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Chemical Engineering Journal 78 (2000) 53–59

Chemical Engineering Journal

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Modelling and predictive control of fed-batch yeast growth on industrial pilot plant scale

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Received 23 February 1999; received in revised form 27 September 1999; accepted 15 November 1999

Abstract

This paper presents an approach for modelling and control of fed-batch yeast growth which requires extensive calculation and a small number of measurements of component concentration. The process model is based on a simplified biochemical reaction description. An adapted predictive control algorithm is based on this model. It provides the future manipulated variable by analytical calculation, avoiding numerical optimisation methods. Experimental performance of this approach is shown for the control of ethanol production during fed-batch growth of *Saccharomyces cerevisiae* on an industrial pilot plant. © 2000 Elsevier Science S.A.

Keywords: Control strategy; Ethanol concentration; Fed-batch yeast growth; Predictive control

1. Introduction

Control of biochemical processes has become an active area of research in recent years. Much attention has been drawn on control of discontinuous bioreactors because of their prevalence in industry. However, this still remains a more difficult task compared to continuous processes because of the transient operation conditions. Moreover, evolution of state variables is often non-linear. Therefore, modelling and control of biochemical processes has been subjected to quite sophisticated methods like extended Kalman filters [1], neuronal networks [2] and fuzzy logic [3]. In addition to intensive off-line or on-line calculation, they often require comprehensive measurements of component concentration [4] which will probably not be available on an industrial scale. Therefore, this paper presents an approach for modelling and control that requires extensive calculation and a small number of measurements of component concentration. The process model is derived from prior knowledge of main system behaviour and is reduced to the representation of essential process features as described in Section 2. It is then introduced in a predictive control algorithm (Section 3). The control algorithm is adapted to the specific approach. Section 4 shows the results obtained on control of fed-batch growth of *Saccharomyces cerevisiae*. The classical control strategy of yeast growth consists in maintaining maximum productivity and preventing ethanol formation by supplying the flow of sugar that keeps the respiratory quotient close to a value of 1 [6]. This means that sugar is used for growth in an optimal way without too much carbon dioxide exhausting the reactor. The respiratory quotient is a sensitive indicator for ethanol production. High values are reached as soon as ethanol is formed in the medium.

A different strategy will be applied in this work, explicitly aiming on the formation of ethanol during yeast growth. Hereby, according to the bottle-neck theory of Sonnleitner and Käppeli, a high productivity of yeast growth is ensured if the ethanol concentration is kept at a constant low value. The objective of this work is to control the production of ethanol during fed-batch yeast growth, so that the measured ethanol concentration in the liquid holdup matches the set-point. The set-point value will be fixed on-line by the control algorithm with respect to process state. At the beginning of a batch no control action is taken and sugar is fed according to a predetermined profile based on experience. The control loop is closed after a certain period of time when the activity of yeast in the reactor is sufficiently high. This is detected by a supervisory routine of the controller, by analysing the ethanol production rate. Then the set-point is set equal to the measured value of ethanol concentration at this specific point of time. In a final stage of culture growth the flow

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PII: S1385-8947(99)00168-0

rate of sugar will be decreased in such a way that sugar and ethanol are oxidised simultaneously, leading to entire consumption of ethanol at the end of culture growth. Hereby the overall yield of transformation from substrate into yeast is improved. Finally the experimental performance of the proposed approach is discussed in Section 5.

2. The process model

The aim of the process model is to give a quantitative relationship between the production of ethanol (model output) and a certain number of process measures (model input).

