ON-LINE STATE OBSERVERS FOR BAKER'S YEAST FERMENTATION

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Abstract –Product quality and productivity improvement of a fermentation process implies, usually, reliable online measurements. Due to the difficulties, high costs and inefficient technology to obtain on-line measurements, several methodologies were developed to overcome these problems. In this work, algorithms for on-line state observation and kinetics parameters estimation applied to fed-batch baker's yeast fermentation process are presented. The algorithms, initially proposed by [12] are used for a brief theoretical study and validated with experimental data. Only two on-line state variables measurements, oxygen and carbon dioxide, are used to estimate all the state and the kinetics parameters. The method shows to be very sensitive to measurements precision, in particular, in the oxygen transfer rate evaluation. A corrective parameter is then introduced and consequently a better performance of the estimators is obtained.

Keywords: baker's yeast fermentation, state observers, kinetics estimators, ODE, simulation.

1. Introduction

Product quality and productivity improvement of a fermentation process implies, usually, reliable on-line measurements. A powerful experimental set-up has, often, prohibitive costs and, even worst, with the technology available some state variables on-line measures cannot be reliable.

In the majority of fermentation industries, control strategies are based on off-line state measurements. This implies a laboratory and a qualified technician, with inherent operation costs, and thus a significant delay in process actuation, with the consequence of a bad control performance [1].

Some problems in measurements and estimation in biological processes are referred in [2], pointing out the methods to reduce the difficulties, such as better sensors, automatic sampler and analysis systems, and on-line estimation of state variables that cannot be physically measured. These three areas are being carefully studied.

Since the beginning of the eighties several numerical techniques for state observation and kinetics estimation have appeared in the literature. As fermentation processes are non-linear, the estimation algorithms must be based on non-linear structures or, at least, must be approximated to a linear model with adaptation.

Developments have been made in non-linear observer techniques with guarantied stability, exploring the non-linear structure of biological processes [3-6]. The developments in techniques for state observers based on models and parameters estimators based on observers may overcome the difficulties in lack of process information and/or reliable measurements.

Also, neural networks applied to fermentation processes are a study focus. The net can easily work with process non-linearity, can dynamically adjust to environment or parameters variations and can infer rules from specified data. An estimator based on neural networks may be applied to state and kinetics estimation of fermentation processes [7-8].

More recently, hybrid model are employed in parameter estimation [9-10]. This formulation combines process models based on material balances with neural nets applied to kinetics estimation [11].

In the present work, some techniques for state and parameters estimation are studied in simulation and experimentally validated.

2. State Observers

In this work we study theoretically and experimentally the algorithms for state observers, initially proposed by [12]. The method is based on the general model for biological reactors,

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

A state observer is an algorithm to calculate the

state variables that cannot be measured on-line, ξ_2 (dimension *n-p*), using the on-line measured state variables, ξ_1 (dimension *p*). It is considered known the yield coefficients (matrix *K* of dimension *nxm*), the dilution rate *D* and the input/output gaseous and liquid flow vectors *F* and *Q*. The reaction rates, $\varphi(\xi)$, are unknown. The number *p* of on-line state variables should be at least equal to the rank of matrix *K* (*Rank* (*K*)=*r*).

The state vector can be then divided in two partitions ξ_1 , ξ_2 :

$$\begin{bmatrix} \xi_1 \\ \xi_2 \end{bmatrix} = L\xi \tag{2}$$

L in a $n \times n$ matrix of line permutation. *K*, *F* e *Q* can be written as:

$$\begin{bmatrix} K_1 \\ K_2 \end{bmatrix} = LK, \begin{bmatrix} F_1 \\ F_2 \end{bmatrix} = LF, \begin{bmatrix} Q_1 \\ Q_2 \end{bmatrix} = LQ \quad (3)$$

where $K_1 \in \mathbb{R}^{p \times m}$, $K_2 \in \mathbb{R}^{(n-p) \times m}$, ξ_1 , F_1 , $Q_1 \in \mathbb{R}^p$ and ξ_2 , F_2 , $Q_2 \in \mathbb{R}^{n-p}$.

