

# Reaction rate reconstruction from biomass concentration measurement in bioreactors using modified second-order sliding mode algorithms

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**Abstract** This paper deals with the estimation of unknown signals in bioreactors using sliding observers. Particular attention is drawn to estimate the specific growth rate of microorganisms from measurement of biomass concentration. In a recent article, notions of high-order sliding modes have been used to derive a growth rate observer for batch processes. In this paper we generalize and refine these preliminary results. We develop a new observer with a different error structure to cope with other types of processes. Furthermore, we show that these observers are equivalent, under coordinate transformations and time scaling, to the classical super-twisting differentiator algorithm, thus inheriting all its distinctive features. The new observers' family achieves convergence to time-varying unknown signals in finite time, and presents the best attainable estimation error order in the presence of noise. In addition, the observers are robust to modeling and parameter uncertainties since they are based on minimal assumptions on bioprocess dynamics. In addition, they have interesting applications in fault detection and monitoring. The observers performance in batch, fed-batch and continuous bioreactors is assessed by experimental data obtained from the fermentation of *Saccharomyces Cerevisiae* on glucose.

**Keywords** Bioreactors · Bioprocess control · Bioprocess observers · Sliding modes

## Introduction

Biotechnological process control and monitoring represent a big challenge because of model uncertainty, unpredictable parameter variations, scarce on-line measurements of most representative variables, etc. For these reasons, extended work has been carried out in the field of parameter and signal estimation in bioreactors [11, 39]. As a result, many software sensors have been developed to estimate variables of interest from the measurement of other process variables. Basically, one may consider two main types of potential variables to be estimated: reaction rates and species concentrations [1]. In this paper we focus on the estimation of reaction rates and, particularly, of specific growth rates. The motivation is that control specifications are often related with the growth rate of microorganisms, whether the objective is to maximize biomass production or to maintain a metabolic steady state [19, 34, 36, 40]. In addition, growth rate estimators provide essential information to monitor the development of microorganisms.

Different methods have been developed to estimate variables and parameters in bioprocesses and the literature is very large. Some of these methods are based on the Kalman or extended Kalman filter [20, 29, 38, 39, 41]. However, they usually result in complex algorithms that in general do not guarantee convergence [10]. Another approach consists in using asymptotic or high-gain observers (see for instance [1, 3, 18, 24]) and the measurement of some key variables. One of the main limitations of observers is their lack of robustness in the estimation of some variables when they rely on a reaction model and/or

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the knowledge of yield coefficients. This problem can be overcome extending the order of the observer to adapt some uncertain parameters (see for instance [14]). When the specific growth rate is the variable to be estimated, very robust adaptive high-gain observers can be designed if on-line biomass concentration measurement is available. With this purpose, some on-line biomass sensors are currently available (see for instance [28] and [21]). This is the approach followed, for instance, in [2, 10, 30] where the specific growth rate is estimated from the biomass growth dynamics without using any reaction model. By this reason, the growth rate can be viewed as an unknown input signal to the biomass dynamics.

During the last decades, considerable research activity has been devoted to design algorithms for unknown input reconstruction. Furthermore, many of them have been originally developed or applied to bioreactors. Some of these algorithms essentially consist in differentiating the output measurement [31]. This approach is used in [6], where the measured signal is filtered over a 20-min window to reduce the underlying noise effects. Another widely extended approach consists in using state observers of measured variables, the estimate error being used to construct or adapt the signal estimate. In some cases, a nominal signal is supposed to be known, which is statically corrected in proportion to the output estimation error [5]. In other cases, the observer dynamics is extended to adapt the signal estimate dynamically [3]. In any case, since the adaptation algorithm is based on the output estimate error, signal reconstruction using continuous observers can be achieved up to a bounded uncertainty, which depends on the magnitude of the signal or/and its time derivatives. Despite this theoretical limitation, these approaches offer comprehensive solutions in many bioreactor applications (see [3] and related papers). However, it should be taken into account that they could introduce some stability problems in closed-loop applications.

The use of discontinuous observers appears to be an attractive alternative. Discontinuous output error injection can be designed to induce a sliding motion on the state estimation error space, thus enforcing the observer to copy the process output despite disturbances and model uncertainties. At the same time, the error is used in some way to reconstruct the unknown signal. Also, sliding observers generate residuals and have interesting applications in fault detection [12]. In the authors knowledge, sliding observers for bioprocesses based on biomass measurement appeared for the first time in [33]. A switching term added to the continuous estimate provides finite time convergence to the unknown signal up to a very high frequency component. More recently, a second-order sliding observer has been presented, which outperforms the previous one [9]. This new observer, which shares some ideas with [25], differs in

the structure of the discontinuous output error injection. Discontinuity appears in the first derivative of the estimate rather than in the estimate itself, thus significantly reducing chattering while the most attractive features of sliding mode observers are preserved.

In this paper, we revisit this approach. We take the second-order sliding observer mentioned above, which has been specifically designed to deal with the nonlinear dynamics of batch processes, as starting point for our last developments. Another observer based on the same sliding mode concepts but with a different error structure is proposed to cope with a larger set of bioprocess dynamics. We also derive some tools to tune the observers. On the other side, we demonstrate that these observers are equivalent under different state and time coordinate transformations to the classical super-twisting differentiator [22, 23]. Consequently, these observers inherit all the attractive features of the super-twisting algorithm. They exhibit finite time convergence to the time-varying unknown signal, which is particularly attractive property in control applications because the observer dynamics do not affect closed-loop stability and performance. Also, they are very robust since they use no model of the reaction. In addition, their off-surface coordinates are signals very sensitive to sensor faults and unpredicted behavior.