Kinetics of *S. cerevisiae* can be described by the model of Sonnleitner and Käppeli [5]. This model subdivides into three major physiological activities:

- production of biomass (R1)
- production of ethanol (and biomass) (R2)
- oxidation of ethanol (R3)

They can be expressed by the following reaction scheme:

$$C_{12}H_{22}O_{11} + \gamma_{5,1}NH_3 + \gamma_{6,1}O_2 \rightarrow \gamma_{1,1}\text{Bio} + \gamma_{2,1}CO_2 + \gamma_{4,1}H_2O \quad \text{(R1)}$$

$$C_{12}H_{22}O_{11} + \gamma_{5,2}NH_3 + \gamma_{4,2}H_2O \rightarrow \gamma_{1,2}\text{Bio} + \gamma_{2,2}CO_2 + \gamma_{3,2}C_2H_5OH \quad \text{(R2)}$$

$$C_2H_5OH + \gamma_{5,3}NH_3 + \gamma_{6,3}O_2 \rightarrow \gamma_{1,3}\text{Bio} + \gamma_{2,3}CO_2 + \gamma_{4,1}H_2O \quad \text{(R3)}$$

According to the bottleneck theory of Sonnleitner and Käppeli sugar in the mixture will be preferably consumed by R1 up to the bottleneck-limit imposed by the respiratory capacity (maximal specific oxygen uptake rate). Any surplus flow of sugar will be metabolised via R2. In addition, the ethanol formed via R2 can also be metabolised oxidatively via R3, if the sugar supply does not exceed the bottleneck-limit. In the original paper Sonnleitner and Käppeli argue that, due to diauxic latency, sugar and ethanol could not be consumed simultaneously. Experimental evidence has shown that for certain strains of *S. cerevisiae* this is not the case [7]. So, in terms of productivity, a policy which allows the production of ethanol followed by a reconsumption of this metabolite could be competitive with classical 'sugar only' one.

Since the objective of this work is to control the concentration of ethanol at a constant value during yeast growth it will be supposed that supplied sugar:

- always exceeds the bottleneck-limit.
- is metabolised via R1 and R2 simultaneously.

In this case it has been shown that sugar uptake can be considered to be instantaneous [7] and the partial material balance of sugar is the following algebraic equation:

$$n_7 = n_{7,1} + n_{7,2} \quad (1)$$

In order to determine the coefficients $\gamma_{i,j}$, measurements of ethanol, carbon dioxide and oxygen concentration as well as sugar flow n_7 are supposed to be available continuously. Hence, the following partial material balances can be derived:

$$\text{carbon dioxide} : \gamma_{2,1} * n_{7,1} + \gamma_{2,2} * n_{7,2} = n_2 \quad (2)$$

$$\text{ethanol} : r_3 = \gamma_{3,2} * n_{7,2} \quad (3)$$

$$\text{oxygen} : n_6 = \gamma_{6,1} * n_{7,1} \quad (4)$$

Additionally the relations

$$\gamma_{2,1} = 12 - \text{Cr} * (\gamma_{6,1} - 12) \quad (5)$$

and

$$\gamma_{3,2} = \text{Cm} * (\text{Ck} + \gamma_{2,2} * \text{Cl}) \quad (6)$$

are derived from atomic balances of R1 and R2. The constants Ck, Cl, Cm and Cr are introduced to facilitate readability of the equations derived from the atomic balances. Their values depend on the composition of biomass as it is noted in Appendix B. In this system of equations (Eqs. (1)–(6)) 6 unknowns ($n_{7,1}$, $n_{7,2}$, $\gamma_{2,1}$, $\gamma_{6,1}$, $\gamma_{2,2}$, $\gamma_{3,2}$) occur. A resulting set of values for these unknowns could not be found due to a singular Jacobian matrix. Thus, the coefficients $\gamma_{i,j}$ cannot be estimated with the on-line available measurements of carbon dioxide, ethanol, oxygen and sugar. Consequently the selectivity of sucrose, represented by the subdivision of total fed sugar n_7 into the flow of sugar reacting via R1 ($n_{7,1}$) and R2 ($n_{7,2}$), cannot be calculated as well. This concerns in particular the prediction of these variables. Therefore, a process model reposing upon the coefficients $\gamma_{i,j}$ of R1 and R2 cannot be employed in a predictive controller.

