Product-based sliding mode observer for biomass and growth rate estimation in Luedeking–Piret like processes

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ABSTRACT

The on-line estimation of non-measurable substances and key process variables is relevant for the real-time monitoring of the bioprocesses. This paper deals with the on-line estimation of microbial growth rate and biomass concentration based on measures of the product concentration. The algorithm does not require a model of the growth rate and it can be applied to batch, fed-batch and continuous processes. The proposed observer, which is based on second-order sliding mode ideas, provides smooth estimates with reduced-order error dynamics. It is also shown that in case of error in the product formation parameters, convergence to an error bound can be achieved. Numerical simulations under different operating conditions are provided and the estimates obtained for lactic acid production are contrasted with documented experimental data.

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1.   Introduction

The utilization of biotechnological processes for the production of metabolites is of special interest for different industries. A large list of commercial bioproducts such as acids, antibiotics and enzymes are produced from various carbon sources using batch, fed-batch, and continuous cultures. Microbial products may be formed as the result of a variety of processes occurring within the cells (Lovitt et al., 1991). From a macroscopic point of view, these products can be classified into growth-associated, non-growth associated and mixed-growth associated according to the kinetics involved in the product formation. The mixed-growth type refers to products for which the rate of formation can be related to both biomass concentration and microbial growth rate. In this case, a widespread mathematical model of the reaction is given by the kinetic expression presented in Luedeking and Piret (1959).

In the development of biotechnological processes, the estimation of non-measurable substances is essential for better understanding and monitoring the process and for the practical implementation of control strategies. Therefore, several observers (software sensors) have been developed to provide an on-line estimation of key variables (Mohd Ali et al., 2015). Particularly, different observers have been proposed for estimating substance concentrations and kinetic rates according to the

available outputs. Algorithms based on gas flow rates, dissolved oxygen, oxygen uptake rate, and other measurements have been used for biomass, substrate and microbial specific growth rate (μ) estimation (Soons et al., 2008; Mohseni et al., 2009; García et al., 2011).

Among the different techniques available for observer design, the sliding mode theory can be used for obtaining algorithms with remarkable properties. Basically, a discontinuous action is used to enforce the state trajectories to reach in finite time the so-called sliding surface and to slide on it from then on (Edwards and Spurgeon, 1998). This sliding surface is defined as function of accessible variables and parameters. These observers provide robust estimates of important variables despite non-linear uncertainties and have been used in a wide range of applications. In biotechnological processes, estimation of μ and substrate concentration from biomass measurements can be found in Picó et al. (2009), while nutrient consumption rate is estimated from substrate in Rahman et al. (2010). Also, second-order sliding mode algorithms for estimation of μ based on biomass and volume measurement can be found in De Battista et al. (2011, 2012), estimation of kinetic rates with possibility of time-varying gains in Vargas et al. (2014) and for estimating a combination of kinetic rates, which is of interest for controlling processes that exhibit overflow metabolism, in Vargas et al. (2013).

In those processes in which an extracellular product is monitored on-line, algorithms based on this measurement could also enhance the information available for process monitoring and control. Although in many cases no direct product measurement is available, there exists indirect methods that can be used in certain industrial fermentations (Payot and Fick, 1997; Peter and Rock, 2012). In Wang et al. (1998), biomass estimation is performed from measurements of the acid production in a fed-batch fermentation of Zymomonas mobilis, where the rate of acid formation is related to the rate of nitrogen assimilation. In Obeid et al. (2010), biomass and substrate concentrations are estimated from measurement of the hydrogen produced in a batch photo-fermentation process but estimation of microbial growth rate is not provided. Obtaining an estimate of μ from the estimated substrate concentration would require knowledge of the structure and the kinetic parameters of μ. Some preliminary results about estimation of biomass and μ based on hydrogen measurement and sliding mode algorithms were presented in Nuñez et al. (2014).

In this work, an estimation algorithm is developed based on product concentration measurement. By considering a Luedeking–Piret relation for the product formation rate, a second-order sliding mode observer is proposed for estimating biomass concentration and the microbial growth rate. Differing from the previous work (Nuñez et al., 2014), the observer structure is modified here for allowing fed-batch and continuous operating modes. Also, a modification of the observer equations permits analyzing the convergence properties in a more suitable form. The advantages of the proposal also include estimation with reduced-order error dynamics and robustness with respect to the model of the growth rate (no kinetic model of μ is required). The present observer also differs from classical asymptotic algorithms since convergence can be achieved even in batch processes.