## Theoretical framework

Let us illustrate with a simple example the use of sliding mode observers for signal reconstruction. Suppose that the problem is the estimation of signal  $u(t)$  from measurement of its integral  $\zeta$ :

$$\dot{\zeta} = u(t) \quad (1)$$

Suppose that the absolute magnitude of  $u(t)$  is bounded by  $|u(t)| < 1$ . Then, the following conventional sliding mode algorithm can be used to reconstruct  $u$ :

$$\begin{cases} \dot{z} = \hat{u} \\ \hat{u} = \alpha \operatorname{sign}(\zeta - z) \end{cases} \quad (2)$$

See that the dynamics of the error  $s \triangleq \zeta - z$  is given by

$$\dot{s} = u(t) - \alpha \operatorname{sign}(s) \quad (3)$$

The solution to (3) for any  $u(t)$  with  $|u(t)| < 1$  satisfies also the differential inclusion<sup>1</sup>

$$\dot{s} \in \Upsilon - \alpha \operatorname{sign}(s) \quad (4)$$

with  $\Upsilon = [-1, 1]$ . For constants  $\alpha > 1$ , any  $u(t) \in \Upsilon$  is dominated by the second term in (3). Then, the state converges in finite time to the surface defined by  $s = 0$ . From

<sup>1</sup> Solutions are understood in the sense of Filippov.

then on, the discontinuous term switches at ideally infinite frequency, establishing a sliding motion on the surface. In sliding mode  $z(t)$  perfectly tracks  $\zeta(t)$ , so  $\hat{u}(t)$  coincides with  $u(t)$  except for a very high frequency error term. The input signal  $u(t)$  can then be reconstructed by filtering the discontinuous estimate. Alternatively, the  $\text{sign}(\cdot)$  function can be replaced with a high-gain continuous function to avoid discontinuity. In both cases, just convergence to a close vicinity can be guaranteed, even in the absence of measurement noise.

Suppose now that the time derivative of  $u(t)$  is bounded by  $|\dot{u}(t)| \leq 1$  while  $u(t)$  is not necessarily bounded. Conventional sliding mode algorithms to reconstruct  $u(t)$  can still be designed. However, more interestingly, second-order sliding mode concepts can be alternatively exploited. Among all the second-order sliding mode algorithms, the super-twisting is the most attractive one for this purpose because it provides a smooth estimate without requiring any further information about  $u(t)$ . The super-twisting algorithm has been proposed in [22]:

$$\begin{cases} \dot{z}_1 = \hat{u} + 2\beta|\zeta - z_1|^{1/2} \text{sign}(\zeta - z_1) \\ \dot{z}_2 = \alpha \text{sign}(\zeta - z_1) \\ \hat{u} = z_2 \end{cases} \quad (5)$$

Note that, differing from the conventional first-order sliding mode algorithm, discontinuity appears in the first derivative  $\dot{\hat{u}}$  rather than on  $\hat{u}$ .

Taking  $s \triangleq \zeta - z_1$  and  $q \triangleq u - z_2$  we obtain the error dynamics

$$\begin{cases} \dot{s} = q - 2\beta|s|^{1/2} \text{sign}(s) \\ \dot{q} = \dot{u} - \alpha \text{sign}(s) \end{cases} \quad (6)$$

The solution to (6) for any  $u(t)$  with  $|\dot{u}(t)| < 1$  satisfies also the differential inclusion

$$\begin{cases} \dot{s} = q - 2\beta|s|^{1/2} \text{sign}(s) \\ \dot{q} \in Y - \alpha \text{sign}(s) \end{cases} \quad (7)$$

Whereas the discontinuous signal dominates the unknown input rate  $\dot{u}(t)$ , finite time convergence to  $s \equiv 0$  is still guaranteed thanks to the continuous, but not Lipschitz, third term in (6). A typical state trajectory converging to the surface  $s = 0$  is plotted in Fig. 1. Originally, stability conditions and convergence rate were derived geometrically from Fig. 1 using majorant curves (see for instance [8, 22]). A Lyapunov approach has been proposed for the first time in [26] and then improved in [27]. After convergence to the surface  $s = 0$ , a sliding regime is established. Once in sliding mode, the invariance condition  $(s, q) = 0$  holds. Then, the state  $z_1$  copies  $\zeta(t)$  and  $u(t)$  is reconstructed. Now,  $\hat{u}(t)$  perfectly tracks  $u(t)$  and is continuous.

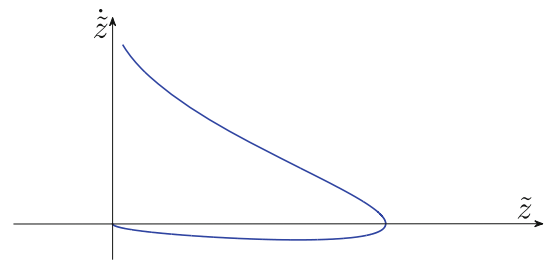


Fig. 1 Typical convergence trajectory of the super-twisting sliding mode algorithm

Algorithm (5) exhibits the following properties:

- convergence in finite time  $T$ ,
- exactness, in the sense that  $\hat{u} \equiv u(t) \forall t > T$  in the absence of noise,
- robustness, in the sense that  $\hat{u}$  tends uniformly to  $u(t)$  as  $z_1$  tends uniformly to  $\zeta$ ,

Moreover, the reconstructor features are not seriously deteriorated by discrete measurement with sufficiently small sampling period, being the estimation error proportional to the sampling time. For the proofs of these statements, the reader is referred, for instance, to [22]. Also, readers unfamiliar with first- and high-order sliding mode observers may consult the works [4, 12, 15, 16, 17].

### Problem statement and main results

#### Bioprocess dynamics

Consider the dynamics of microorganism growth in a bioreactor

$$\begin{cases} \dot{x} = (\mu - F_i(t)/v)x \quad x(0) > 0 \\ \dot{v} = F_i(t) - F_0(t) \quad v(0) > 0 \end{cases} \quad (8)$$

where  $x$  is the biomass concentration,  $v$  is the liquid volume in the bioreactor,  $\mu$  is the specific growth rate of microorganisms,  $F_i \geq 0$  and  $F_0 \geq 0$  are the inlet (free of biomass) and outlet flow rates, respectively. Suppose that  $x$  is measured, and that  $v$  and  $F_i(t)$  are known or measured. Thus, the objective is to estimate  $\mu$  under the assumption that  $x$  and  $v$  remain strictly positive. Note that this will be always true in practice. Otherwise, the problem does not make sense. On the other hand, biomass concentration is bounded because of the mass balance principle.

