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Observer Design for Saccharomyces Cerevisiae Fermentations

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Abstract: The estimation of the states of a *Saccharomyces cerevisiae* (*S. cerevisiae*) biomass production process using glucose as carbon and energy source in CSTR-type bioreactor cultures was investigated. The biomass estimation was evaluated numerically by implementing the observer in the bioreactor model at a continuous regime. The observer was designed with two terms, one error proportional (Ke) and one sign type (Ksign(e)f(e)). The performance of the proposed observer was compared with the observer published by Bastin and Dochain. For different dilution rates, both observers showed equal performance for the same numerical conditions. Moreover, their response was evaluated for different initial conditions of the model and the estimators with a better performance index of the proposed observer.

Keywords: Software sensors, Observer design, State estimation, Nonlinear systems, batch fermentation

1. INTRODUCTION

The yeast *S. cerevisae* is a eukaryotic, heterotrophic microorganism belonging to the fungi kingdom (Parapouli et al., 2020), non-pathogenic, and considered to be a generally safe organism (GRAS) (Ostergaard et al., 2000). This yeast has been known for a long time for its use in the fermentation of beverages such as beer, cider, sake, and wine (Jacobus et al., 2021) and even in the production of bread (Lahue et al., 2020) in different processes of the pharmaceutical industry (Ostergaard et al., 2000). This species has been widely studied because it is used as a biological model in basic research and in biotechnological applications such as the production of alcoholic beverages on an industrial scale, the production of biofuels from cellulose sources (Parapouli et al., 2020), and as an input for animal and human food because it contains a high protein content (Parapouli et al., 2020). An advantage of working with this microorganism is its susceptibility to genetic modification by means of recombinant DNA technology or by random mutagenesis or the crossing of two strains (Ostergaard et al., 2000).
Due to the wide knowledge and use of this yeast in the scientific community (academic and industrial), as well as the need to control and optimize these bioprocesses in batch, fed-batch, or continuous bioreactors, the design and implementation of estimators and/or controllers to observe and control the process variables, respectively, continues due to the nonlinear nature of the microorganism and the operating conditions. According to the authors Bastin and Dochain (Chen et al., 1990b, 1990a; Dochain, 2003), software sensors are programs that apply process models and estimation algorithms to estimate variables and parameters that are not easily measured or that are available. Software sensors use online data to estimate these variables. Different applications of observers in bioprocesses are presented in Table 1 for different modes of bioreactor operation, i.e., batch and fed-batch and continuous. Therefore, in this work a nonlinear observer is designed and numerically implemented to observe the biomass concentration in *S. cerevisiae* cultures in a continuous bioreactor, considering the modeling errors in the proposed observer. The performance of the proposed observer was compared with the observer published by Chen et al. (1990a, 1990b).

Table 1. Biotechnological processes by *Saccharomyces cerevisiae* are observed and/or regulated through the implementation of observers and controllers.

Bioreactor type	Methods	Ref.
Fed-batch	Flow cytometry (FCM)	(Palomba et al., 2021)
Fed-batch	Calorimetry-based control	(Kottelat et al., 2021)
Batch	UDE-based hybrid model approach and the deep hybrid model proposed by Bangi and Knon	(Bangi et al., 2022)

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Batch	First-order sliding mode observer, proportional sliding mode observer and high-order sliding mode observer	(Alvarado-Santos et al., 2022)
Fed-batch	Hybrid observer (an asymptotic observer combined with an extended Kalman filtrer applied to high-density cultures of S. cerevisiae)	(Bárzaga-Martell et al., 2021)
Batch	Unsecented Kalman Filter (UKF)	(Yousefi-Darani et al., 2021)
Fed-batch	PID, system identification and parameter optimization.	(Hu et al., 2021; Qin & Zhai, 2024)
Batch	Sliding mode observer	(Spurgeon, 2008)