The rest of the paper is organized as follows. The next section describes the general state-space representation of the bioprocess, the proposed observer is introduced and its convergence properties are analyzed. Section 3 presents numerical results for batch and fed-batch processes where product signals similar to real process data are considered. Finally, conclusions are given in Section 4.

2. Observer design and analysis

2.1. Process model

The following state equations, based on mass balance principles, describe the dynamics of the process (Nielsen et al., 2003)

$$\dot{x} = r_x - Dx, \tag{1}$$

$$\dot{s} = -r_s + D(S_{in} - s), \tag{2}$$

$$\dot{p} = r_p - Dp, \tag{3}$$

where x, s and p are the concentrations of biomass, substrate and product, respectively, S_{in} is the substrate concentration in the feed, r_s the substrate consumption rate and $D \geq 0$ stands for the dilution rate. The biomass formation rate is $r_x = \mu x$ where μ is the microbial specific growth rate. The rate of product formation given by

$$r_p = \alpha r_x + \beta x, \tag{4}$$

is referred to as the Luedeking–Piret model. The parameters α and β are coefficients for growth- and non-growth-associated production, respectively. Expression (4) was presented in Luedeking and Piret (1959) for describing the formation of lactic acid in batch cultivation of *Lactobacillus delbrueckii*. Since then, Luedeking–Piret like expressions have been reported for the specific product formation of diverse metabolites

including acetic, butyric, lactic and succinic acids (Song et al., 2008, 2010; Zhao et al., 2010), penicillin acylase, bacteriocins and other antibiotics (Ramírez et al., 1994; Elibol and Mavituna, 1999; Guerra et al., 2007). Also, the microbial formation of hydrogen has been described with expressions of this type and modified to consider different effects on the hydrogen formation rate (Gadhamshetty et al., 2008; Obeid et al., 2009). In many cases, these models have been extended to include fed-batch and continuous operation by including terms associated to the dilution rate. The Luedeking–Piret relationship has also been used for relating the oxygen uptake rate with biomass concentration and microbial growth rate in aerobic processes (Jenzsch et al., 2004).

The aim of this work is to estimate biomass concentration and the specific growth rate since these process variables provide valuable information about the time-evolution of the culture. To this end, the following assumptions are made:

Assumption 2.1. Product concentration is monitored on-line.

Assumption 2.2. The coefficients of Eq. (4) are known.

Assumption 2.3. The function r_x is non-negative and bounded with a bounded time-derivative.

It is worth noting that no modeling of r_x, r_s is assumed and no measurement of the substrate concentration is required. This is advantageous to the application of the observer in a wide range of processes that satisfy the general model (1)–(4).

2.2. Sliding mode observer design

In order to estimate biomass concentration and its specific growth rate, the variables $(\widehat{p}, \widehat{r}_x, \widehat{x})$ denote the estimation of (p, r_x, x), respectively. Since no model of μ is required, Eq. (2) is not needed. Then, the estimation error is defined as

$$\widetilde{p} = p - \widehat{p}, \tag{5}$$

$$\widetilde{r}_x = r_x - \widehat{r}_x, \tag{6}$$

$$\widetilde{x} = x - \widehat{x}. \tag{7}$$

In sliding mode observers the available measurements are used to construct the so-called switching function (σ). By using a discontinuous action, the objective is to reach the surface defined by $\sigma = 0$ in finite time and to stay on this surface from then on. If this is achieved, it results in a reduced-order dynamics system from which estimations can be obtained despite certain nonlinearities and uncertainties (Edwards and Spurgeon, 1998). From the previous assumptions, the only available measurement is the product concentration. Then, the switching function is defined as

$$\sigma = \widetilde{p}. \tag{8}$$

Thus, if $\sigma = 0$ is reached in finite-time $\widehat{p}$ copies the dynamics of the product formation rate and estimates of x and μ can be obtained. To this end, the following algorithm is considered.