The specific growth rate  $\mu$  is function of the concentration of several nutrients in the bioreactor as well as on environmental conditions. Our purpose is to design robust observers for  $\mu$  not relying on models of the reaction kinetics and nutrient dynamics, which are only barely known in real world. That is why we treat  $\mu$  as an external

unknown input. Like in Luenberger-like adaptive observers [3, 14, 32], an upper-bound on  $|\dot{\mu}|$  is needed to tune the sliding mode algorithms. Mainly, two types of upper-bounds are usually considered for  $|\dot{\mu}|$ . In continuous bioreactors, it is reasonable to use absolute bounds. However, in batch and fed-batch bioreactors, where biomass grows significantly during the process, it makes more sense to consider a biomass-proportional bound on  $|\dot{\mu}|$ . This is corroborated in the Appendix where expressions of these bounds for a typical reaction model are derived.

### Problem formulation

Biomass dynamics in the first line of (8) can be rewritten as

$$\dot{x} = f(x, t) + x\mu(t) \quad x(0) = x_0 > 0 \quad (9)$$

where the state  $x \in \mathfrak{R}$  is measured,  $\mu \in \mathfrak{R}$  is unknown and time-varying, and function  $f(x, t) = -xF_1(t)/v(t)$  is known. Note that  $f(x, t)$  is well-defined under the assumption that  $v > 0$ . Hereinafter,  $\mu(t)$  is considered an unknown input to system (9).

The bioprocess dynamics (9) is bilinear in  $x$  and  $\mu$ . Conventional first- and high-order sliding mode input reconstructor algorithms are conceived for systems linear in the input. So, a first approach consists of reconstructing the product  $x\mu$ , and then divide by  $x$  to obtain  $\mu$ . However, this straightforward application of the existing algorithms is not optimum in terms of noise. In this paper we evaluate the use of high-order sliding mode ideas to reconstruct  $\mu$  directly from (9). We consider two algorithms actually, which deal with the bilinearity of (9) and are applicable to growth rates with biomass-proportional and absolute derivative bounds.

**Definition 1** Let  $\mathcal{U}$  be the set of inputs for which a solution to (9) exists. Let  $\mathcal{X}$  be the set of solutions to (9) for all  $\mu \in \mathcal{U}$ .

A solution  $x(t)$  is said to be strictly positive and bounded if there exist constants  $\underline{x} > 0$  and  $\bar{x} > 0$  such that  $\underline{x} \leq x(t) \leq \bar{x} \forall t \in \mathfrak{R}^+$ .

**Definition 2** Let  $\mathcal{U}^+ \subset \mathcal{U}$  be the set of all inputs such that the solution  $x(t)$  to (9) is strictly positive and bounded. Let  $\mathcal{X}^+ \in \mathcal{X}$  be the set of all bounded and strictly positive solutions.

**Definition 3** Let  $\overline{\mathcal{U}}^+(\cdot)$  be the set of inputs in  $\mathcal{U}^+$  with bounded time derivative. That is  $\overline{\mathcal{U}}^+(\rho) = \{\mu \in \mathcal{U}^+ : |\dot{\mu}| < \rho\}$ .

In batch processes, the growth rate derivative is proportional to biomass concentration and accepts a biomass-proportional bound. The same may occur in fed-batch

processes when substrate is supplied in proportion to biomass (see the Appendix). Obviously, for bounded solutions, an absolute bound also exists but it may be too conservative. So, we define the following set of input signals:

**Definition 4** Let  $\overline{\mathcal{U}}_x^+(\cdot)$  be the set of inputs in  $\overline{\mathcal{U}}^+$  with state-proportional bounded time derivative. That is  $\overline{\mathcal{U}}_x^+(\rho) = \{\mu \in \overline{\mathcal{U}}^+(\rho) : |\dot{\mu}| < \rho x/\bar{x}\}$ .

Then, the task is to estimate the unknown input  $\mu \in \overline{\mathcal{U}}^+(\cdot)$  or  $\mu \in \overline{\mathcal{U}}_x^+(\cdot)$  to (9) from measurement of  $x \in \mathcal{X}^+$ .

### Sliding observer for growth rates with biomass-proportionally bounded time derivative

Consider the process

$$\mathcal{P}_{x\rho} : \begin{cases} \dot{x} = f(x, t) + x\mu(t) & x(0) = x_0 > 0 \\ \mu \in \overline{\mathcal{U}}_x^+(\rho) \end{cases} \quad (10)$$

Then,

$$\mathcal{O}_{\text{SMI}} : \begin{cases} \dot{z}_1 = f(x, t) + \left(z_2 + 2\beta|\zeta|^{\frac{1}{2}}\text{sign}(\zeta)\right)\rho x \\ \dot{z}_2 = \frac{x}{\bar{x}}\text{sign}(\zeta) \\ \zeta = (\rho\bar{x})^{-1}(x - z_1) \\ \hat{\mu} = \rho z_2 \end{cases} \quad (11)$$

is an observer for (10) that converges in finite time for suitable gains  $\alpha$  and  $\beta$ . Convergence of this observer has been already investigated in [9]. There, the observer error dynamics is transformed to a polytopic one, and LMIs were used to assess on its stability. Here, we present an alternative approach to the problem. We show that the observer is equivalent, after a time scale transformation, to the standard super-twisting algorithm. Then, all the classical results about stability and tuning of the super-twisting algorithm can be applied [22, 27].

