dt} &= -\mu_o \cdot X - \frac{F}{V}O + OTR \\
\frac{dV}{dt} &= F
\end{aligned}
\qquad (11)
$$

where μ_X is the biomass specific growth rate, μ_{PP} the specific production rate, μ_s the substrate specific consumption rate, μ_o the oxygen specific growth rate and ε a constant modelling the hydrolysis phenomenon (e.g. $\varepsilon = 0$ for the yeast production and $\varepsilon = 1$ for the penicillin culture).

3.2 Simplified specific growth rates

From this 5 ODE model, the second step examines the identification of specific growth rates kinetics common and well known by the biologists (e.g. Monod, Haldane, Contois kinetics). For that, a general methodology has been developed which is presented and compared below for understanding facilities only on the two particular cases (the yeast and the penicillin production). The approach aims at finding the simplest specific growth rates kinetics, which could be similar to many kinds of bioreactors.

Simplified specific growth rate of biomass

The equation describing the evolution of biomass for both the yeast and the penicillin production is given by the first differential equation of (11). Attention is focused inside this equation on the biomass specific growth rate. This specific growth rate for the yeast culture is fairly complex, given by:

$$\mu = k_1 r_1 + k_2 r_2 + k_3 r_3 \qquad (12)$$

It depends on the glucose, ethanol and oxygen variables. Simulations performed with parameters of Table 1 provide variations given in Figures 2 and 3.

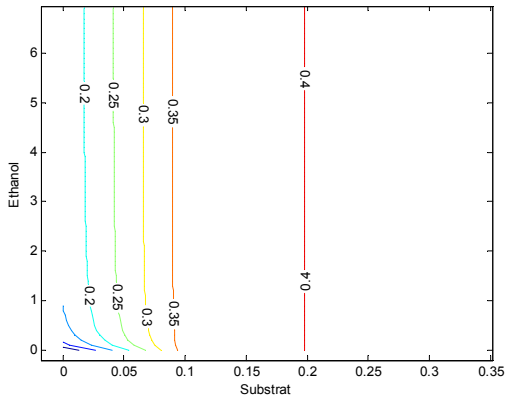

Fig. 2. Biomass specific growth rate as a function of substrate and ethanol concentrations.

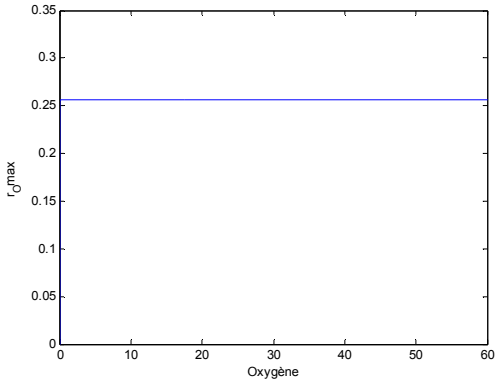

Fig. 3. Biomass specific growth rate as a function of oxygen concentration.

As shown in Figure 2 (resp. 3), the effect of ethanol (resp. oxygen) on the biomass specific growth rate can be neglected, and the most influent variable is glucose. The simplified form of this specific growth rate can thus be the following Monod kinetics:

$$\mu_X = \mu'_x \frac{S}{S + K'_x} \tag{13}$$

where μ'_x and K'_x are unknown constants which will be determined later.

However, based on equation (8), the specific growth rate of biomass for penicillin appears to follow a Contois kinetics. For generic model purposes, this kinetics is also approximated by a Monod law. The generic biomass specific growth rate is thus given by (13). This approximation will be discussed later in Section 4.

Simplified substrate specific consumption rate

The equation describing the evolution of the substrate for both the yeast and the penicillin production is given by the second differential equation of (11). It is widely mentioned in the literature, and not only for the two examples here, that the Monod law can usually model the specific consumption rate of substrate. The generic substrate specific consumption rate is thus as follows:

$$\mu_s = \mu'_s \frac{S}{S + K'_s} \tag{14}$$

Simplified specific production rate

The equation describing the evolution of the product for both the yeast and the penicillin production is given by the third differential equation of (11). The specific production rate of penicillin follows a Haldane kinetics (9) and can not be modelled by a Monod law without a significant loss of accuracy.

The generic specific production rate will be thus characterized by this Haldane kinetics (9), even if this could lead for some bioreactors to a Monod scheme.