Instead, the process model is derived from a summarising description of main process behaviour, giving just the minimum of information required for adequate control. Respectively, only essential process features will be represented by the model. They can be expressed by the following process knowledge:

- biomass takes up a flow of sugar ($n_{7,\text{a}}$) for maintenance of cell-activity and growth.
- biomass transforms a surplus flow of sugar ($n_{7,\text{b}}$) into ethanol.

In fact this is a more 'biological' process description of macroscopic organism behaviour compared to the rather 'chemical' one — describing components transformation as reaction scheme — implied by R1 and R2. In particular, transformation of sugar into ethanol is no longer considered to be linked with further production of biomass.

With the aim of avoiding the estimation of biomass and respiratory capacity it is supposed that the flow of sugar taken up for maintenance of cell-activity and growth $n_{7,\text{a}}$ is proportional to oxygen consumption n_6:

$$n_{7,\text{a}} = \lambda_6 * n_6 \quad (7)$$

Hereby the kinetics of yeast growth and the actual growth rate are inherently taken into account. Note that for the considered state 'production of ethanol' $n_{7,a}$ does not depend on the future set-point profile, so that the prediction of $n_{7,a}$ can be achieved by extrapolation of an empirical law. For this study experimental evidence has shown that the following polynomial type is appropriate

$$n_{7,a} = \lambda_6 * \sum a_i * d^i, \quad i = 0, 1, 2, \ldots, k \tag{8}$$

The parameters a_i of Eq. (8) are identified on-line at each sampling period for a set of past values of n_6, respectively, d being the number-relative to the current sampling period-of the past or future cycle at which n_6 has been measured or is to be predicted for. Parameter k has to be chosen according to the expected evolution of n_6 during culture growth.

The flow of sugar transformed into ethanol is expressed by

$$n_3 = \lambda_3 * n_{7,b} \tag{9}$$

with

$$n_3 = r_3 + n_{3,l} \tag{10}$$

Nomenclature of partial sugar flows has been changed from $n_{7,1}/n_{7,2}$ to $n_{7,a}/n_{7,b}$ to indicate that they do not refer to the reaction scheme R1/R2. Respectively, λ_3 and λ_6 are empirical parameters and have to be determined a priori or estimated on-line.

The ethanol loss flow $n_{3,1}$ results from aeration of the bioreactor and is calculated as product of air outlet stream and ethanol concentration $C_{3,s}$ in the gaseous phase.

$$n_{3,l} = v_s * C_{3,s} \tag{11}$$

The value of $C_{3,s}$ is provided by a database according to the respective operating conditions. This database was build up by measuring $C_{3,s}$ for a set of experiments, covering the relevant operating conditions.

2.1. Model error compensation

Since appropriate values of λ_3 and λ_6 have to be determined empirically, a model mismatch caused by the choice of these variables is quite probable. Therefore, a model error compensation has been implemented to cope with this problem. Any error caused by the choice of λ_3 and λ_6 will result in inequality between the quantity of ethanol predicted to be present in the reactor at a future point of time $t+\Delta t$ and the quantity that will have been measured after the time increment Δt has passed by. This inequality can be taken into consideration by introducing a coefficient f_3:

$$\Delta N_{3,mes} = f_3 * \Delta N_{3,p} \tag{12}$$

By attributing any model error to the initial choice of λ_3 the model error can be compensated by calculating:

$$\lambda_3 = f_3 * \lambda_{3,0} \tag{13}$$

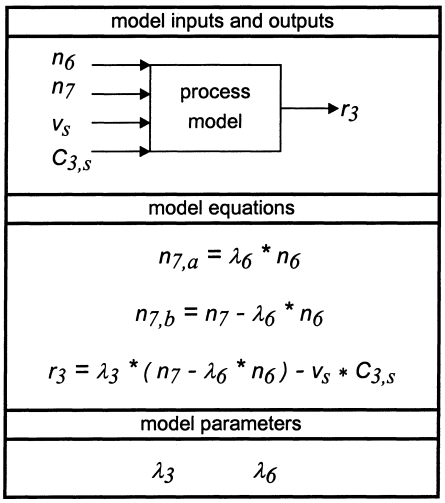

Fig. 1. Summary of the process model.