Proposed algorithm: The proposed observer is given by the following equations

$$\dot{\widehat{p}} = \alpha(\widehat{r}_x + M_2|\widetilde{p}|^{1/2}sign(\widetilde{p})) + \beta\widehat{x} - Dp, \tag{9a}$$

$$\dot{\widehat{r}}_x = M_1 sign(\widetilde{p}), \tag{9b}$$

$$\dot{\widehat{x}} = \widehat{r}_x - D\widehat{x}, \tag{9c}$$

where M_1 and M_2 are positive parameters and $sign(\cdot)$ is the signum function. Notice that Eq. (9a) consists of a copy of Eq. (3) with the addition of a corrective term. Also the biomass formation rate is estimated in (9b) with a discontinuous term in the time derivative and therefore, it results in a continuous estimate of the variable r_x.

The error dynamics follow from differentiating (5)–(7):

$$\dot{\widetilde{p}} = \alpha\widetilde{r}_x + \beta\widetilde{x} - \alpha M_2|\widetilde{p}|^{1/2}sign(\widetilde{p}), \tag{10}$$

$$\dot{\widetilde{r}}_x = \frac{d}{dt}(r_x) - M_1 sign(\widetilde{p}), \tag{11}$$

$$\dot{\widetilde{x}} = \widetilde{r}_x - D\widetilde{x}. \tag{12}$$

By applying the change of variables $e_1 = \widetilde{p}/\alpha$, $e_2 = \widetilde{r}_x$, the equations can be written in the following form

$$\dot{e}_1 = e_2 - k_2|e_1|^{1/2}sign(e_1) + \rho_1(t), \tag{13a}$$

$$\dot{e}_2 = -\frac{k_1}{2}sign(e_1) + \rho_2(t), \tag{13b}$$

$$\dot{\widetilde{x}} = e_2 - D\widetilde{x}, \tag{13c}$$

with $(k_1, k_2) = (2M_1, \alpha^{1/2}M_2)$. The perturbations that affect the error coordinates (e_1, e_2) are

$$\rho_1(t) = \frac{\beta}{\alpha}\widetilde{x}, \tag{14a}$$

$$\rho_2(t) = \frac{d}{dt}(r_x). \tag{14b}$$

Clearly, perturbation ρ_1 is bounded for a bounded error in biomass concentration, whereas ρ_2 will always be bounded due to the physical meaning of the variable. A problem with ρ_1 is that the perturbation may be not zero for $e_1 = 0$. According to Eq. (13a), this means that e_2 may be not zero when the sliding surface is reached. However, it will be shown that the perturbation ρ_1 vanishes with first order dynamics when the system evolves in sliding mode regime.

To ensure that a sliding regime is established in finite time, positive parameters k_1 and k_2 (M_1 and M_2) should be properly chosen. A Lyapunov approach with quadratic-like functions was introduced in Moreno and Osorio (2008). Since then, several generalizations of the second order algorithms were developed in order to deal with different type of perturbations (see for instance ((Moreno, 2009; Picó et al., 2013))). For the unperturbed system (13), where $\rho_i = 0$, the solution of the Lyapunov equation

$$A^T P + PA = -Q, \tag{15}$$

provides suitable parameters for the observer, where $A = \begin{pmatrix} -k_2 & 1; -k_1 & 0 \end{pmatrix}$, $P = P^T > 0$ and $Q = Q^T > 0$ (see Moreno (2012) for more details). In case of absolute bounds for the perturbations, $|\rho_1(t)| < \bar{\rho}_1$ and $|\rho_2(t)| < \bar{\rho}_2$, a condition for finite-time convergence to a neighborhood of the origin is given in Moreno (2012) (Theorem 4.3, p. 123). Given the Eq. (13), the convergence

condition is valid for an arbitrary bound $\bar{\rho}_1 > 0$ but $\bar{\rho}_2$ small enough is required such that

$$\bar{\rho}_2 < \frac{1}{4}\frac{\lambda_{\min}(Q)}{\lambda_{\max}(P)}, \tag{16}$$

where $\lambda_{min}(Q)$, $\lambda_{max}(P)$ are the minimum and the maximum eigenvalues of Q and P, respectively. Eq. (15) can be solved analytically or with numerical methods.