First, let us apply the coordinates transformation  $(\zeta(x, z_1), \varphi(u, z_2))$  with  $\varphi = \frac{\mu}{\rho} - z_2$ :

$$\begin{cases} \dot{\zeta} = \frac{x}{\bar{x}}(\varphi - 2\beta|\zeta|^{\frac{1}{2}}\text{sign}(\zeta)) \\ \dot{\varphi} = \frac{\mu}{\rho} - \alpha\frac{x}{\bar{x}}\text{sign}(\zeta) \end{cases} \quad (12)$$

Consider now the time scale transformation  $\mathfrak{T} : \mathfrak{R}^+ \mapsto \mathfrak{R}^+$

$$\tau = \mathfrak{T}(t) \triangleq \int_0^t \frac{x(\xi)}{\bar{x}} d\xi. \quad (13)$$

Note that  $\mathfrak{T}$  exists and is invertible for any  $x \in \mathcal{X}^+$ . Moreover, (12) is independent of  $x$  after the time scaling (13). In fact, the observer error dynamics in the new time scale is

$$\begin{cases} \zeta' = \varphi - 2\beta|\zeta|^{\frac{1}{2}}\text{sign}(\zeta) \\ \varphi' = \frac{\mu'}{\rho} - \alpha\text{sign}(\zeta) \end{cases} \quad (14)$$

where ' denotes differentiation with respect to  $\tau$ . Considering that  $|\mu'| < \rho \forall \mu \in \overline{\mathcal{U}}_x^+(\rho)$ , it follows that  $\overline{\mathcal{U}}_x^+(\rho) \subset \{\mu : \mu' \in \rho\Upsilon, \forall \tau\}$ , where  $\Upsilon = [-1, +1]$ . Then, any solution to (14) for  $\mu \in \overline{\mathcal{U}}_x^+(\rho)$  satisfies also the differential inclusion

$$\begin{cases} \zeta' = \varphi - 2\beta|\zeta|^{\frac{1}{2}}\text{sign}(\zeta) \\ \varphi' \in \Upsilon - \alpha\text{sign}(\zeta) \end{cases} \quad (15)$$

Note that inclusion (15) is independent of the original system (10) and of  $\mu$ . Moreover, it represents the family of sliding surface coordinate dynamics of the super-twisting sliding algorithm (see (7)). Finite-time convergence of (15) has been already demonstrated for suitable gains  $\alpha$  and  $\beta$  (see for instance [22] and [27]). That is, after a finite time  $\tau^*$  a sliding regime is established on surface  $\zeta = 0$ . The sliding mode invariance condition  $\zeta(\tau) \equiv 0$ , i.e.  $\zeta(t) \equiv 0$ , implies  $z_1(t) = x(t)$  and  $\hat{\mu}(t) = \mu(t) \forall t > \mathfrak{T}^-(\tau^*)$ .

Sliding observer for growth rates with absolutely bounded time derivative

Consider now the process

$$\mathcal{P}_\rho : \begin{cases} \dot{x} = f(x, t) + x\mu(t) & x(0) = x_0 > 0 \\ \mu \in \overline{\mathcal{U}}^+(\rho) \end{cases} \quad (16)$$

where  $\hat{\mu}$  accepts an absolute bound  $\rho$ . Then,

$$\mathcal{O}_{SM2} : \begin{cases} \dot{z}_1 = \left(\frac{f(x,t)}{x} + \rho z_2 + 2\rho\beta|\sigma|^{\frac{1}{2}}\text{sign}(\sigma)\right)z_1 \\ \dot{z}_2 = \alpha\text{sign}(\sigma) \\ \sigma = \rho^{-1}\ln(x/z_1) \\ \hat{\mu} = \rho z_2 \end{cases} \quad (17)$$

with  $z_1(0) > 0$ , is proposed as an observer for (16), where  $z_1$  is the estimated biomass and  $\hat{\mu}$  is the estimated growth rate. Furthermore, we will demonstrate that this observer converges in finite time. Note that a logarithmic law  $\sigma = \rho^{-1}\ln(x/z_1)$  is used as observer error. This error signal is well-defined since  $x$  is strictly positive and  $z_1$  diverges from 0. In fact,  $\sigma \rightarrow \infty$  and  $z_2$  is increasing as  $z_1 \rightarrow 0^+$ . Therefore, the right hand side of the first equation in (17) becomes positive for  $z_1 > 0$  small enough.

This nonlinear definition of the observer error allows us, after a smooth change of coordinates, to transform (17) into the standard super-twisting sliding algorithm.

In fact, apply the coordinates transformation  $(\sigma(x, z_1), \phi(u, z_2))$  with  $\phi = \frac{u}{\rho} - z_2$ :

$$\begin{cases} \dot{\sigma} = \phi - 2\beta|\sigma|^{\frac{1}{2}}\text{sign}(\sigma) \\ \dot{\phi} = -\frac{\dot{u}}{\rho} - \alpha\text{sign}(\sigma) \end{cases} \quad (18)$$

Considering that  $\overline{\mathcal{U}}^+(\rho) \subset \{\mu : \dot{\mu} \in \rho\Upsilon, \forall t\}$ , any solution to (14) for  $u \in \overline{\mathcal{U}}^+(\rho)$  satisfies also the differential inclusion

$$\begin{cases} \dot{\sigma} = \phi - 2\beta|\sigma|^{\frac{1}{2}}\text{sign}(\sigma) \\ \dot{\phi} \in \Upsilon - \alpha\text{sign}(\sigma) \end{cases} \quad (19)$$

Inclusion (19) represents the family of sliding surface coordinate dynamics of the super-twisting sliding mode algorithm (see (7)). This proves finite-time convergence of (19) for suitable gains  $\alpha$  and  $\beta$  [22, 27]. That is, after a finite time  $t^*$ , a sliding regime is established on surface  $\sigma = 0$ . The sliding mode invariance condition  $\sigma(t) \equiv 0$  implies  $z_1(t) = x(t)$  and  $\hat{\mu}(t) = \mu(t) \forall t > t^*$ .

*Remark 1* The sliding surface coordinates  $\zeta$  and  $\sigma$  not only indicate convergence of the algorithm but also divergence caused by unexpected fast growth rate variations. Therefore, they are effective residuals to indicate bioreactor malfunctions, sensor faults or changes in microorganism behavior (both abrupt and gradual).

*Remark 2* The proposed observers can be used to estimate the kinetic rate  $r(t)$  in any reaction of the form

$$\dot{p} = r(p, t)p + g(p, t) \quad (20)$$

provided analogous assumptions to the ones made here are fulfilled.