2. METHODOLOGY

2.1 Basic methodology for writing model equations

Led us write the balances for bioreactor as:

$$Rate\ accumulation = rate\ in - rate\ out \ (1)$$

$$rate\ in = bulk\ flow\ into\ the\ volume\ + generation\ with\ the\ volume + transfer\ into\ the\ volume\ across\ the\ boundaries\ other\ than\ by\ bull\ flow.$$

$$rate\ out = bulk\ flow\ out\ the\ volume\ + generation\ with\ the\ volume + transfer\ out\ the\ volume\ across\ the\ boundaries\ other\ than\ by\ bull\ flow.$$

We can now write the balance equations for the quantity.

$$\frac{d[Vy_i]}{dt} = [F_{in}y_{in} + Vr + VN] \ (2)$$

Batch fermentation processes ($F_{in}y_{in} - F_{out}y_{out} = 0$) for *Saccharomyces cerevisiae*

A general form of these equations is given by:

$$\frac{d[Vx]}{dt} = R_x \ (3)$$

$$R_x = r_x V \ (4)$$

$$r_x V = x\mu(x, s, T = Cte., pH = Cte.)V;\ x(t = 0) = x_0 \ (5)$$

$$\frac{d[Vs]}{dt} = R_s = -r_s V = -\frac{r_x V}{Y_{xs}} = -\frac{x}{Y_{xs}}\mu(x, s, T, pH, \dots)V;\ s(t = 0) = s_0 \ (6)$$

where x is the biomass concentration (g/L); s represents the substrate concentration (g/L); Y_{xs} represents the yield coefficient of substrate in grams per gram of biomass (g/g); and $\mu(\cdot)$ is the growth rate (or specific growth rate). μ_{max} is the maximum specific growth rate (1/h)

2.2 Observer design (proposed observer)

Yeast cells (*S. cerevisiae*=X)

$$\frac{d\hat{X}}{dt} = \mu(\hat{X}, \hat{S})\hat{X} + D \cdot [X_{in} - \hat{X}] + k_1\varepsilon + \zeta_1(\hat{S},\ \varepsilon) \ (7)$$

Carbon substrate (Glucose=S)

$$\frac{d\hat{S}}{dt} = -Y\mu(\hat{X}, \hat{S})\hat{X} + D \cdot [S_{in} - \hat{S}] + k_2\varepsilon + \zeta_2(\hat{S},\ \varepsilon) \ (8)$$

Initial conditions

$\hat{X}_0\ (time\ zero) = 0.15\ g/L$

$\hat{S}_0\ (time\ zero) = 10\ g/L$

In this work, we design of the gains $\zeta_1(\hat{S},\ \varepsilon)$ and $\zeta_2(\hat{S},\ \varepsilon)$ with the following functions:

$\zeta_1(\hat{S},\ \varepsilon) = \gamma_1 sign(\varepsilon)\left(1 - \left(\frac{\varepsilon}{\beta_1}\right)^2\right)$, $\zeta_2(\hat{S},\ \varepsilon) = \gamma_2 sign(\varepsilon)\left(1 - \left(\frac{\varepsilon}{\beta_2}\right)^2\right)$ and with $\varepsilon = S - \hat{S}$. Also, with $\zeta_1(\hat{S},\ \varepsilon), \zeta_2(\hat{S},\ \varepsilon) \in \Re_{\pm}: \{-1, 0, +1\}$ and $\gamma_1, \gamma_2, X_{in}, S_{in}, D,\ Y, k_1, k_2$and $\beta \in \Re_+$.

Remark 1. Functions $\zeta_i(\hat{S},\ \varepsilon)\ \{i = 1, 2\}$:

$\left|\zeta_1(\hat{S},\ \varepsilon)\right| = \left|\gamma_1 sign(\varepsilon)\left(1 - \left(\frac{\varepsilon}{\beta_1}\right)^2\right)\right| \leq \gamma_1$ but if $\gamma_1 \leq 1$,

so that, $\zeta_1(\hat{S},\ \varepsilon)$ is bounded, and this assumption, gives opportunity to Assumption 1 (A1)

A1 $\left|\zeta_1(\hat{S},\ \varepsilon)\right| \leq 1$

Analogously for the function $\zeta_2(\hat{S},\ \varepsilon)$

$\left|\zeta_2(\hat{S},\ \varepsilon)\right| = \left|\gamma_2 sign(\varepsilon)\left(1 - \left(\frac{\varepsilon}{\beta_2}\right)^2\right)\right| \leq \gamma_2$ but if $\gamma_2 \leq 1$,

so that, $\zeta_2(\hat{S},\ \varepsilon)$ is bounded, and this assumption gives opportunity to Assumption 2 (A2).