The process model is summarised in Fig. 1.

3. The predictive controller

Predictive control has shown very good results in various applications [8–10]. Its success led to the development of a certain number of different algorithms [11]. They all consist of the same three basic elements:

1. a dynamic model for the on-line simulation (prediction) of the future system behaviour.
2. a reference trajectory $\boldsymbol{C}_{3,r}(d)$ which describes the smooth transition of the target variable from its current value to the future set-point profile $\boldsymbol{C}_{3,sp}(d)$ within a horizon of prediction Hp_y. This trajectory can be interpreted as desired behaviour of the closed loop system.
3. an objective criterion $J(\boldsymbol{n}_7,\boldsymbol{e})$ as a function of the future values of the manipulated variable $\boldsymbol{n}_7(d)$ and the future controller error $\boldsymbol{e}(d)$.

By means of the objective criterion an optimal profile for the future values of the manipulated variable

$$\boldsymbol{n}_{7,opt}(d) = [n_7(d), n_7(d+1), \ldots, n_7(d+\mathrm{nu})] \tag{14}$$

is calculated for the horizon of prediction of the manipulated variable Hp_u that guides the predicted target variable as close as possible to the reference trajectory. It is

$$\mathrm{nu} = \frac{Hp_u}{\Delta t} \tag{15}$$

and

$$\Delta t = t(d+1) - t(d) \tag{16}$$

This calculation is based on:

- the future values of the target variable predicted by the dynamic model

$$\boldsymbol{C}_{3,p}(d) = [C_{3,p}(d), C_{3,p}(d+1), \ldots, C_{3,p}(d+\mathrm{ny})] \tag{17}$$

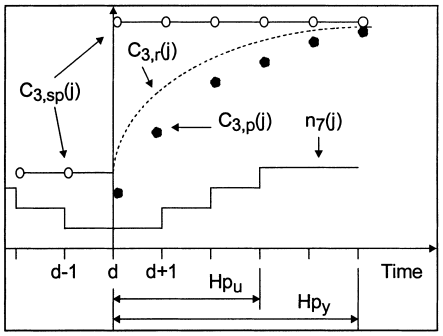

Fig. 2. Principle of predictive control.

with

$$\mathrm{ny} = \frac{\mathrm{Hp_y}}{\Delta t} \tag{18}$$

- a given set-point profile

$$\boldsymbol{C}_{3,\mathrm{sp}}(d) = [C_{3,\mathrm{sp}}(d), C_{3,\mathrm{sp}}(d+1), \ldots, C_{3,\mathrm{sp}}(d+\mathrm{ny})] \tag{19}$$

- and the future controller error

$$\boldsymbol{e}(n) = [\Delta C_3(d), \Delta C_3(d+1), \ldots \Delta C_3(d+\mathrm{ny})] \tag{20}$$

with

$$\Delta C_3(d+j) = [C_{3,\mathrm{r}}(d+j) - C_{3,\mathrm{p}}(d+j)] \tag{21}$$

as depicted in Fig. 2.

For each period Δt a value of the future manipulated variable will have to be calculated. Consequently nu values have to be determined for the optimal profile. In practical applications nu typically ranges from 20 to 50, therefore it will be difficult to calculate the optimal profile analytically. Instead, in standard predictive control algorithms the objective criterion is introduced. Its numerical minimisation leads to the optimal profile of the manipulated variable. Its first element $n_{7,\mathrm{opt}}(d)$ is applied on the process. After a one step shift of the data arrays the calculation is repeated with a new set of process measures at the next period.