### Experimental results

Three experiments were carried out to assess the observers performance in real world. Experimental results obtained from the fermentation of the industrial strain *Saccharomyces Cerevisiae* T73 (wild type) are presented in this section. Processes in batch, continuous and fed-batch modes were run. Biomass measurement was carried out using the sensor described in [28]. This sensor took samples every 12 s and returned a filtered value over a window of 2 min. These measures ( $x_m$ ) were injected to the proposed sliding observers to estimate  $\mu(t)$ . As suggested above, observer (11) was used in the batch and fed-batch processes, whereas (17) was used in the continuous one. Both observers have been tuned as a compromise between convergence and sensitivity. We have found  $\alpha = 1.1$  and  $\beta = 1.8$  suitable for this application. Nevertheless, the key parameter is  $\rho$  (or the product  $\rho\alpha$ ). Note that the higher the gain  $\rho$ , the faster the observer will converge, but the higher the noise sensitivity will be. Also, robustness against uncertainties in  $\max_t(|\dot{\mu}(t)|)$  will increase with  $\rho$ , but sensitivity to faults will decrease in the same manner. Preliminary bounds on  $|\dot{\mu}|$  used to tune  $\rho$  were obtained following the derivations in the Appendix. Then, these

bounds were finely tuned based on previous experimental data and our own experience in the process. Alternatively,  $\rho$  can be continuously adapted to improve robustness without unnecessarily increasing output noise following some of the procedures recently presented in [7, 13, 35, 37].

For comparative purposes, we obtain another  $\mu$ -estimate by numerical differentiation of the measured signal:

$$\mu_d = \frac{\dot{x}_m}{x_m} + \frac{F_i}{v} \quad (21)$$

Although quite crude, this method provides an exact estimate under noiseless conditions. Obviously, because of measurement noise and the way it is constructed,  $\mu_d$  is highly corrupted with noise. Of course, the real growth rate is not available to compare with the sliding observer outputs. Nevertheless, in the figures shown below, the real  $\mu$  can be guessed behind the noisy  $\mu_d$ .

### Batch reactor

The first process was run in batch mode. The initial condition was  $x(0) = 0.1$  g/L. Biomass concentration and growth followed time-varying profiles. The process finished when the nutrients in the bioreactor, initially in excess, were consumed. The growth rate of microorganisms was estimated with observer (11) tuned with  $\alpha = 1.1$ ,  $\beta = 1.8$  and  $\rho = 0.5$ .

Figure 2 shows the experimental results. Figure 2a plots the measured biomass concentration ranging from 0.1 to 3.5 g/L. As it is typical in batch processes, measurement was not reliable and highly corrupted with noise during the first hours because of the low initial biomass. Consequently, the growth rate estimation  $\mu_d$  varied randomly between unacceptable large limits (see Fig. 2b). It is also observed in Fig. 2a that growth dropped abruptly at  $t = 14$  h, most probably due to the depletion of some essential substrate. Growth stopped at  $t = 24$  h when nutrient exhausted. Figure 2c zooms out the plot of  $\mu_d$  and shows also the estimate provided by the observer, which was initialized at  $((\hat{x}(0), \hat{\mu}(0)) = (0, \mu_m))$ . Since a biomass-proportional bound was used, the observer output evolved slowly at the beginning preventing the estimate from large and infeasible deviations. After convergence, the sliding observer provided a smooth estimate that closely tracked the real growth rate. Of course, there is a trade-off in the selection of  $\rho$  between noise sensitivity and convergence rate. Figure 2d depicts the sliding surface coordinate. It is seen that the observer took 6 h to converge.

### Fed-batch reactor

The second fermentation was run in fed-batch mode in a biostat B5 bioreactor. The initial conditions, obtained from

a previous batch phase, were  $x(0) = 2.6$  g/L and  $v(0) = 1$  L. The inlet substrate concentration was  $s_i = 20$  g/L. The specific growth rate of microorganisms was reconstructed with observer (11) tuned with  $\alpha = 1.1$ ,  $\beta = 1.8$  and  $\rho = 0.15$ .

Figure 3 shows the experimental results. Figure 3a plots the exponential-like input flow, which was proportional to biomass population ( $F_i(t) = \lambda xv$ ). Figure 3b depicts the biomass concentration measures  $x_m$ , and the estimate  $\hat{x} = z_1$  provided by the observer. During approximately 1 h, between  $t = 11.7$  h and  $t = 12.8$  h, the pump of the biomass sensor run unprimed, thus leading to erroneous measures and abrupt changes. On the other hand, the observer provided a smooth estimate  $\hat{x}$ . Figure 3c displays the measured volume used to determine the feeding law. The process was interrupted when the volume reached 3 L. Figure 3d shows the specific growth rate estimation  $\mu_d$  obtained according to (21) and  $\hat{\mu}$  provided by the sliding observer. Recall that, assuming that the biomass sensor works properly,  $\mu_d$  represents the real growth rate to which a large high-frequency noise signal is added. It is observed that  $\mu_d$  was very noisy and became unstable when the fault in the biomass sensor occurred. On the contrary, the sliding observer provided a much smoother estimate that converged rapidly at the beginning of the process (the initial biomass concentration was much higher than in the batch process) and remained stable in the presence of the fault. Furthermore, the sliding estimate was almost insensitive to such abrupt perturbation. Meanwhile, the sliding surface coordinate was very sensitive to the sensor fault. Effectively, it is seen in Fig. 3e that the observer diverged at  $t = 12.8$  h because of the sensor fault and converged again 1 h after the fault was cleared. Far from being a drawback of the observer, its divergence indicated the occurrence of the fault, an abrupt fault in this case.