A2 $\left|\zeta_2(\hat{S},\ \varepsilon)\right| \leq 1$

Let us derive the general structure of state observers. Consider the following nonlinear system representation for *S. cerevisiae* fermentations in a bioreactor:

$$\dot{x} = f(x, u) + \Delta\ell;\ y = h(x) = Cx \ (9)$$

With $f(\hat{x}, u) = \begin{bmatrix} f_1(X, S) \\ f_2(X, S) \end{bmatrix} = \begin{bmatrix} \mu(X, S)X + D \cdot [X_{in} - X] \\ -Y\mu(X, S)X + D \cdot [S_{in} - S] \end{bmatrix}$; $y = \begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X \\ S \end{bmatrix}$; $x = \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} = \begin{bmatrix} X \\ S \end{bmatrix}$; $C = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$; and $\dot{x} = \begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \end{bmatrix} = \begin{bmatrix} \dot{X} \\ \dot{S} \end{bmatrix}$

where $x \in \Re_+^{1\times 2}$is the vector of the state variables; $u \in \Re_+^{1\times 2}$ is the control input vector; $f(x, u): \in \Re_+^{1\times 2+1\times 2} \rightarrow \Re_+^{2\times 1}$is a nonlinear smooth vector function and Lipschitz in and uniformly bounded in u; $\Delta\ell$ is the modeling error; and $y \in \Re_+^{1\times 2}$is the vector of measured states. This proposed (13) presents an estimation technique for systems subject to modeling errors $\Delta\ell$ (parameter uncertainties), which is a realistic process situation.

Remark 2. The modeling error:
$|\Delta\ell| \leq \alpha$, so that, $\Delta\ell$ is bounded, and this assumption, gives opportunity to Assumption 3 (A3)
A3 $|\Delta\ell| \leq \alpha$
For this purpose, it is proposed the following observer's structure and its corresponding convergence analysis.
The general structure of the state observer for system (9) is:

$$\dot{\hat{x}} = f(\hat{x}, u);\ \hat{y} = h(\hat{x}) = C\hat{x} \ (10)$$

Usually is required, at least that $|\varepsilon| = |x - \hat{x}| = |S - \hat{S}| = 0$, as $t \to \infty$.

Proposition 1. The following dynamic system is an observer for system (9)

$$\dot{\hat{x}} = f(\hat{x}, u) + K\varepsilon + \gamma sign(\varepsilon)\left(1 - \left(\frac{\varepsilon}{\beta}\right)^2\right);\ \hat{y} = h(\hat{x}) = C\hat{x} \ (11)$$

Where

$$\varepsilon = x - \hat{x} \ (12)$$

The main advantage of this observer's structure is to couple a class of function bounded to 1, i.e., $sign(\varepsilon)[1 - (\varepsilon/\beta)^2] \leq 1$with a discontinuous sign function in order to provide smoothness to the corresponding output injection; besides, a proportional term $K \cdot \varepsilon$ is considered in order to provide stability to the estimation procedure, which increases the robustness in the states observing.

2.3 Sketch of proof of proposition 1

For the demonstration of proof of convergence of the error to zero, the error dynamics are considered as follows:

$$\dot{\varepsilon} = \dot{x} - \dot{\hat{x}} = f(x, u) - f(\hat{x}, u) + \Delta\ell - K\varepsilon - \gamma sign(\varepsilon)\left(1 - \left(\frac{\varepsilon}{\beta}\right)^2\right) \ (13)$$

Taking norm to maximize Eq. (13):

$$|\dot{\varepsilon}| \leq |f(x, u) - f(\hat{x}, u)| + |\Delta\ell| - K|\varepsilon| - \gamma\left|sign(\varepsilon)\left(1 - \left(\frac{\varepsilon}{\beta}\right)^2\right)\right| \ (14)$$

Now, taking into account the following assumption and the corresponding function properties:

Assumption 4 (A4)
$|f(x, u) - f(\hat{x}, u)| \leq L|\varepsilon|$ (15)
The Lipschitz constant is $L > 0$
Therefore Eq. (13) considering assumptions A1-A4, can be expressed as:

$$|\dot{\varepsilon}| \leq (L - K)|\varepsilon| + (\alpha - \gamma) \ (16)$$

By solving the above differential inequality:

$$|\varepsilon| \leq \varepsilon_0 e^{(L-K)t} + \frac{(\alpha - \gamma)}{L}\left[1 - e^{(L-K)t}\right] \ (17)$$

Considering the matrix $(L - K)$ as a Hurwitz stable matrix.
For $t \to \infty$.
Then, eq. 17 yields

$$|\varepsilon| \leq \frac{(\alpha - \gamma)}{L} \ (18)$$

Remark 3. Note that the proportional term of the observer structure provide, as usual, stability to the observer, the observer's gain K acts as a convergence rate parameter to lead to the estimation error to the closed-ball with radius proportional to $(\alpha - \gamma)$, moreover the estimation error can be made as small as desired if $\alpha \approx \gamma$, and the property of $e^{(L-K)t}$: if $e^{(L-K)t}$ is nonsingular , $\left(e^{(L-K)t}\right)^{-1} = e^{-(L-K)t}$.

3. RESULTS AND DISCUSSIONS

3.1 S. cerevisiae batch and continuous mode cultures

Different fermentations ($n = 3$) were developed in a batch reactor, controlling temperature and pH through the instrumentation systems in the bioreactor. A glucose consumption rate of $0.60\ gS/L$ was observed during the first 15 hours of the culture. Biomass production was $0.645\ gX/Lh$ with a maximum cell growth rate of 0.4147 1/h. After the estimation process of the kinetic parameters, a value of 0.5080 1/h was obtained for the maximum cell growth rate (Figure 1). For batch cultures, parameter values were obtained by nonlinear regression (data not shown here), giving a fit as shown by the solid line in Fig. 2. With the nominal value of these parameters, the model was extended to continuous operation mode considering the operating parameters $D = 0.1$, 0.15, and 0.20 1/h with $Sin = 5$ and 10 g/L for different time intervals. The numerical results are shown in Figure 3 for D = 0.10, 0.15, and 0.20 1/h, with $Sin = 5\ mg/L$.

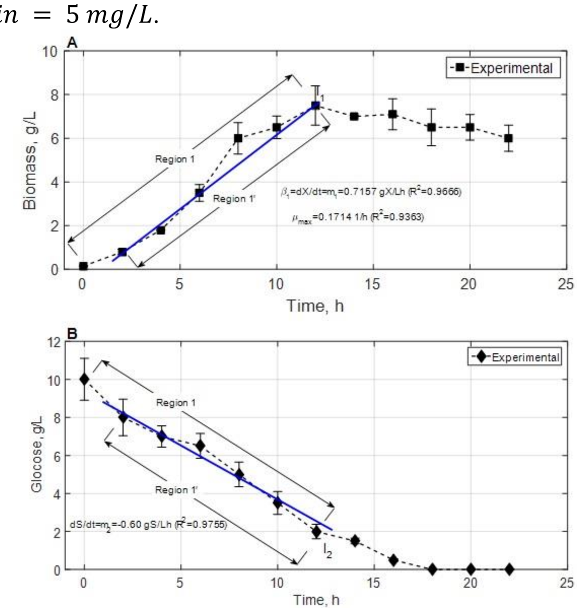

Figure 1. Plot of experimental, data yeast fermentation biomass concentration A and glucose concentration B.

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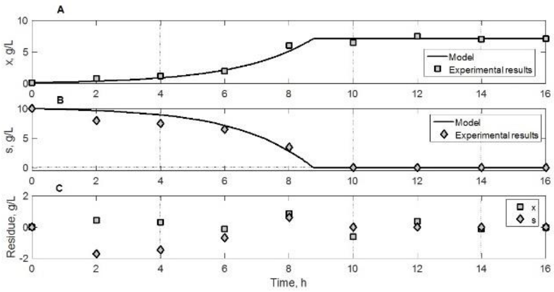

Figure 2. Comparison of model with experimental results: biomass concentration A, substrate concentration B, and residues C.