In the present case the control algorithm provides the future values of the manipulated variable n_7 so that the process follows a given set-point profile $C_{3,\mathrm{sp}}$ of the target variable C_3 (ethanol concentration in the liquid holdup). According to the process model the future manipulated variable n_7 is

$$n_7(d+j) = n_{7,\mathrm{a}}(d+j) + n_{7,\mathrm{b}}(d+j) \tag{22}$$

where $n_{7,\mathrm{a}}(d+j)$ is already known by extrapolating Eq. (8)

$$n_{7,\mathrm{a}}(d+j) = \lambda_6 * \sum a_i * (d+j)^i, \quad i = 0, 1, 2, \ldots, k \tag{23}$$

Likewise $n_{7,\mathrm{b}}$ is represented by a polynomial law. This corresponds to structuring the manipulated variable as it has been proposed by Richalet [12].

$$n_{7,\mathrm{b}}(d+j) = \sum b_i * (d+j)^i, \quad i = 0, 1, 2, \ldots, m \tag{24}$$

The parameter m has to be chosen according to the expected evolution of $n_{7,\mathrm{b}}$ during culture growth. Eq. (24) determines the structure of $n_{7,\mathrm{b}}$ so only the coefficients b_i have to be calculated in order to obtain the future manipulated variable.

By introducing Eqs. (10) and (24) in Eq. (9) and integrating the resulting equation we obtain the control law:

$$N_{3,\mathrm{p}}(d+\mathrm{Pc}) + N_{3,\mathrm{l,p}}(d+\mathrm{Pc}) = \lambda_3 * \sum \left(\frac{1}{i+1}\right) * b_i * (d+\mathrm{Pc})^{i+1}, \quad i = 1, 2, \ldots, m \tag{25}$$

With the aim of determining the parameters b_i the control law (25) is solved for each point of coincidence Pc. A point of coincidence is understood as a future point of time where the process state matches the desired evolution. The solution of Eq. (25) requires the prediction of the future ethanol loss $N_{3,\mathrm{l,p}}(d+\mathrm{Pc})$ via the air-outlet flow (see Appendix A) and the prediction of the quantity of ethanol in the liquid holdup at a future point of time $N_{3,\mathrm{p}}(d+\mathrm{Pc})$. The reference trajectory $C_{3,\mathrm{r}}(d+\mathrm{Pc})$ (see Appendix A) represents the desired future evolution of ethanol concentration. Supposing the future ethanol concentration to match the desired evolution, $N_{3,\mathrm{p}}(d+\mathrm{Pc})$ can be calculated by:

$$N_{3,\mathrm{p}}(d+\mathrm{Pc}) = C_{3,\mathrm{r}}(d+\mathrm{Pc}) * V_{\mathrm{p}}(d+\mathrm{Pc}) \tag{26}$$

The prediction of the future holdup volume is

$$V_{\mathrm{p}}(d+\mathrm{Pc}) = V(d) + \Delta V_{7,\mathrm{a}}(d+\mathrm{Pc}) + \Delta V_{7,\mathrm{b}}(d+\mathrm{Pc}) \tag{27}$$

where $\Delta V_{7,\mathrm{a}}$ and $\Delta V_{7,\mathrm{b}}$ correspond to the increase of holdup volume due to the future flow of sugar. The calculation of these variables is reported in Appendix A.

The unknown parameters b_i of Eq. (25) are calculated by introducing $m+1$ points of coincidence on the reference trajectory. Since the reference trajectory is the desired future evolution of the process, Eq. (25) has to be fulfilled for every point of coincidence. By setting up Eq. (25) for every point of coincidence we obtain a system of $m+1$ linear equations. In general $m+1$ is significantly inferior to nu, so that the parameters b_i are available analytically for linear process models by solving the system of $m+1$ equations. This is an advantage compared to standard predictive control because no objective criterion has to be resolved by a numerical optimisation method which would require intensive calculation while convergence to the global minimum is not always guaranteed.