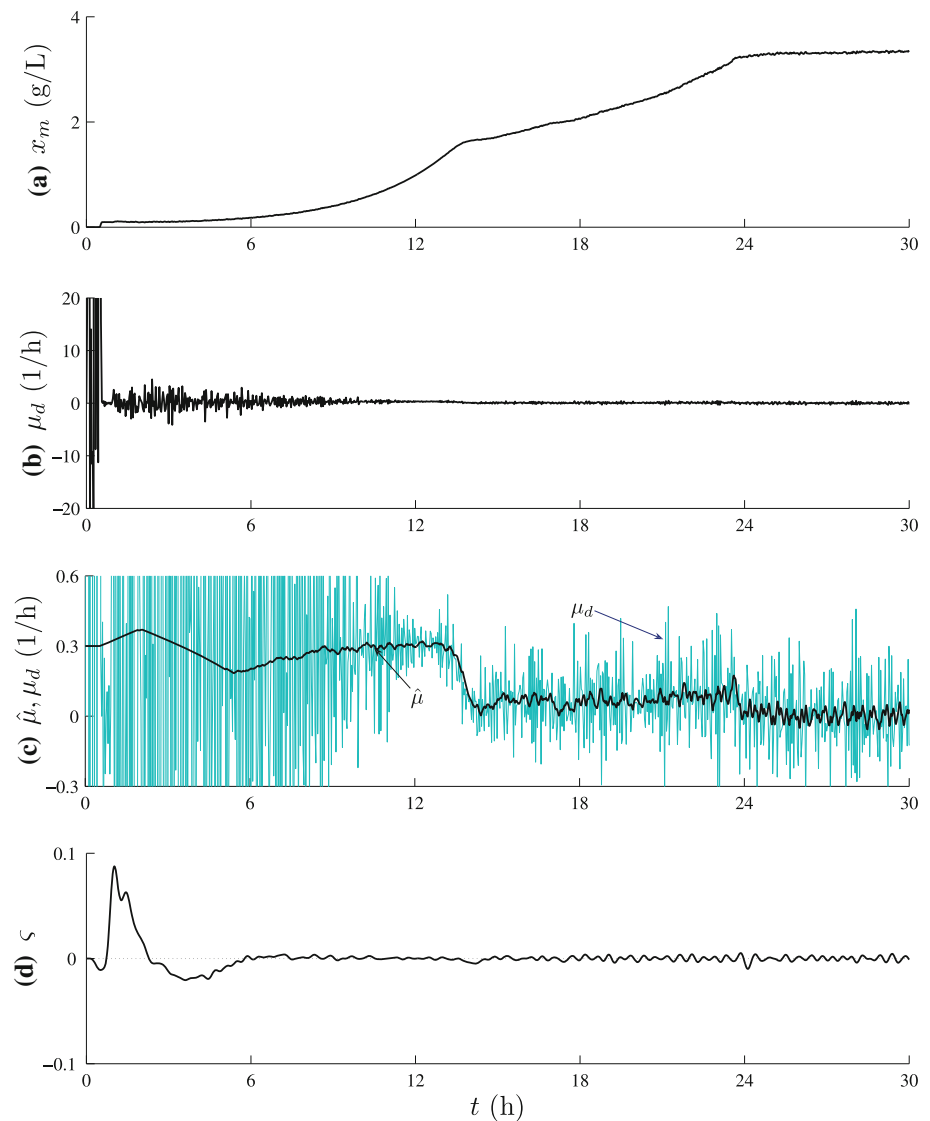
### Continuous reactor

The third experiment took place in a chemostat. The initial conditions were  $x(0) \simeq 11.7$  g/L and  $s(0) \simeq 0$  g/L. The inlet substrate concentration was  $s_i = 10$  g/L, whereas the reactor volume was  $v = 3$  L. The input flow  $F_i(t) = \mu_r v$  was piecewise constant. The initial set-point was  $\mu_r = 0.18$  h<sup>-1</sup>, and a reference step  $\Delta\mu_r = 0.04$  h<sup>-1</sup> was applied at time  $t = 25$  h.

The growth rate of microorganisms was estimated with observer (17), which used a biomass-independent upper-bound on  $\hat{\mu}$ . The observer was tuned with  $\alpha = 1.1$ ,  $\beta = 1.8$  and  $\rho = 0.5$ .

Figure 4 shows experimental data collected during the first 54 h of the process. The top plot displays the piecewise constant feeding profile. Figure 4b depicts the biomass concentration measures  $x_m$ , which were used to

**Fig. 2** Experimental results for the batch process. **a** Measured biomass concentration. **b** Estimated growth rate using measurement differentiation. **c** Growth rate estimates using measurement differentiation (*out of scale*) and sliding observer. **d** Sliding surface coordinate