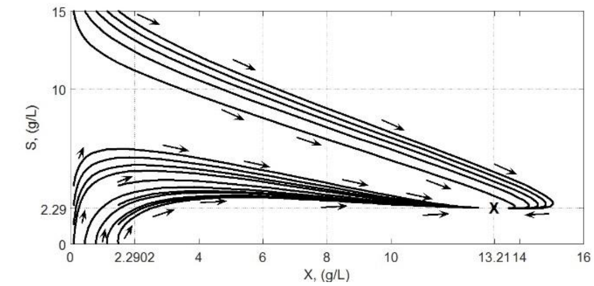

Figure 4. Plot-phase for Contios model, Case I conditions x=stable steady-state

The dynamic response of the variables in (9) in continuous mode is shown in Figure 3 for three dilution rates D, 0.1, 0.15, and 0.2 1/h. It is observed that the variables reach the equilibrium state for t = 30 hours. This is verified graphically for D=0.2 1/h and D=0.25 1/h with a substrate feed concentration value of 10 g/L (Figure 3A). For these operating conditions, the observability matrix was full-range. Also, for the same initial conditions, the substrate feed concentration in the feed stream was perturbed from 10 to 5 g/L using a step function for a time frequency of 5 hours at 2) < t<3 (Figure 3B) in order to evaluate the performance of the observers in (11) and the appendix.

For D=0.1 h-1 was used to generate the following phase-plane plot for model in (9) under case I conditions (see Figure 4). Notice that all initial conditions converge to equilibrium point x=$(\bar{X},\bar{S})$=($13.2\ 1/h, 2.29\ mg/L\ h$). Thus, $\lambda_1 < 0$ and $\lambda_2 < 0$, i.e., $\lambda_1 = -\mu(\bar{X},\bar{S}) = -0.1$ and $\lambda_2 = -Y\dot{\mu}(\bar{X},\bar{S}) = -0.3515$

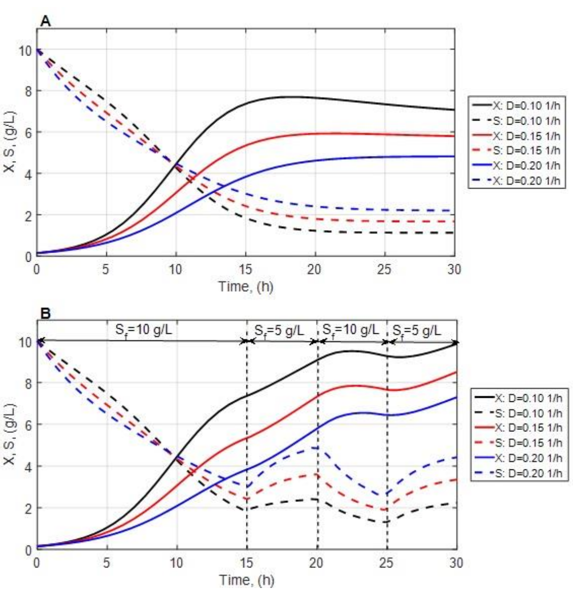

Figure 3. Numerical simulation of the system (23) for different initial conditions.

Finally, experimentally for yeast growth in a continuous reactor with a dilution rate of D = 0.2 1/h, the steady state is reached after twenty hours, i.e., after two dilution times (Figure 5). The values of the kinetic parameters of the model in equation (12) obtained by using the *fminsearch* function of MatLab are those reported previously in this section.

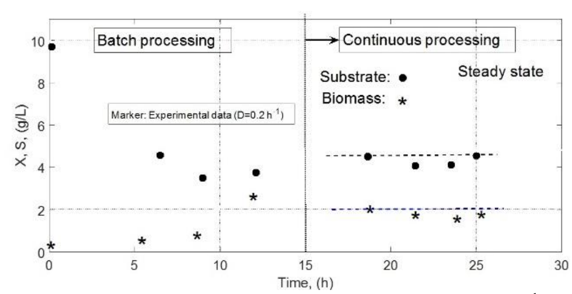

Figure 5. Contonuous processing: Dilution rate D=0.2 h^{-1} and concentration of feed $S_f = 5 g/L$ and V=1 L.