If adequate parameters are chosen for the observer, the sliding surface $\widetilde{p} = 0$ is reached and by combining (10) and (12) with the invariance condition $\widetilde{p} = \dot{\widetilde{p}} = 0$ (Edwards and Spurgeon, 1998), the sliding mode dynamics yields

$$\alpha(\dot{\widetilde{x}} + D\widetilde{x}) + \beta\widetilde{x} = 0. \tag{17}$$

Then, the reduced-order dynamics for biomass estimation results in

$$\dot{\widetilde{x}} = -\left(\frac{\beta}{\alpha} + D\right)\widetilde{x}. \tag{18}$$

According to Eq. (18), the estimation error of biomass concentration converges to zero with first-order dynamics and from Eq. (12), $\widetilde{r}_x$ also vanishes. Therefore, an estimation of the specific growth rate can be defined as

$$\widehat{\mu} = \frac{\widehat{r}_x}{\widehat{x}}. \tag{19}$$

It is worth noting that there is a main difference with classical asymptotic observers since convergence of $\widehat{x}$ and $\widehat{r}_x$ can be achieved even in batch cultures (i.e. $D = 0$) for processes that satisfy $\beta > 0$. On the contrary, this convergence rate depends on process variables and may result slow if β/α is small. In such a case, the algorithm would only be useful for continuous and fed-batch cultivations.

Observe also that, the design of the observer is independent of the structure of μ and no modeling of this nonlinear and uncertain variable is required, except for a bound on the time-derivative of the biomass formation rate r_x. This parameter can be obtained from previous experience about the microbial strain (e.g. kinetic parameters) and process simulation.

The structure of the observer (9) differs from the previous product-based second-order algorithm presented in Nuñez et al. (2014). Here, biomass formation rate r_x is estimated instead of estimating only μ. This allowed writing the error dynamics in the form (13) and (14) which is more suitable for designing the observer parameters. Also dilution terms are included for application in fed-batch and continuous processes.

Analysis of parameter uncertainties: In Eq. (17), perfect knowledge of the product formation parameters was considered. Analysis for errors in the Luedeking–Piret coefficients can be performed by considering that the parameters used in the observer are different from the actual process values. Now, the observer parameters are denoted as $\alpha_o = a_1\alpha$ and $\beta_o = a_2\beta$, where the coefficients a_i are not equal to unity.

If a constant error in α is considered, the error dynamics of $\widetilde{p}$ (Eq. (10)) yields

$$\dot{\widetilde{p}} = \alpha\widetilde{r}_x - \alpha_o M_2|\widetilde{p}|^{1/2}sign(\widetilde{p}) + \beta\widetilde{x} + \alpha(1 - a_1)\widehat{r}_x. \tag{20}$$

Therefore, the last term also appears now in the perturbation $\rho_1 = \frac{\beta}{\alpha}\widetilde{x} + (1 - a_1)\widehat{r}_x$ described in Eq. (13). Clearly if $a_1 = 1$, Eqs.

(10) and (14a) are recovered. Recall that convergence conditions described in the previous section hold for $\bar{\rho}_1 > 0$ and the constraint (16) is required for ρ_2. From Eq. (20) and under sliding mode regime, the resulting dynamics is now given by

$$\alpha(\dot{\widetilde{x}} + D\widetilde{x}) + \beta\widetilde{x} + \alpha(1 - a_1)\widehat{r}_x = 0. \tag{21}$$

Thus, although asymptotic convergence is not obtained, the error $\widetilde{x}$ converges to a ball around the origin defined by

$$\widetilde{x} \to \mathcal{B}\left(\frac{|(a_1 - 1)|\bar{r}_x}{(\beta/\alpha) + D_{\min}}\right), \tag{22}$$

where $D_{min} \geq 0$ and the upper bound given for r_x is considered for $\widehat{r}_x$.

A similar analysis can be performed if mismatch in β is considered. Under sliding regime the dynamics would be

$$\alpha(\dot{\widetilde{x}} + D\widetilde{x}) + \beta\widetilde{x} + \beta(1 - a_2)\widehat{x} = 0, \tag{23}$$

and the biomass estimation error would converge to

$$\widetilde{x} \to \mathcal{B}\left(\frac{(\beta/\alpha)|(a_2 - 1)|\bar{\widehat{x}}}{(\beta/\alpha) + D_{\min}}\right). \tag{24}$$

Results (22) and (24) can be combined in case of mismatch in both parameters. It is worth noting here that the dilution rate has a positive effect by reducing the size of the error ball.