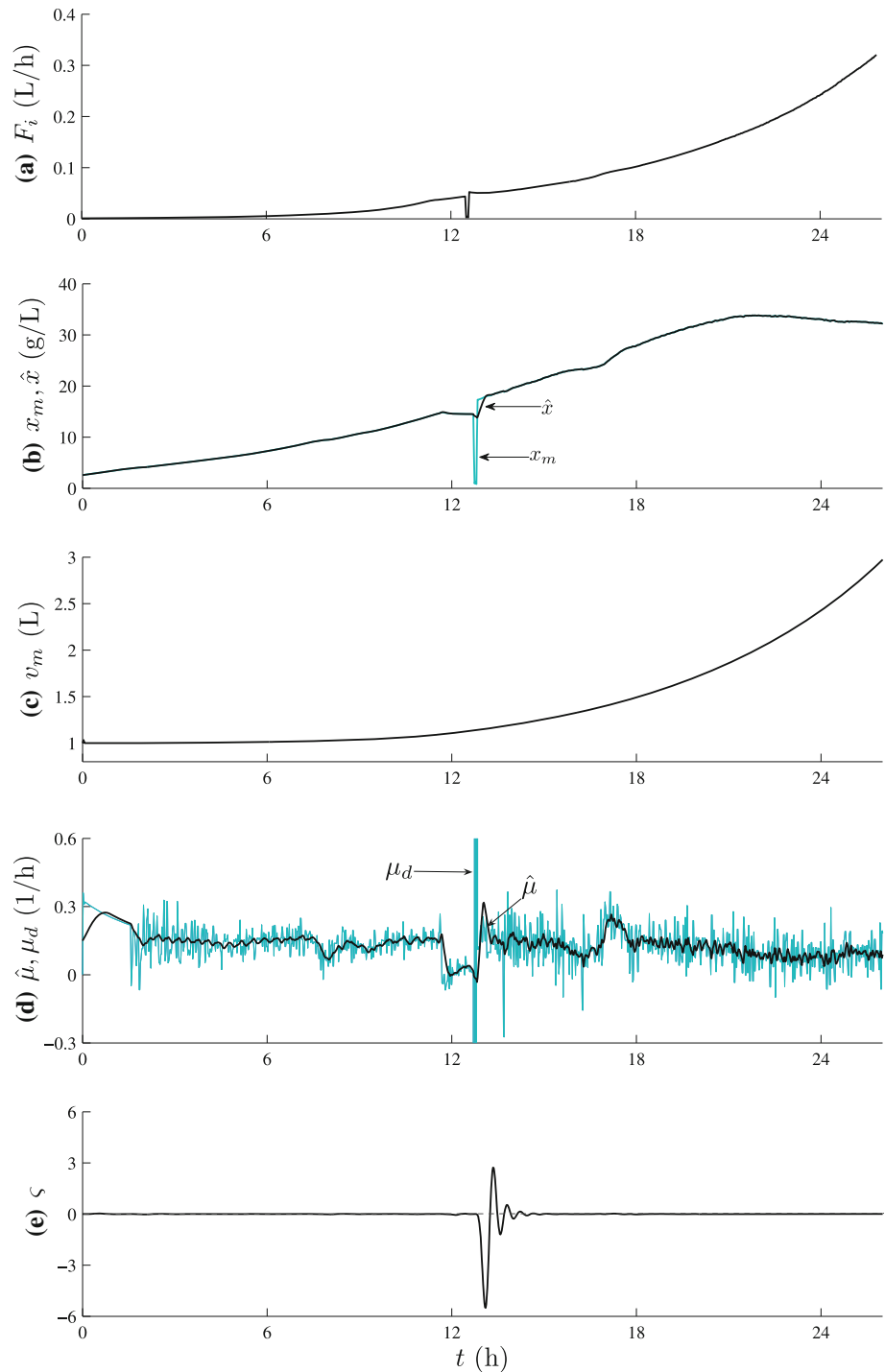
estimate  $\mu$ . A 1-h drift fault was induced in the biomass sensor at  $t \approx 40$  h. Figure 4c displays the specific growth rate estimation  $\mu_d$  obtained according to (21) and  $\hat{\mu}$  provided by the sliding observer. The drift fault in the bioreactor caused the pulse observed in  $\mu_d$  that did not correspond with reality. Finally, the bottom plot depicts the observer sliding coordinate  $\sigma$ . Effectively, it is seen in Fig. 4d that the observer converged for the first time in less than 2 h. From then on, the growth rate estimate perfectly tracked the real growth rate with much less noise than  $\mu_d$ . At  $t \approx 40$  h, the observer diverged since it was not able to track the drastic—and unreal—increase in  $\mu$ . This fast variation largely exceeded the admissible rate of change  $\rho$  of the observer output. Note the importance of the sliding surface coordinate  $\sigma$  to determine the observer convergence and, therefore, the estimate reliability. Continuous observers do not provide such information.

*Comment* This sort of variation in  $\mu$  may also be caused by other reasons like variations in physicochemical conditions or metabolic changes. The observer may be used with the aim of detecting these changes or to track  $\mu(t)$  despite them. In the latter case, the parameter  $\rho$ , or  $\alpha$ , can be increased to gain in robustness at the cost of higher noise and lower sensitivity against sensor faults. Alternatively,  $\rho$ -adaptation can be implemented as mentioned at the beginning of this section to avoid increasing noise unnecessarily.

## Conclusions

A pair of modified second-order sliding mode observers have been evaluated for signal reconstruction in bioreactors. They have been specifically designed to estimate the

**Fig. 3** Experimental results for a fed-batch process. **a** Input flow. **b** Measured and estimated biomass concentration. **c** Measured volume. **d** Growth rate estimates using sliding observer ( $\hat{\mu}$ ) and measurement differentiation ( $\mu_d$ ). **e** Sliding surface coordinate



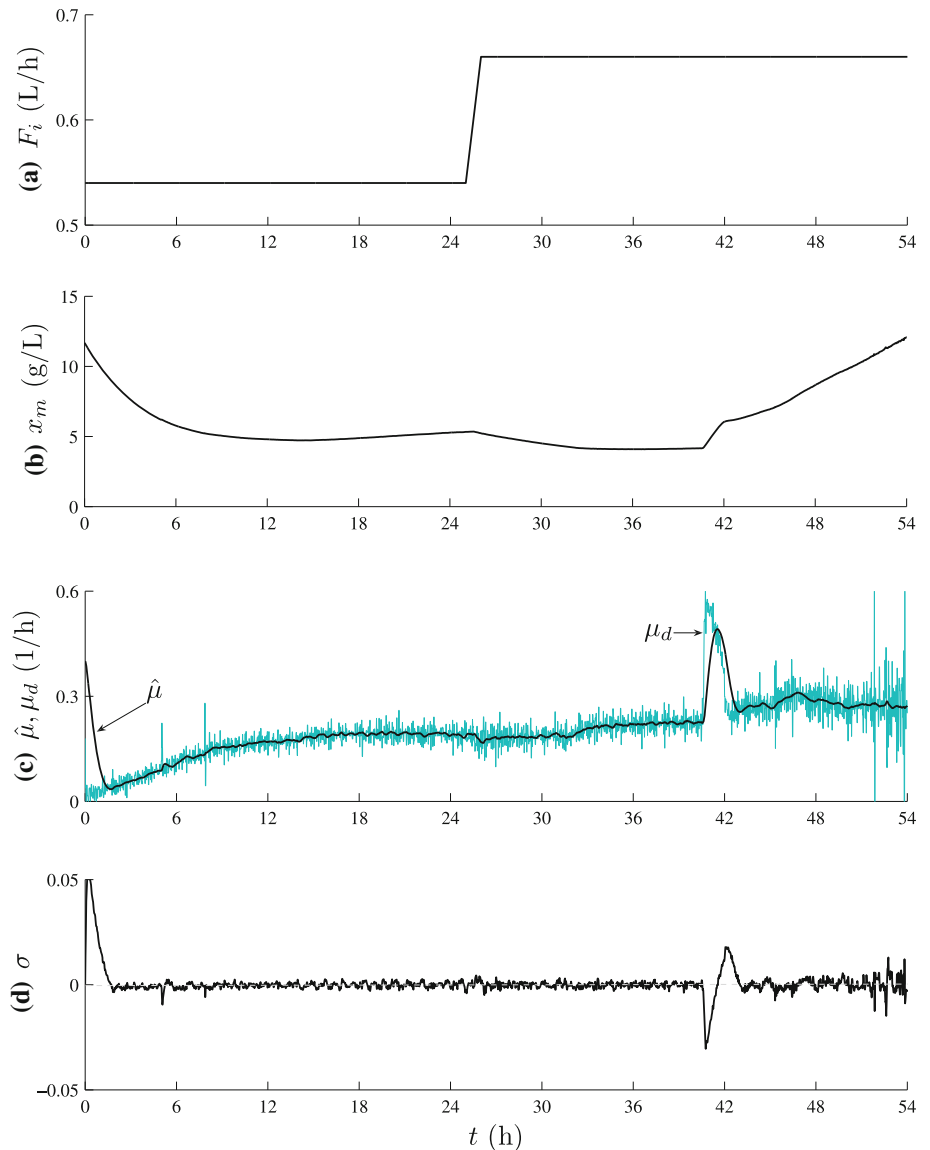
specific growth rate of microorganisms based on biomass measurement. One of them has been conceived for batch and fed-batch processes, whereas the other one is more suitable in continuous process applications. Their design is not based on any model for the kinetics of the reaction, which may be monotonic or not. Just an upper-bound on its time derivative is required to tune the observer parameters.