3.2 Proposed observer performance

In this section, the numerical results of the implementation of the observer proposed in (11) to the *S. cerevisiae* biomass production bioprocess (9) are presented and compared with the Bastin and Dochain observer (Chen et al., 1990b) (see appendix) to estimate the biomass concentration by measuring the substrate (glucose) concentration. Specifically, equilibrium state three, i.e., only case 3, is considered.

Figs. 6 and 7 illustrate the performance of both observers and the system in a simulation performed under the following conditions (continuous process): $\mu_{max} = 0.5080\ \text{h}^{-1}$; $K_C = 0.7039$; $Y = 0.5838$ A square wave influent substrate concentration from 5 to 10 g/L and a constant value for dilution rate (operation parameters). Initial conditions for system (process model Eq. (9)) $X_0 = 0.15\ g/L$ and $S_0 = 10\ g/L$ for system Eq. (11) (asymptotic observer) $\hat{X}_0 = 0.15\ g/L$ and $\hat{S}_0 = 10\ g/L$ with three sets of eigenvalues λ_1 and $\lambda_2$proposed observer (system Eq. (11)) $\hat{X}_0 = 0.15\ g/L$ and $\hat{S}_0 = 10\ g/L$. The numerical values used in our simulations for the steady-state solution are: Equilibrium point 1 (steady-state solutions): Case 1 Low dilution rate

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with D = 0.10 1/h and $S_{in} = 10\ g/L$ for nontrivial steady-state $\bar{x} = (\bar{X}, \bar{S}) = colum[13.2137\ g/L \quad 2.2902\ g/L]$ and the eigenvalues $\lambda_1 = -0.1\ 1/h$ and $\lambda_2 = -0.3515\ g/Lh$ with trivial steady-estate $\bar{x} = (\bar{X}, \bar{S}) = colum[0\ g/L \quad 10\ g/L]$; Equilibrium point 2 (steady-state solutions): Case 2 Medium dilution rate with $D = 0.15$ 1/h and $S_{in} = 10\ mg/L$ for nontrivial steady-state $\bar{x} = (\bar{X}, \bar{S}) = colum[11.1858\ g/L \quad 3.4719\ g/L]$ and the eigenvalues $\lambda_1 = -0.15\ 1/h$ and $\lambda_2 = -0.3050\ g/Lh$; and with trivial steady-estate $\bar{x} = (\bar{X}, \bar{S}) = colum[0\ g/L \quad 10\ g/L]$; Case 3 Low dilution rate with $D = 0.20$ 1/h and $S_{in} = 10\ mg/L$ for $\bar{x} = (\bar{X}, \bar{S}) = colum[9.5094\ g/L \quad 4.4486\ g/L]$ and the eigenvalues $\lambda_1 = -0.20\ 1/h$ and $\lambda_2 = -0.2711\ g/Lh$; and with nontrivial steady-estate $\bar{x} = (\bar{X}, \bar{S}) = colum[0\ g/L \quad 10\ g/L]$. For simulation purposes to evaluate the performance of the proposed observer, case 3 was considered.

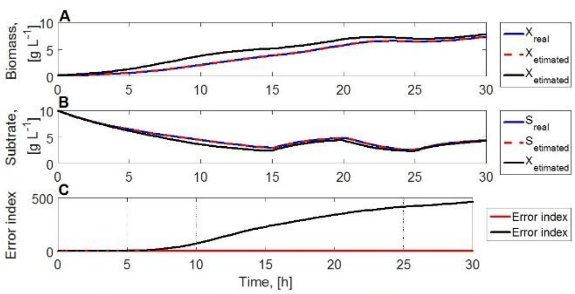

Figure 6. Application of proposed observer with $y = S$ (Eq. (11)) and asymptotic observer (Bastin and Dochain) for Case 3 with low dilution rate D=0.2 h^{-1} and initial conditions $\hat{X}_0 = 0.15\ g/L$ and $\hat{S}_0 = 10\ g/L$: comparison between systems (x) and (x) and the curve for biomass (A), substrate (B), and error index (C).