The general dynamical model is re-written as:

$$\frac{d\xi_1}{dt} = K_1 \varphi(\xi_1, \xi_2, t) - D\xi_1 + F_1 - Q_1 \qquad (4)$$
$$\frac{d\xi_2}{dt} = K_2 \varphi(\xi_1, \xi_2, t) - D\xi_2 + F_2 - Q_2 \qquad (5)$$

Additionally, the condition of the number of measured variables, p, being equal to the number of reaction rates, m, makes K_1 a complete square matrix.

Introducing the state transformation, corresponding to a linear change of coordinates:

$$Z = A\xi_1 + \xi_2$$
(6)
where $A \in \Re^{(n-r)xr}$ is the solution of

$$AK_1 + K_2 = 0 \tag{7}$$

$$A = -K_2 K_1^{-1} \tag{8}$$

eliminating ξ_{2} , we obtain:

$$\frac{dZ}{dt} = -DZ + A(F_1 - Q_1) + (F_2 - Q_2)$$
(9)

We must point out that in (9) the term of reaction rates is not included, permitting the on-line state observation without knowing the kinetics.

3. Kinetics Estimators

In order to estimate the kinetics we consider a class of problems (baker's yeast is included) defined as:

$$\varphi(\xi, t) \equiv H(\xi)\rho(\xi, t) \tag{10}$$

where $H(\xi)$ is a $m \times r$ matrix of known state functions and $\rho(\xi)$ is a *r* vector of unknown state functions.

A kinetics estimator is an algorithm to determine

 $\rho(\xi,t)$, considering known the vector of state variables ξ (on-line measured or obtained by an observer algorithm), the on-line measured *D*, *F* and *Q*, the yield coefficients (matrix *K*) and the matrix $H(\xi)$.

The kinetics algorithm proposed, based on the general dynamical model with state observers is:

$$\frac{d\xi}{dt} = KH(\xi)\hat{\rho}(t) - D\xi + F - Q - \Omega_1\left(\xi - \hat{\xi}\right)$$
(11)

$$\frac{d\hat{\rho}}{dt} = \Omega_2 \Big(\xi - \hat{\xi}\Big) \tag{12}$$

where $\hat{\rho}$ is the on-line estimation of $\rho(\xi, t)$.

The term $(\xi - \hat{\xi})$, (12), reflects the difference between ρ and $\hat{\rho}$. This adaptation law is a variation of gradient method [13]. Selecting the gain matrix Ω_1 and Ω_2 , which guarantees the algorithm stability and convergence, completes the estimator design.

Applying the transformation $\psi \equiv K_s^{-1}\xi_s$ to (11) and (12), to decouple the equations with respect to the specific growth rate, the second order dynamics estimator (EDSO) is obtained:

$$\frac{d\psi}{dt} = H\hat{\rho} - D\psi + K_s^{-1} (F_s - Q_s) - \Omega_s (\psi - \hat{\psi})$$
(13)
$$d\hat{\rho} = \pi(z) + (z) + (z)$$

$$\frac{d\rho}{dt} = H^{T}(\xi)\Gamma_{s}(\psi - \hat{\psi})$$
(14)

where $\Omega_2 \equiv [KH(\xi)]^{T}$ and subscript *s* means the subgroup of *r* equations of the complete state model with the condition of including all the *r* parameters that need to be estimated.

If $H(\xi)$ is a diagonal matrix and the gain matrix are defined as $\Omega_s \equiv diag\{-\omega_i\}, \ \Gamma_s \equiv H^{-1}(\xi).diag\{\gamma_i\}$ we have :

$$\tau_i^2 \frac{d^2 \hat{\rho}_i}{dt^2} + 2\zeta_i \tau_i \frac{d \hat{\rho}_i}{dt} + \hat{\rho}_i = \rho_i \quad i=1,...,r \quad (15)$$

with $\tau_i = (\gamma_i h_i)^{-0.5}$ and $\zeta_i = 0.5\omega_i (\gamma_i h_i)^{-0.5}$, where h_i are the diagonal elements of $H(\xi)$.

The estimation of each parameter follows a typical second order response in changes of true parameters, with natural period of oscillation τ_i and damping factor $\zeta_{i,}$, which are functions of the state system, so time dependent.

As a restriction, $H(\xi)$ must be an invertible diagonal matrix, which is the case for most fermentation processes.