3.　Results and discussion

In this section, numerical results on batch and fed-batch processes are given in order to evaluate the performance of the observer. Photo-fermentation for biohydrogen production (batch mode) and a lactic acid production process (batch and fed-batch modes) are considered.

According to the main assumptions given in Section 2, the practical application of the proposed observer at industrial scale requires availability of product measures. In certain processes, the observer can be implemented with direct measures of the product (e.g. biohydrogen) whereas in other industrial fermentations indirect methods can be considered. For example, lactic acid concentration can be determined with conductivity measures of the fermentation broth (Payot and Fick, 1997) or by combining on-line pH and base added measures (Peter and Rock, 2012). Also, a previous study of the fermentation kinetics can be performed in order to estimate the coefficients of the Luedeking–Piret expression and to obtain a bound of the maximum rate of change of r_x. Then, according to the parameters obtained for Eq. (4) and the process dilution rate, the observer can be evaluated. The algorithm introduced in Section 2.2 was tuned with parameters $k_1 = 12, k_2 = 6$. These gains guarantee convergence for $|\dot{r}_x| = |\rho_2| < 0.187$ g/L/h^2 and were obtained for the matrices $Q = 4I$ and $P = \begin{pmatrix} 4.33 & -2; -2 & 1.36 \end{pmatrix}$ by solving Eq. (15). Although the same parameters are used in the three following cases, the observer can also be tuned according to the bound on $|\dot{r}_x|$ known for each case.

3.1.　Batch photo-fermentation of hydrogen

First, we consider biomass and growth rate estimation in a biohydrogen photo-fermentation process with the bacteria
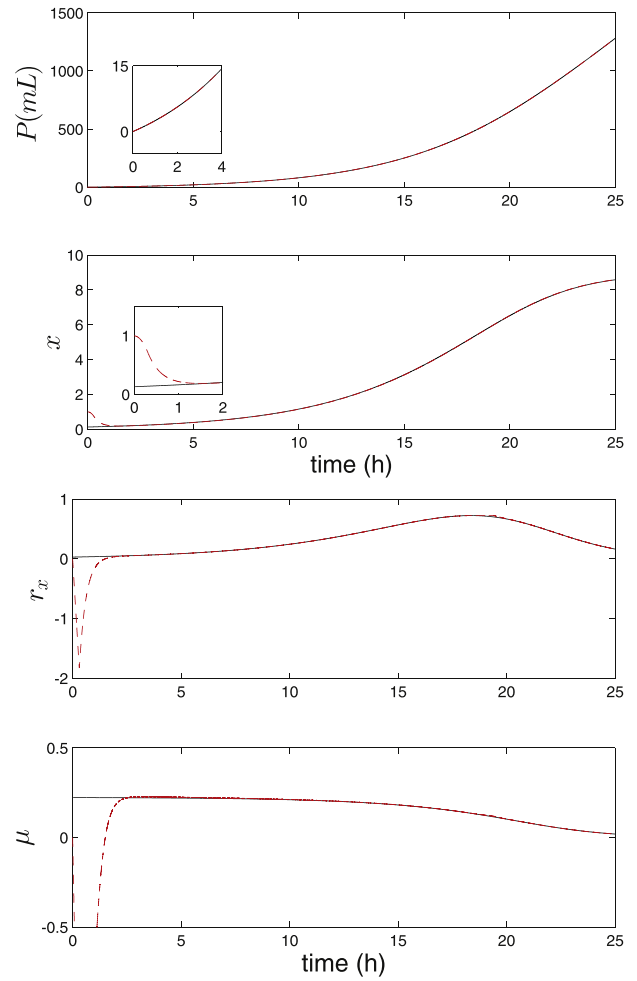
Fig. 1 – Results in batch photo-fermentation of hydrogen from *R. capsulatus*: simulated process variables (solid line) and observer variables (dashed line).