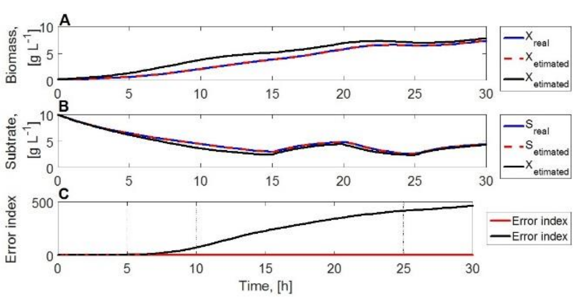

Figure 7. Application of proposed observer with $y = X$ in (11) and asymptotic observer (Bastin and Dochain) for Case 3 with low dilution rate D=0.2 h^{-1} and initial conditions $\hat{X}_0 = 0.135\ g/L$ and $\hat{S}_0 = 10\ g/L$: comparison between systems (x) and (x) and the curve for biomass (A), substrate (B), and error index (C).

4. CONCLUSIONS

A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

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Appendix A. (Chen et al., 1990a, 1990b)

Yeast cells

$$\frac{d\hat{X}}{dt} = \mu(\hat{X},\hat{S})\hat{X} + D\cdot\left[X_{in} - \hat{X}\right] + \gamma_1(\hat{X},\hat{S})\cdot\varepsilon$$

Carbon substrate

$$\frac{d\hat{S}}{dt} = -Y\mu(\hat{X},\hat{S})\hat{X} + D\cdot\left[S_{in} - \hat{S}\right] + \gamma_2(\hat{X},\hat{S})\cdot\varepsilon$$

Where

$$\varepsilon = x - \hat{x}$$

Initial conditions

$\hat{X}_0\ (time\ zero) = 0.15$

$\hat{S}_0\ (time\ zero) = 10$

Remark 1. Measured on-line. Assume the substrate concentration s is measured on-line.

Hence, we are in the situation where:

$\varepsilon = S - \hat{S}$ is the error and $\gamma_1$and γ_2 are the gains.

$r_x(X,S) = \mu(X,S)X\ = \frac{\mu_{max}S}{K_cX+S}X$ is reaction rate, so that, it governed by the Contois law;

Definition 2. Model of the specific growth rate. Dependence on the substrate concentration $s(t)$ and on the biomass concentration $x(t)$: $\mu(x,s)$.

Contois (1959)

$$\mu(X,S) = \frac{\mu_{max}S}{K_cX + S}$$

With Eq. (1), the experimental data, we assume that the specific growth rate $\mu(x,s)$ obeys the Contoins law. Where μ_{max} and k_s are constant kinetic coefficients.

Definition 1. The gains $\gamma_1(\hat{x},\hat{s})$ and $\gamma_2(\hat{x},\hat{s})$

$$\gamma_1(\hat{x},\hat{s}) = -\lambda_1 - \lambda_2 - Y(\tilde{r}_x)_s + (\tilde{r}_x)_x - \delta D$$

$$\gamma_2(\hat{x},\hat{s}) = \frac{1}{Y(\tilde{r}_x)_x}\{-\lambda_1\lambda_2 - (\lambda_1+\lambda_2)(D-(\tilde{r}_x)_x)^2 + Y(\tilde{r}_x)_x(\tilde{r}_x)_s\}$$

Where $(\tilde{r}_x)_s \triangleq \frac{\partial\tilde{r}_x}{\partial s}\Big|_{\xi=\hat{\xi}} = \frac{\mu_{max}k_s x^2}{(k_s\hat{x}+\hat{s})^2}$ and $(\tilde{r}_x)_x \triangleq \frac{\partial\tilde{r}_x}{\partial s}\Big|_{\xi=\hat{\xi}} = \frac{\mu_{max}x^2}{(k_s\hat{x}+\hat{s})^2}$

Remark 2. According with the gain $\gamma_2(\hat{x},\hat{s})$, note that the estimated value $\hat{s}$ must be allowed to be zero in order to avoid division by zero in $\gamma_2(\hat{x},\hat{s})$.