Simplified specific growth rate of oxygen

The equation describing the evolution of oxygen for both the yeast and the penicillin production is given by the fourth differential equation of (11). In the case of the penicillin production, the specific growth rate of oxygen is as follows (Bajpai and Reuss, 1980):

$$\mu_O = \frac{\mu}{Y_{x/o}} + \frac{\mu_{pp}}{Y_{p/o}} + m_o \tag{15}$$

and it appears to be even more complicated in the case of the yeast production. Therefore, a Monod kinetics is selected as the simplest structure and will be further validated:

$$\mu_o = \mu'_o \frac{S}{S + K'_o} \tag{16}$$

Table 1 below summarizes the structures selected to build these generic specific growth rates kinetics. Some are justified by the evolution laws, or by existing results in the literature, others have been chosen to provide simple models and will be validated in the following Section. The procedure selected to identify the unknown parameters appearing in the generic kinetics (9), (13), (14) and (16) is based on the nonlinear least square method. For that, the routine LSQCURVEFIT has been used within the MATLAB™ environment.

Table 1. Kinetics of the generic specific growth rates

Specific rates	Law
Specific growth rate of biomass	Monod
Specific consumption rate of substrate	Monod
Specific production rate	Haldane
Specific growth rate of oxygen	Monod

4. SIMULATIONS AND VALIDATION

The generic kinetics are validated in this section comparing results obtained with the simplified structure and with the complete specific growth rates, for the two cases of the yeast and penicillin cultures.

Tests have been performed only in fed-batch conditions, since the specific rates kinetics do not depend of the operating mode (batch or fed-batch). The results obtained under these conditions are provided below. Concentrations displayed in Figures 4 to 19 are in g/l and plotted versus time in hour.

4.1 Validation in the yeast culture case

The generic specific rates are first identified in the respiro-fermentative regime, providing the relations:

$$\mu_X = 0.458 \frac{S}{S + 0.036};\ \mu_s = 3.5 \frac{S}{S + 0.1}$$
$$\mu_{pp} = 1.5926 \frac{S}{S + 0.6375 + \frac{S^2}{340.4928}} \tag{17}$$
$$\mu_o = 0.2553 \frac{S}{S + 0.0004}$$

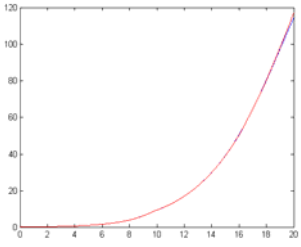

Fig. 4. Biomass

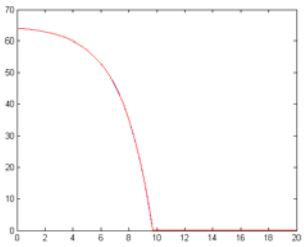

Fig. 5. Substrate

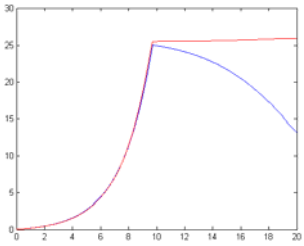

Fig. 6. Ethanol

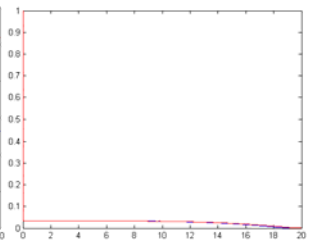

Fig. 7. Oxygen

Figures 4 to 7 show that the process variables behaviour looks similar for the complex and generic models as long as the regime remains the same (left part of the plots). Another model has been identified for the respirative regime, with the kinetics (18), which validates the generic approach, Figures 8 to 11. The negative sign in the expression of μ_{PP} may indicate ethanol consumption instead of production; this has still to be further investigated.