4. Experimental

The experiments have been carried out on an industrial pilot plant in fed-batch mode. The controller has been implemented as independent application, providing at the same time a software-interface that integrates the controller into

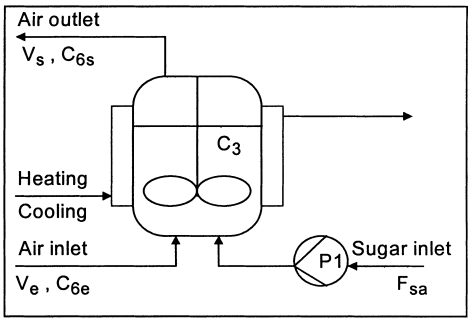

Fig. 3. General principle of a fed-batch bioreactor.

the supervision system (MFCS/win, B. Braun Biotech lnternational GmbH) of the industrial site.

As it is depicted in Fig. 3, sugar is fed by pump P1. Aeration of the bioreactor is assured by an air-stream through the reactor. Its evolution as a function of time is pre-determined by experience and managed by the supervision system. Oxygen concentration in the gaseous phase is measured at inlet and outlet. Ethanol concentration is obtained using a probe immersed into the liquid holdup. These concentrations are measured on-line.

The system with closed control loop is depicted in Fig. 4. For a given set-point of ethanol concentration the controller computes the required feed of sugar F_{sa} which is an aqueous solution of the sucrose flow n_7 computed by the controller. The conversion of n_7 to F_{sa} is reported in Appendix C. The feed of sugar F_{sa} is then applied to the process. Resulting ethanol concentration is measured and fed back to the controller.

In the following all values $W_{i,r}$ referring to x- or y-axis are reduced with respect to a reference value ($W_{i,r}=W_{i,mes}/W_{i,ref}$). Unit of x-axis is always reduced time t_r. As air-flow its output value is given which nearly equals the input flow.

Fig. 5 shows fed-batch growth of *S. cerevisiae* with a constant set-point. No model error compensation has been active. Thus, model mismatch leads to a continuously increasing control error, up to 3%. Nevertheless, the disturbance caused by varying air-flow which affects ethanol loss through the gaseous phase is quite well rejected.

Fig. 6 depicts the effects of model error compensation. In a first time the controller is operating with the same configuration as in Fig. 5 and control error increases continuously, up to 2.08%.

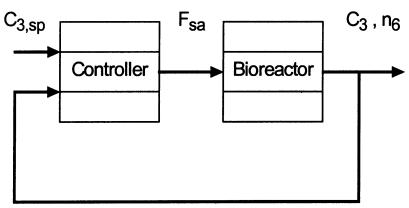

Fig. 4. Closed control loop.

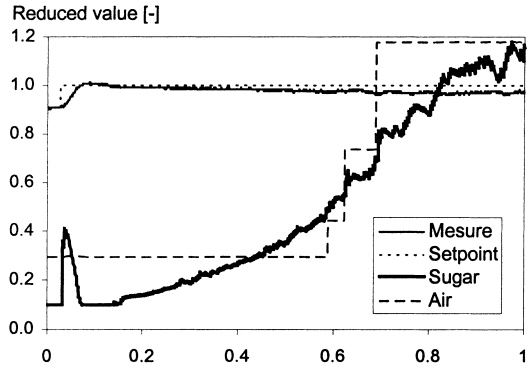

Fig. 5. Ethanol concentration, sugar- and air-during fed-batch growth (without model error compensation).

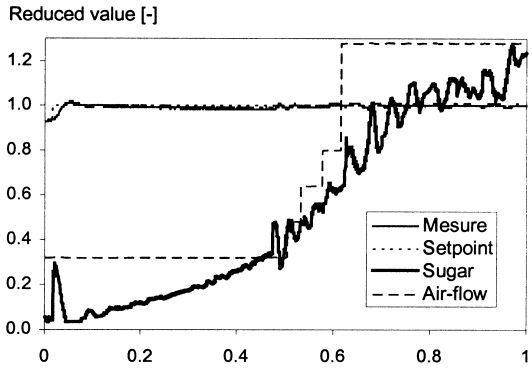

Fig. 6. Ethanol concentration, sugar- and air-flow during fed-batch growth (with model error compensation).