The stability analysis for the estimator, concerning convergence dynamics and tuning, is presented in [6].

4. Application to baker's yeast fermentation

Yeast growth is characterized by two metabolic pathways: respiratory and fermentative growth on glucose with ethanol production (respiro-fermentative regime, RF) and respiratory growth on both glucose and ethanol (respirative regime, R) [14].

The dynamical model for fed-batch baker's yeast production is written as two partial models:

Respiro-fermentative model, RF

$$\frac{d}{dt} \begin{bmatrix} X \\ S \\ E \\ O \\ C \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \\ -k_5 & 0 \\ k_7 & k_8 \end{bmatrix} \begin{bmatrix} \mu_s^o \\ \mu_s^r \end{bmatrix} X - D \begin{bmatrix} X \\ S \\ E \\ O \\ C \end{bmatrix} + \begin{bmatrix} 0 \\ DS_e \\ 0 \\ OTR \\ 0 \end{bmatrix} - \begin{bmatrix} 0 \\ 0 \\ 0 \\ OTR \\ 0 \end{bmatrix} - \begin{bmatrix} 0 \\ 0 \\ 0 \\ CTR \end{bmatrix}$$
(16)

Respirative model, R

$$\frac{d}{dt}\begin{bmatrix} X\\S\\E\\O\\C \end{bmatrix} = \begin{bmatrix} 1 & 1\\-k_1 & 0\\0 & -k_4\\-k_5 & -k_6\\k_7 & k_9 \end{bmatrix} \begin{bmatrix} \mu_s^o\\\mu_e^o \end{bmatrix} X - D\begin{bmatrix} X\\S\\E\\O\\C \end{bmatrix} + \begin{bmatrix} 0\\DS_e\\0\\OTR\\0 \end{bmatrix} - \begin{bmatrix} 0\\0\\0\\CTR\\0 \end{bmatrix} - \begin{bmatrix} 0\\0\\0\\CTR\\0 \end{bmatrix} - \begin{bmatrix} 0\\0\\0\\CTR\\0 \end{bmatrix} - \begin{bmatrix} 0\\0\\0\\0\\TTR\\0 \end{bmatrix} - \begin{bmatrix} 0\\0\\0\\TTR\\0 \end{bmatrix} - \\TTR\\0 \end{bmatrix} - \begin{bmatrix} 0\\0\\0\\TTR\\0 \end{bmatrix} - \\TTR\\0 \end{bmatrix} - \\TTR\\0 \end{bmatrix} - \\TTR\\0 \\TTR\\0 \end{bmatrix} - \\TTR\\0 \\TTR\\0 \end{bmatrix} - \\TTR\\0 \\TT$$

The proposed observer must considerer regime alternation of baker's yeast. The decision rule for the transition of regimes is thus: from respiro-fermentative to respirative when the specific growth rate on the fermentative growth on glucose is $\mu_s^r \leq 0$; from respirative to respiro-fermentative when the specific growth rate on the respirative growth on ethanol is $\mu_e^0 \leq 0$.

Being O-C the on-line measures considered (Fig. 1) we have the partitions $\xi_1^{T} = [O \ C]$ and $\xi_2^{T} = [X \ S \ E]$ with the corresponding $(F_1 - Q_1)^{T} = [OTR \ -CTR]$, $(F_2 - Q_2)^{T} = [0 \ DS_e \ 0]$ for each model. The corresponding partitions in matrix *K* are in Table 1.





For each regime, the state transformation defined by Z,

(9), is

Respiro-fermentative model, RF

$$\frac{dZ_{RF}}{dt} = -D\hat{Z}_{RF} + A_{RF}U_1 + U_2$$
(18)

$$\hat{\xi}_{2,RF} = \hat{Z}_{RF} - A_{RF}\xi_1 \tag{19}$$

$$\frac{d\hat{Z}_{R}}{dt} = -D\hat{Z}_{R} + A_{R}U_{1} + U_{2}$$
(20)

$$\hat{\xi}_{2,R} = \hat{Z}_R - A_R \xi_1$$
(21)

here, $U_1 = (F_1 - O_1) \quad U_2 = (F_2 - O_2)$

where
$$U_1 = (F_1 - Q_1), U_2 = (F_2 - Q_2),$$

 $A_{\text{RF}} = -K_{2,\text{RF}}K_{1,\text{RF}}^1 \text{ and } A_{\text{R}} = -K_{2,\text{R}}K_{1,\text{RF}}^1$