Rhodobacter capsulatus from organic acids, where hydrogen formation rate is given by Eq. (4) with

$$\alpha = \frac{\phi(I)}{y_{x/p}}. \tag{25}$$

In this expression, $\phi(I)$ is a term dependent on the light intensity applied to the bioreactor (Obeid et al., 2009). In this process, μ is represented with a Monod model

$$\mu = \mu_{\max}\frac{s}{s + k_s}, \tag{26}$$

where the kinetic parameters are $\mu_{max} = 0.4\,\text{h}^{-1}$ and $k_s = 10\,\text{g/L}$. The substrate consumption is represented with $r_s = r_x/y_{x/s}$ where the substrate to biomass yield is $y_{x/s} = 0.7$ g/g, and the Luedeking–Piret coefficients are $\alpha = 5$ and $\beta = 16$. The initial conditions for the batch cultivation were $x(0) = 0.13$ g/L, $s(0) = 12.57$ g/L and $p(0) = 0$ mL. The observer was tuned with initial conditions $\widehat{p}(0) = p(0)$ mL, $\widehat{r}_x(0) = 0$ g/L/h and $\widehat{x}(0) = 1$ g/L.

The results for biomass and growth rate estimation are presented in Fig. 1. The top plot shows the produced hydrogen, which is a gaseous product, and the estimated variable almost overlapped. The estimation of the biomass formation rate is shown in Fig 1c, where after a finite-time transient, the convergence rate depends on $\beta/\alpha = 3.2\,\text{h}^{-1}$. Estimation of biomass concentration is shown in Fig 1b where it is observed a relatively fast convergence to the actual biomass concentration.

Finally, Fig. 1d exhibits the estimate of μ defined in Eq. (19). At the end of the process, the reduction of μ may occur due to the depletion of substrate. Thus, the end of the batch process can also be estimated.

3.2.   Batch fermentation of lactic acid

Batch fermentation of lactic acid by *Lactobacillus rhamnosus* could also be described with a Luedeking–Piret kinetic expression. To emulate on-line measurement of the product concentration, the experimental fermentation data recently presented in Choi et al. (2014) was fitted to the logistic function

$$P(t) = \frac{P_{\max}}{1 + e^{-G(t - t_M)}}, \tag{27}$$

with $P_{max} = 98.325$ g/L, $G = 0.466$ h^{-1},   and $t_M = 9.32$ h (RMSE = 0.715).

The results of biomass concentration and μ estimation are presented in Fig. 2. The observer was tested with the coefficients $\alpha = 2.573$, $\beta = 0.412$ h^{-1} given in Choi et al. (2014) and the initial conditions were $\widehat{p}(0) = p(0) = 2.1$ g/L, $\widehat{r}_x(0) = 0$ g/L/h and $\widehat{x}(0) = 1$ g/L. The biomass estimation was compared with values reported by dry cell weight measurement. As can be seen in Fig. 2a, the estimation resulted in good correlation with experimental data during the growth phase. Since the biomass growth is inhibited by lactic acid accumulation, a decreasing μ is expected during the cultivation. The result of growth rate estimation is presented in Fig. 2b and is compared with estimates calculated from a Monod model with product inhibition given by

$$\mu = \mu_{\max} \frac{s}{s + k_s} \left(1 - \frac{p}{P_{\max}}\right)^c, \tag{28}$$

with $\mu_{max} = 0.511$ h^{-1}, $k_s = 0.3$ g/L, $P_{max} = 101.6$ g/L and $c = 2$ (Choi et al., 2014). Given the ratio $\beta/\alpha \approx 0.16$ h^{-1}, a relatively low convergence rate was obtained as it is seen from the comparison of μ with estimates calculated with Eq. (28). It is worth noting that at the end of the process the assumption of constant β seems not to be valid. As the time-derivative of p goes to zero this would cause a negative μ estimation with a consequent reduction in the estimated biomass concentration. Despite this, the estimation during the growing phase was satisfactory and can be considered for process monitoring. A possible variation to overcome the problem can be to consider a known time-varying function instead of a constant β in the structure of the observer This case will be considered in the next example.

3.3.   Fed-batch fermentation of lactic acid

The aim is to verify the convergence of the observer with a simulation under realistic conditions in fed-batch mode. Then, the fed-batch cultivation of *L. rhamnosus* with lactic acid production is considered. In Choi et al. (2014), it is reported a fed-batch case in which the bioreactor is fed with a modified exponential law that provides the carbon source (fructose and glucose from Arabic date juice). Also, a second flow rate of ammonia solution is applied in the time interval [0, 74] h. The initial volume of the culture was $v(0) = 2$ L.