At t_r=0.45 model error compensation is activated without docking procedure. Therefore, the manipulated variable changes abruptly but continues to grow steadily until further disturbance is introduced by varying air-flow. In general system behaviour becomes more oscillating as it is shown by evolution of error compensation coefficient f_3 in Fig. 7. However, this does not effect the performance of model error compensation and average control error is reduced to 0.02%.

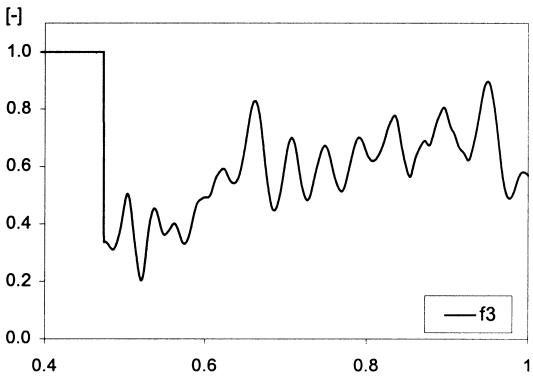

Fig. 7. Evolution of model error compensation coefficient f_3.

According to Eq. (12) coefficient f_3 represents the mismatch between the quantity of ethanol in the mixture predicted by the process model and the measured value. Supposing the structure of the process model to be appropriate, any deviation of f_3 from the unity means that model parameters have not been set to the right values. Therefore, the step of f_3 from 1.0 to 0.32 when activating model error compensation indicates that the choice of model parameters λ_3 and λ_6 has not been close to their actual values.

5. Discussion and conclusion

Predictive control of ethanol production during fed-batch yeast growth was based on a process model containing a simplified biochemical reaction description. The influence of biomass growth is introduced by oxygen uptake. This offers, in a first time, the advantage that no biomass estimation is necessary. Secondly, the fact that growth of yeast might be limited either by sugar supply or oxygen transfer capacity of the bioreactor is inherently taken into consideration. As a resuming conclusion, control of a constant set-point profile was well achieved for a wide range of system state evolution. The implemented model error compensation dealt out to be quite effective, so that precise knowledge of model parameters is not necessarily required a priori. On the other hand the controller becomes more sensible to disturbances which will be subject to further improvement. This might be achieved by replacing the model error compensation based on the coefficient f_3 by an approach for on-line estimation of the model parameters λ_3 and λ_6.

Further research will also be directed to the extension of the process model and the control algorithm to the oxidation of ethanol in order to be able to apply the entire process control strategy described in introduction. Moreover, the optimisation of the set-point profile will be subject to future work.

6. Nomenclature

Δ	increment
ε	parameter of reference trajectory
γ	stoechiometric coefficient
λ	specific uptake coefficient
a	polynomial coefficient
b	polynomial coefficient
C	concentration (mol/m^3)
d	process time given in number of cycles
e	control error
f	coefficient
F	feed (kg/s)
Hp	horizon of predictions (s)
J	objective criterion
K	specific transformation coefficient from mol to m^3 (m^3/mol)
n	molar flow (mol/s)
nu	number of periods comprised in the horizon of prediction of the manipulated variable
ny	number of periods comprised in the horizon of prediction
N	quantity (mol)
Pc	point of coincidences (s)
r	accumulation rate in holdup (mol/s)
S	set-point
t	times (s)
u	manipulated variable
v	volumetric flow (m^3/s)
V	volume (m^3)
y	target variable

Indices

0	initial
a	maintenance of cell-activity and reproduction
b	transformation of sugar into ethanol
e	inlet
i	components index; i=1, biomass; i=2, carbon dioxide; i=3, ethanol; i=4, water; i=5, ammonia; i=6, oxygen; i=7, sucrose
i,j	components index, reaction index; j=1, reaction R1; j=2, reaction R2; j=3, reaction R3
l	loss
mes	measured
opt	optimal
p	prediction
r	reference
ref	reference for reduced values
s	outlet
sa	sugar
sp	set-point
t	total
u	manipulated variable
y	target variable

Acknowledgements

Financial support of the project by TEPRAL, Centre de Recherche Branche Boissons, Group Danone is gratefully acknowledged.