Table1 Partition in matrix K					
Model K ₁		1	<i>K</i> ₂		
RF	$\begin{bmatrix} -k_5 \\ k_7 \end{bmatrix}$	$\begin{bmatrix} 0\\ k_8 \end{bmatrix}$	$\begin{bmatrix} 1\\ -k_1\\ 0 \end{bmatrix}$	$\begin{bmatrix} 1 \\ -k_2 \\ k_3 \end{bmatrix}$	
R	$\begin{bmatrix} -k_5 \\ k_7 \end{bmatrix}$	$-k_6$ k_9	$\begin{bmatrix} 1 \\ -k_1 \\ 0 \end{bmatrix}$	$\begin{bmatrix} 1 \\ 0 \\ -k_4 \end{bmatrix}$	

When a change of regime occurs, the transformation variable (\hat{z}) and the state estimate $(\hat{\xi}_2)$, calculated by the other partial model are used in the new partial model, described as:

 $RF \rightarrow R$ change:

$$\hat{\xi}_{2,RF} = Z_{RF} - A_{RF}\xi_{1,RF}$$
(22)
$$\hat{Z}_{R} = \hat{\xi}_{2,RF} + A_{R}\xi_{1,R}$$
(23)

 $Z_{\rm R} = \xi_{2,\rm RF} + A_{\rm R}\xi_{1,\rm R}$ with $\mu_{\rm s,\rm R}^{\rm o} = \mu_{\rm s,\rm RF}^{\rm o}$ and $\mu_{\rm s,\rm R}^{\rm r} = 0$

$$\frac{R \to RF \text{ change:}}{\underset{\land}{\xi_{2,R}} = \underset{\land}{Z_R} - A_R \xi_{1,R}}$$
(24)

$$Z_{RF} = \xi_{2,R} + A_{RF}\xi_{1,RF}$$
(25)

with $\mu_{s,RF}^{o} = \mu_{s,R}^{o}$ and $\mu_{e,RF}^{o} = 0$.

The assimptotic observer for estimation of X, S and E for each regime is defined by two systems (26) and (27):

Respiro-fermentative model, RF

$$\begin{aligned} \frac{dZ_1}{dt} &= -D\hat{Z}_1 + ((k_8 - k_7)OTR + k_5CTR)\frac{1}{K_{RF}} \\ \frac{d\hat{Z}_2}{dt} &= -D\hat{Z}_2 + ((k_2k_7 - k_1k_8)OTR - k_2k_5CTR)\frac{1}{K_{RF}} + DS_e \\ \frac{d\hat{Z}_3}{dt} &= -D\hat{Z}_3 + (-k_3k_7OTR + k_3k_5CTR)\frac{1}{K_{RF}} \end{aligned}$$

$$\hat{X} = \hat{Z}_{1RF} - [(k_8 - k_7)O - k_5C]/K_{RF}$$

$$\hat{S} = \hat{Z}_{2RF} - [(k_2k_7 - k_1k_8)O + k_2k_5C]/K_{RF}$$

$$\hat{E} = \hat{Z}_{3RF} - (-k_3k_7O - k_3k_5C)/K_{RF}$$
with $K_{PF} \equiv k_5k_8$. (26)

$$\frac{Respirative \ model, \ R}{d\hat{Z}_1} = -D\hat{Z}_1 + ((k_9 - k_7)OTR - (k_6 - k_5)CTR)\frac{1}{K_R}$$

$$\frac{d\hat{Z}_2}{dt} = -D\hat{Z}_2 + (-k_1k_9OTR + k_1k_6CTR)\frac{1}{K_R} + DS_e$$

$$\frac{d\hat{Z}_3}{dt} = -D\hat{Z}_3 + (k_4k_7OTR - k_4k_5CTR)\frac{1}{K_R}$$

$$\hat{X} = \hat{Z}_{1R} - [(k_9 - k_7)O + (k_6 - k_5)C]/K_R$$

$$\hat{S} = \hat{Z}_{2R} - (-k_1k_9O - k_1k_6C)/K_R$$

$$\hat{E} = \hat{Z}_{3R} - (k_4k_7O + k_4k_5C)/K_R$$
with $K_R \equiv k_5k_9 - k_6k_7$.