Product concentration data was fitted with a smoothing spline function according to the parameters reported in Choi et al. (2014). This product signal plus measurement noise (of zero mean and $\sigma_p = 0.05$ g/L) was the input to the observer.
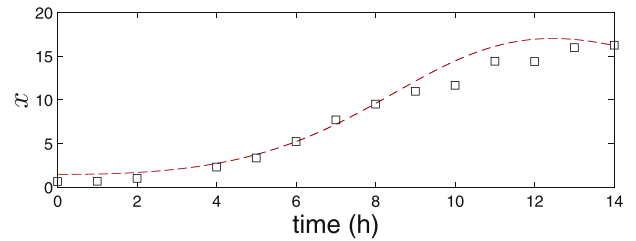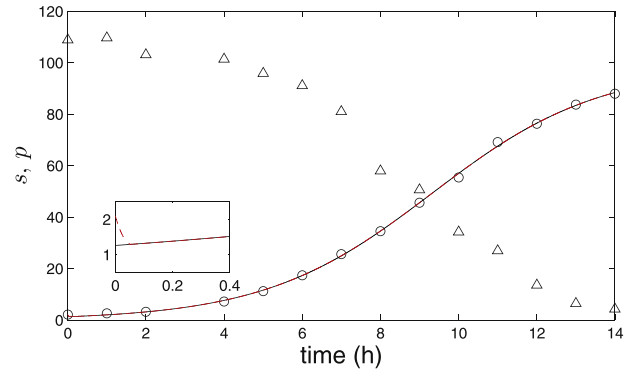
(a) Results for biomass estimation (dashed line) from lactic acid concentration (solid line). Batch data: substrate concentration (open triangle), lactic acid concentration (open circle) and biomass concentration (open square).
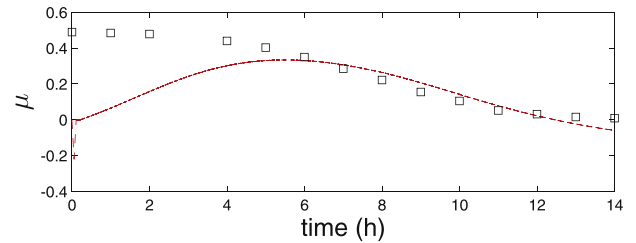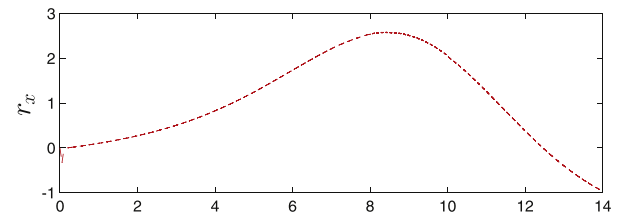
(b) Results for μ estimation: specific growth rate from batch data and eq. (28) (open square) vs estimated $\widehat{\mu} = \widehat{r}_x / \widehat{x}$ (dashed line).
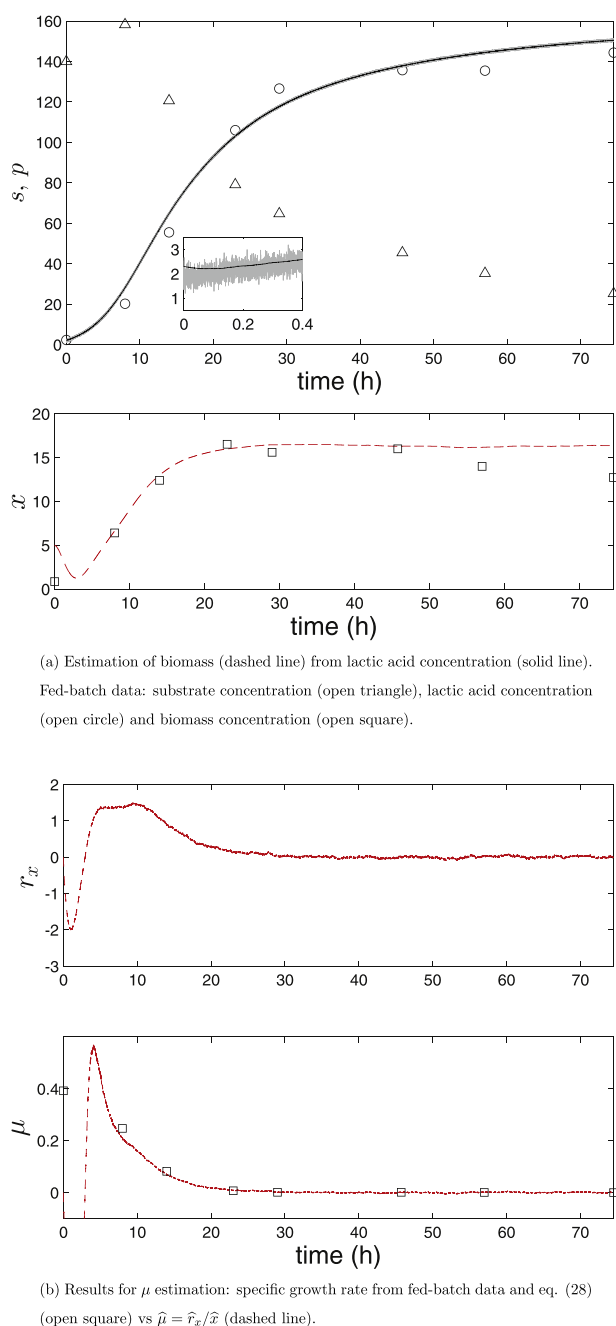
Fig. 2 – Results in batch culture for lactic acid production (experimental data from Choi et al. (2014)).