$$\mu_X = 165.8645\,\frac{S}{S+11.797};\mu_s = 3.5\,\frac{S}{S+0.1}$$

$$\mu_{pp} = -0.2550\,\frac{S}{S+93.3507+\dfrac{S^2}{19.718}} \qquad (18)$$

$$\mu_o = 159.4209\,\frac{S}{S+14.0046}$$

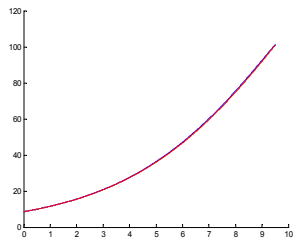

Fig. 8. Biomass

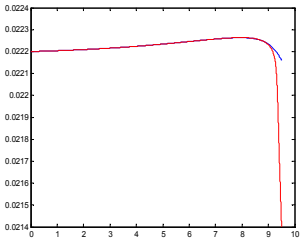

Fig.9. Substrate

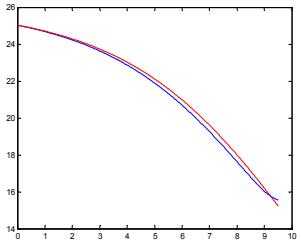

Fig. 10. Ethanol

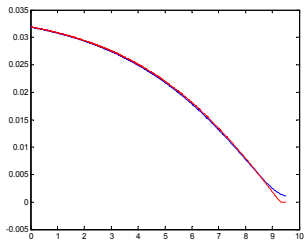

Fig. 11. Oxygen

4.2 Validation in the penicillin culture case

The same approach is used now to identify the generic kinetics of the penicillin production process:

$$\mu_x = 0.098\,\frac{S}{S+1.359};\mu_s = 0.2306\,\frac{S}{S+0.873}$$

$$\mu_{pp} = 0.0026\,\frac{S}{S+4.556+\dfrac{S^2}{0.176}} \qquad (19)$$

$$\mu_o = 2.8801\,\frac{S}{S+0.679}$$

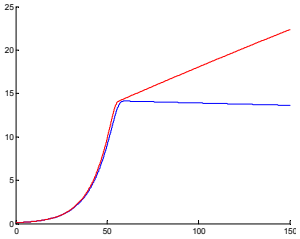

Fig. 12. Biomass

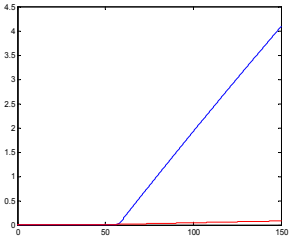

Fig.13. Penicillin

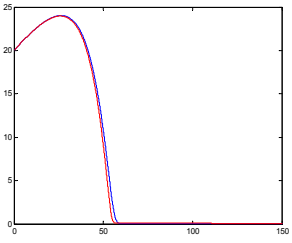

Fig. 14. Substrate

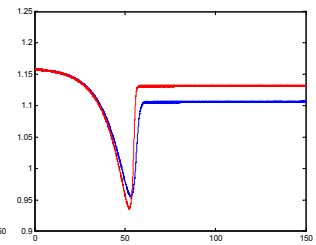

Fig. 15. Oxygen

Figures 12 to 15 show that for small variations of the biomass concentration, the two models are similar, i.e. at the beginning of the culture. But when important biomass concentrations are reached, the variables behaviour changes. In other words, simplifications performed in the biomass kinetics (Contois to Monod) are not entirely relevant for important values of the biomass concentration. Further tests have been conducted with an additional term in the expression of the biomass specific growth rate, identified as follows:

$$\mu_x = \left(1.5413\,\frac{S}{S+3.0381}\right)\cdot\left(\frac{1.3492}{1.3492+X}\right)$$

$$\mu_{pp} = 0.0023\,\frac{S}{S-0.0118-\dfrac{S^2}{400.7091}} \qquad (20)$$

$$\mu_s = 0.0462\,\frac{S}{S+0.041};\mu_o = 0.7502\,\frac{S}{S+0.015}$$

With this adjustment, Figures 16 to 19 show a good agreement between the complex rates and the generic formulation. Of course, for small values of the biomass concentration, the additional term can be removed, leading to the initial generic Monod law.