Regarding kinetics estimator, by introducing the transformation $\psi \equiv K_s^{-1}\xi_s$ and making $\xi_s^{T} = \xi_1^{T} = [O \ C]$, the measured variables considered, it is obtained for each regime the following equations:

$$\Psi_{RF} \equiv \begin{bmatrix} \Psi_{1,RF} \\ \Psi_{2,RF} \end{bmatrix} \equiv \begin{bmatrix} k_8 & 0 \\ -k_7 & -k_5 \end{bmatrix} \begin{bmatrix} O \\ C \end{bmatrix} = \begin{bmatrix} k_8 O \\ (-k_7 O - k_5 C) \end{bmatrix} (28)$$
$$\Psi_R \equiv \begin{bmatrix} \Psi_{1,R} \\ \Psi_{2,R} \end{bmatrix} \equiv \begin{bmatrix} k_9 & k_6 \\ -k_7 & -k_5 \end{bmatrix} \begin{bmatrix} O \\ C \end{bmatrix} = \begin{bmatrix} (k_9 O + k_6 C) \\ (-k_7 O - k_5 C) \end{bmatrix} (29)$$

The systems for kinetics estimator for each partial regime are then written:

Respiro-fermentative model, RF

$$\frac{d\hat{\psi}_{1,RF}}{dt} = \hat{\mu}_{s,RF}^{o}\hat{X} - D\psi_{1,RF} + k_{8}OTR + \omega_{11}(\psi_{1,RF} - \hat{\psi}_{1,RF})
\frac{d\hat{\psi}_{2,RF}}{dt} = \hat{\mu}_{s,RF}^{r}\hat{X} - D\psi_{2,RF} - k_{7}OTR + k_{5}CTR + \omega_{12}(\psi_{2,RF} - \hat{\psi}_{2,RF})
\frac{d\hat{\mu}_{s,RF}^{o}}{dt} = \omega_{21}(\psi_{1,RF} - \hat{\psi}_{1,RF})$$
(30)
$$\frac{d\hat{\mu}_{s,RF}^{r}}{dt} = \omega_{22}(\psi_{2,RF} - \hat{\psi}_{2,RF})
\frac{Respirative model, R}{dt}
\frac{d\hat{\psi}_{1,R}}{dt} = \hat{\mu}_{s,R}^{o}\hat{X} - D\psi_{1,R} + k_{9}OTR - k_{6}CTR + \omega_{11}(\psi_{1,R} - \hat{\psi}_{1,R})
\frac{d\hat{\psi}_{2,R}}{dt} = \hat{\mu}_{e,R}^{o}\hat{X} - D\psi_{2,R} - k_{7}OTR + k_{5}CTR + \omega_{13}(\psi_{2,R} - \hat{\psi}_{2,R})
\frac{d\hat{\mu}_{s,R}^{o}}{dt} = \omega_{21}(\psi_{1,R} - \hat{\psi}_{1,R})$$
(31)
$$\frac{d\hat{\mu}_{e,R}^{o}}{dt} = \omega_{23}(\psi_{2,R} - \hat{\psi}_{2,R})$$

Since the kinetics algorithm needs biomass

concentration value (which is not experimentally available on-line) it is necessary to estimate it by using the observer just mentioned before.

The estimator gains tuning follows a second order dynamics response, where:

$\omega_{11,k} = 2\zeta_1/\tau_1$	(32)
$\omega_{121} = 2\zeta_2/\tau_2$	(33)

$$\omega_{13,k} = 2\zeta_3 / \tau_3 \tag{34}$$

$$\omega_{21\,k} = (X_k \tau_1^2)^{-1} \tag{35}$$

$$\omega_{22\,k} = (X_k \tau_2^2)^{-1} \tag{36}$$

$$\omega_{23,k} = (X_k \tau_3^2)^{-1}$$
(37)

5. Results and Discussion

Both state and kinetics estimators are simulated in a *Fortran90* software. Different runs are taken in order to test their performance [13].