Since product inhibition may reduce the non-growth associated term, the expression

$$\beta(p) = \beta_0 \left(1 - \frac{p}{P'_{\max}}\right)^d, \tag{29}$$

was considered in the observer structure. The parameters for the process are $\alpha_0 = 1$, $\beta_0 = 1.078$ h^{-1}, $d = 1.75$ and $P'_{\max} = 166$ g/L (Choi et al., 2014). The initial conditions for the observer were $\widehat{p}(0) = p(0) = 2.3$ g/L, $\widehat{r}_x(0) = 0$ g/L/h and $\widehat{x}(0) = 5$ g/L.

The product signal and $\widehat{p}$ are shown overlapped in the top plot of Fig. 3a. In the bottom plot, the estimation of biomass concentration is compared with the off-line biomass data. A relatively fast convergence is shown (approx. 1 h), and also a good correlation with biomass experimental measures was obtained (particularly, during the first 45 h). During the stationary phase given by the product inhibition, the biomass concentration resulted in overestimated values

(a) Estimation of biomass (dashed line) from lactic acid concentration (solid line). Fed-batch data: substrate concentration (open triangle), lactic acid concentration (open circle) and biomass concentration (open square).

(b) Results for μ estimation: specific growth rate from fed-batch data and eq. (28) (open square) vs $\widehat{\mu} = \widehat{r}_x/\widehat{x}$ (dashed line).

Fig. 3 – Results in fed-batch culture for lactic acid production (experimental data from Choi et al. (2014)).

when compared with the measured data. The estimates of the biomass formation rate and specific growth rate are exhibited in Fig. 3b. Again, $\widehat{\mu}$ was compared with estimates given by Eq. (28) for the reported values of substrate and product concentrations. In the fed-batch culture, the parameters for (28) were $\mu_{max} = 0.415\,\mathrm{h}^{-1}$, $k_s = 0.3\,\mathrm{g/L}$, $P_{max} = 167.2\,\mathrm{g/L}$ and $c = 4$ (Choi et al., 2014). Since μ is a decreasing function of p, a nearly zero growth rate was obtained during the second part of the cultivation due to the higher concentration of product reached in fed-batch mode. It is worth noting that a better estimate of μ was obtained with the inclusion of the time-varying β given in Eq. (29).

4. Conclusion

In this work, biomass and specific growth rate were estimated from product concentration signals. The algorithm is

applicable to processes with a Luedeking–Piret like expression for product formation rate. Distinctive properties of the proposal are that it is applicable to batch, fed-batch and continuous processes, it does not depend on the kinetic model of μ and both variables can be tracked with reduced-order error dynamics. For batch cultures, the convergence dynamics depends on the ratio of the product formation coefficients and processes of slow and relatively fast convergence were considered. For processes with an input flow rate, the dilution term ensures convergence, at least to an error ball around the origin, even in case of a low β/α ratio. The estimates exhibited a good agreement with documented experimental data.

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