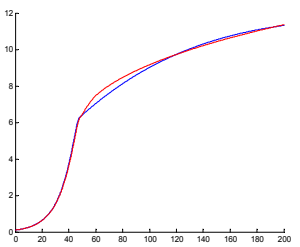

Fig. 16. Biomass

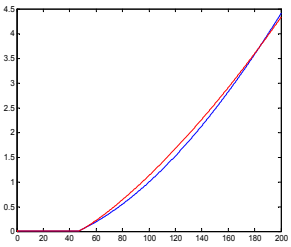

Fig. 17. Penicillin

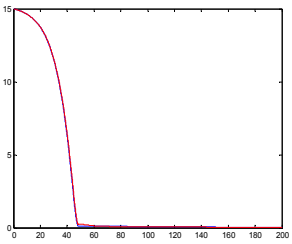

Fig. 18. Substrate

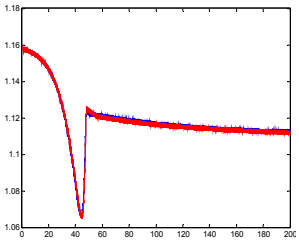

Fig. 19. Oxygen

5. CONCLUSION

This paper focuses on the elaboration of a simplified 5 ODE model of bioreactors with generic specific growth rates kinetics, appropriate for further use during the design phase of robust controllers. The main conclusion comes from the fact that the specific rates of biomass, substrate and oxygen can be approximated by Monod kinetics (with an additional term for the biomass kinetics), while a Haldane kinetics is more appropriate to model the specific production rate. This generic model only depends on the culture regime. Monod kinetics must be systematically replaced by Haldane ones in case of any other inhibiting phenomenon modelled.

REFERENCES

Bajpai, R. and M. Reuss (1980). A mechanistic model for penicillin production. *Journal of Biotechnology 30*, 330-344.

Bastin, G. and D. Dochain (1990). On-line estimation and adaptive control of bioreactors (Elsevier science publishers B.V.), 379, Amsterdam-Oxford- New York-Tokyo.

Hasar, H. and C. Kinaci (2004). Empirical model representing microbial activity in a submerged MBR treating strength wastewater. *Desalination 170,* 161-167.

Montague, G., A. Morris, A. Wright, M. Aynsley and A. Ward (1986). Growth monitoring and control through computer-aided on-line mass balancing in fed-batch penicillin fermentation. *Canadian Journal of Chemical. Eng. 64*, 567-580.

Renard, F., A. VandeWouwer, S. Valentinotti and D. Dumur (2006). Control of yeast fed-batch cultures using minimal a priori process knowledge and measurement information. *Journal of process control.*

Stephan, M., U. Beshay, K. Friehs and E. Flaschel (2003). Influence of medium composition on growth behaviour of Dictyostelium discoideum for cultivation on axenic media. *Process Biochemistry 39*, 333-343.

Sonnleitner, B. and O. Käppeli (1986). Growth of Saccharomyces cereivisiae is controlled by its limited respiratory capacity: formulation and verification of a hypothesis. *Biotechnology Bioengineering 28*, 927-937.

Veglio, F., F. Beolchimi and S. Ubaldini (1998). Empirical models for oxygen mass transfer: a comparison between shake flask and lab-scale fermentor and application to manganiferous ore bioleaching. *Process biochemistry 33*, 367-376.

APPENDIX

Table 2. Parameters values, model (7)

Parameters	Value	Units
k_1	0.49	g of X/g of S
k_2	0.05	g of X/g of S
k_3	0.72	g of X/g of P
k_4	0.48	g of P/g of S
k_5	0.39	g of O/g of C
k_6	1.10	g of X/g of S
k_7	0.58	g of C/g of S
k_8	0.42	g of C/g of S
k_9	0.62	g of X/g of P
μ_O	0.256	g of O/g of X/h
μ_S	3.5	g of S/g of X/h
K_O	0.0001	g of O/l
K_S	0.1	g of S/l
K_P	0.1	g of P/l
S_{in}	500	g /l

Table 3. Initial conditions, model (7)

Variables	X	S	P	O	V
Value	0.1	64.08	0	1	5
Units	g/l	g/l	g/l	g/l	l

Table 4. Parameters values, model (10)

Parameters	Value	Units
$Y_{x/s}$	0.45	g of X/g of S
$Y_{x/o}$	0.04	g of X/g of O
$Y_{p/s}$	0.9	g of P/g of S
$Y_{p/o}$	0.2	g of P/g of O
m_x	0.014	l/h
m_o	0.467	l/h
α_1	0.143	mmol C/g X
α_2	4.10^{-7}	mmol C/g X h
α_3	10^{-4}	mmol C/l h
μ_x	0.092	g of S/g of X/h
μ_P	0.005	g of P/g of X/h
K_x	0.15	g /l
K_I	0.1	g /l
K_P	0.0002	g /l
S_{in}	600	g /l

Table 5. Initial conditions, model (10)

Variables	X	S	P	O	V
Value	0.1	15	0	1.16	100
Units	g/l	g/l	g/l	g/l	l