Several experimental data are then considered to validate observer and kinetics estimator algorithms. Only one run is presented and extensively studied here, named EXPA08. It is a closed loop experiment where the input flow rate profile, F, is automatically calculated by a control law (Fig. 2). Notice some noise, particularly in F profile (interfering in D evaluation, D=F/V).



Fig.2. EXPA08 – Feed rate F, dilution rate D and fermenter liquid volume V experimental profiles

Fig. 3 shows the biomass, X, glucose, S and ethanol, E obtained experimentally (points), obtained by the theoretical model (thin line) and applying the observer algorithm (thick line); also it is presented the biomass growth rates in the three metabolic pathways (respirative in glucose and ethanol and fermentative in glucose): theoretical (thin line) and estimated (thick line).

There are in fact significant differences between experimental values and estimated/observed ones. It can even be said that model values approximate better the experimental results than do the estimator/observer algorithms.



Fig. 3 EXPA08 – (a) Biomass X, glucose S and ethanol E profiles: experimental values (points), theoretical model (thin line) and observed values (thick line); (b) biomass growth rates profiles: theoretical (thin line) and estimated (thick line)

Previous results [13] show the importance of the online measure for gaseous transfer rates, especially for oxygen (OTR): the observer needs an accurate measure. Its experimental determination is extremely delicate. Analysing Fig. 4, we can see that in the first 12 hours the difference between gaseous oxygen composition in the input and output flows, OTR values, are zero (or negative). So, OTR on-line evaluation may be the origin on the differences obtained in the results.

The oxygen transfer rate is experimentally determined by (making α equals to 1):

$$OTR = \frac{G_e M_{O_2}}{V} \left(y_{e,O_2} - \alpha \frac{y_{e,N_2} y_{s,O_2}}{y_{s,N_2}} \right)$$
(38)

with

- G_e gas molar flow input
- $y_{e,i}$ molar fraction of component i in the gaseous flow input
- $y_{s,i}$ molar fraction of component i in the gaseous flow output
- M_i molar mass of component i
- *V* fermenter volume

Being α equals to 1, it is often verified a subtraction

of similar numbers, which is a potential cause of errors. That is the reason for the introduction of parameter α .



Fig. 4 EXPA08 - OTR and CTR experimental (thick line) and theoretical (thin line) profiles

Fig. 5 shows the OTR experimental profile, with α =0.975 (thick line), and the theoretical value (thin line).



Fig. 5. EXPA08 - OTR and CTR experimental with α =0.975 (thick line) and theoretical (thin line) profiles

Fig. 6 shows (a) the biomass, X, glucose, S and ethanol, E profiles obtained experimentally (points), obtained by the theoretical model (thin line) and applying the observer algorithm, with α =0.975 (thick line); (b) the biomass growth rates in the three metabolic pathways (respirative in glucose and ethanol and fermentative in glucose): theoretical (thin line) and estimated (thick line).

Comparing Fig. 3 and Fig. 6, the introduction of parameter α improves both state observed values and kinetics estimates. In order to improve the kinetics estimates, α is slightly reduced to 0.97. However, the kinetics estimates are improved in opposite to the worst results for the state observers.

6. Conclusions

In this paper, the algorithms for state observers and kinetics estimator applied to baker's yeast fed-batch fermentation are presented.

Tests are first taken in a *Fortran90* based program and then experimentally validated.

The on-line measures of oxygen and carbon dioxide (O-C) are used to observe state variables and estimate

the kinetics. The study shows that the algorithms tested are very sensitive to measures precision, in particular, the on-line measure of oxygen transfer rate (OTR). This is due to the subtraction of small values in OTR evaluation, (38), that causes error propagation. In order to overcome this situation a corrective parameter α is introduced. In the absence of alternatives for on-line measurement of oxygen transfer rate, this parameter works as a corrector.

The estimator algorithms are particularly sensitive and not very robust to measurement errors (more than the observer model). The method implies then a precise experimental set-up.



Fig. 6 EXPA08 – (a) Biomass X, glucose S and ethanol E profiles: experimental values (points), theoretical model (thin line) and observed values (thick line); (b) biomass growth rates profiles: theoretical (thin line) and estimated (thick line), considering α =0.975

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