Appendix A. Formulae

$$N_{3,\mathrm{l,p}}(d+\mathrm{Pc}) = \int_{d}^{d+\mathrm{Pc}} v_\mathrm{s} * C_{3,\mathrm{s}}(\tau)\,\mathrm{d}\tau \quad (A.1)$$

The first order exponential reference trajectory $C_{3,\mathrm{r}}(d+\mathrm{Pc})$ is given by:

$$C_{3,\mathrm{r}}(d+\mathrm{Pc}) = C_3(d) + [C_{3,\mathrm{sp}}(d+\mathrm{Pc}) - C_3(d)] * \psi \quad (A.2)$$

with

$$\psi = \left[1 - \exp\left(\frac{\mathrm{Pc} * \ln(1-\varepsilon)}{\mathrm{Hp_y}}\right)\right] \quad \text{(A.3)}$$

and

$$\varepsilon = \frac{C_{3,\mathrm{r}}(d + \mathrm{HP_y})}{C_{3,\mathrm{sp}}(d + \mathrm{HP_y})}, \quad \varepsilon < 1 \quad \text{(A.4)}$$

A constant value of ε=0.95 has been chosen a priori. This corresponds to a first order model with $t_{95\%}$ equal to $\mathrm{Hp_y}$ ($t_{95\%}$ corresponding to the time when the model output matches 95% of the total variation after a change in input).

$$\Delta V_{7,\mathrm{a}} = K * \lambda_6 * \int_d^{d+\mathrm{Pc}} \sum a_i * \tau^i \, \mathrm{d}\tau, \quad i = 0, 1, 2, \ldots, k \quad \text{(A.5)}$$

$$\Delta V_{7,\mathrm{b}} = K * \int_d^{d+\mathrm{Pc}} \sum b_i * \tau^i \mathrm{d}\tau, \quad i = 0, 1, 2, \ldots, m \quad \text{(A.6)}$$

Eq. (A.6) can be transformed to

$$\Delta V_{7,\mathrm{b}} = K * \sum \frac{b_i}{(1+i)} * [(d + \mathrm{Pc})^{i+1} - t^{i+1}] \quad \text{(A.7)}$$

Appendix B. Constants

Bio	biomass: $CH_{\alpha_1} O_{\alpha_2} N_{\alpha_3}$ (mol); α_1=1.68, α_2=0.54, α_3=0.14
Ck	−2.16
Cl	4.18
Cm	3.64
Cr	−0.9569

$$\mathrm{Ck} = 22 + 12 * (3 * \alpha_2 - \alpha_1) - 2 * (11 - 12 * \alpha_3) \quad \text{(B.1)}$$

$$\mathrm{Cl} = 2 * (2 - \alpha_3) - (3 * \alpha_2 - \alpha_1) \quad \text{(B.2)}$$

$$\mathrm{Cm} = 6 + 2 * (3 * \alpha_2 - \alpha_1) - 2 * (1 - 2 * \alpha_3) \quad \text{(B.3)}$$

$$\mathrm{Cr} = \frac{4}{\alpha_2 * (3 - \alpha_1/\alpha_2 - 2 * (2/\alpha_2 - \alpha_3/\alpha_2))} \quad \text{(B.4)}$$

Appendix C. Conversion of sugar feed

$$F_{\mathrm{sa}} = \frac{n_7}{x_{\mathrm{sa}}} * M_{\mathrm{sa}} \quad \text{(C.1)}$$

Mass fraction of sucrose in the feed:

x_{sa}= 0.222.

Molar weight of sucrose:

$M_{\mathrm{sa}} = 0.342\,\mathrm{kg/mol}$.

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