It was shown that these observers are equivalent, after some coordinate and time scale transformations, to the so-

called super-twisting sliding algorithm, thereby inheriting its attractive features. In contrast with continuous observers, perfect tracking after finite convergence time can be achieved in the absence of noise, whereas chattering caused by noise is substantially reduced in comparison with conventional sliding observers. This theoretical property, i.e. finite time convergence, is very attractive in real-world control applications since the separation principle can be applied to design observer and controller independently.



**Fig. 4** Experimental results for a continuous process. **a** Input flow. **b** Measured biomass concentration. **c** Growth rate estimates using sliding observer ( $\hat{\mu}$ ) and measurement differentiation ( $\mu_d$ ). **d** Sliding surface coordinate



Furthermore, an internal variable of the observers determines the convergence time that can be used to decide when to close the loop. In addition, this internal variable is also an effective residual to indicate reactor malfunction, sensor faults, etc.

The observers performance has been assessed experimentally by means of fermentation of *Saccharomyces Cerevisiae* on glucose. The results confirmed their distinctive properties, namely fast convergence, excellent tracking, robustness and effectiveness in fault detection and monitoring.

Future research will be conducted to estimate several time-varying reaction rates from the measurement of several species concentrations. The main challenge is that an extra unknown function should be incorporated to avoid too conservative bounds, implying further modifications of the super-twisting algorithm.

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**Appendix**

Consider the following bioreaction model, which is typically used to describe the growth of *Saccharomyces Cerevisiae* on glucose:

$$\begin{cases} \dot{s} = -y\mu x + \frac{F_i(t)}{v}(s_i - s) & s(0) \geq 0 \\ \mu = m(s) \triangleq \mu_{m\frac{s}{s+k}} \end{cases} \quad (22)$$

being  $s$ : substrate concentration;  $y$ : yield coefficient;  $s_i$ : substrate influent concentration;  $F_i$ : feeding flow;  $\mu_m$ : maximum specific growth rate;  $k$ : half saturation constant.

From (22), an expression for the  $\mu$ -dynamics is readily obtained:

$$\dot{\mu} = \frac{dm(s)}{ds} \dot{s} = \frac{(\mu_m - \mu)^2}{\mu_m k} \left( -y\mu x + \frac{F_i(t)}{v} (s_i(t) - m^{-1}(\mu)) \right) \quad (23)$$

From (23), upper-bounds for  $|\dot{\mu}|$  under different scenarios are derived next. These bounds can be used to tune observers (11) and (17). In any case, these bounds can be adjusted up and down according to previous experience about the process.

#### Batch operation mode

In batch mode ( $F_i = 0$ ), biomass concentration and growth follow time-varying profiles. From (23), it follows that  $\dot{\mu}$  is minimum with respect to  $s$  at  $k/2$ . Therefore,

$$|\dot{\mu}(t)| \leq \rho_b x(t) = \frac{4}{27} \frac{y\mu_m^2}{k} x(t). \quad (24)$$

#### Fed-batch operation mode

Fed-batch processes are usually fed in proportion to biomass population ( $F_i = \lambda xv$ ), in order to achieve growth at constant rate. The value of  $\lambda$  that is compatible with a given growth rate  $\mu_r$  can be easily obtained from (22):

$$\lambda = \frac{\mu_r y}{s_i - s} \quad (25)$$

resulting in a substrate dynamics

$$\dot{s} = -y(\mu - \mu_r)x \quad (26)$$

From (23), it then follows that  $\dot{\mu}$  can be bounded by

$$|\dot{\mu}(t)| \leq \rho_{fb} x(t) = \frac{4}{27} \frac{y(\mu_m - \mu_r)^3}{k\mu_m} x(t). \quad (27)$$

#### Continuous operation mode

Although closed-loop control strategies can be implemented, chemostats are usually operated—at least during the initial phase—in open loop ( $F_i = F_0 = \mu_r v$ ). They reach their steady states when the extraction of reaction medium equals the substrate flow rate. After a set-point step  $\Delta\mu_r$  or a control reaction of the same amplitude,  $\mu$  evolves to its new equilibrium with bounded time derivative

$$|\dot{\mu}(t)| \leq \rho_c = \frac{(\mu_m - \mu_r)^2}{\mu_m k} |\Delta\mu_r| s_i \quad (28)$$

This is an absolute bound independent of biomass, so the use of (17) is recommended.

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