

Specific Kinetic Rates Regulation in Multi-Substrate Fermentation Processes. ^{*}

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Abstract: The regulation of the biomass specific growth rate is an important goal in many biotechnological applications. To achieve this goal in fed-batch processes, several control strategies have been developed employing a closed loop version of the exponential feeding law, an estimation of the controlled variable and some error feedback term. Moreover, in some bioprocesses there is more than one feeding flow entering the bioreactor and supplying different nutrients or substrates. Hence, the problem of estimating multiple substrate consumption rates together with the specific growth rate of the microorganism becomes relevant. In this context, the dynamic behavior of fed-batch processes with multiple substrates and Haldane kinetics is further investigated. In particular, a nonlinear PI control law based on a partial state feedback with gain dependent on the output error is used. Then, with a recent developed algorithm for several kinetic rates estimation based on second-order sliding mode (SM) ideas, we extend the mentioned control strategy to a multi-substrate fed-batch bioprocess. The observer provides smooth estimates that converge in finite time to the time-varying parameters and allows independent design of the observer and controller dynamics. The features of the proposed estimation and control strategies are assessed by simulation in different scenarios.

Keywords: Nonlinear PI control; multiple kinetic rate estimators; process control; high order sliding modes; bioreactors; multi-substrate fermentation.

1. INTRODUCTION

The expanding biotechnological industry is demanding more efficient, reliable and safe processes to optimize production and improve quality. Control engineers have to overcome a large number of obstacles to control fed-batch fermentations. They must deal with complex dynamic behavior of microorganisms, strong modeling approximations, external disturbances, nonlinear and even inherently unstable dynamics, scarce on-line measurements of most representative variables, etc. In (Smets et al., 2004) a description of the history and state of the art in the field of fermentation fed-batch process control is presented.

From a biological standpoint, the control of a biotechnological process would be to make microorganisms reach a (possibly time-varying) metabolic state at which their physiological behavior is appropriate for the desired goals: e.g. production of a given metabolite or protein. These metabolic states are usually related to growth rate (Ihssen and Egli, 2004; Gnoth et al., 2008). Also growth rate is

related to substrate consumption rate. Thus, tuning the feed rate to achieve either constant substrate concentration in the broth or constant metabolite production rate are common strategies in the area (Valentinotti et al., 2003; Oliviera et al., 2004; Jenzsch et al., 2006).

Current availability of more on-line reliable biomass and volume measurement devices allow direct control of specific growth rate. This is especially true for small and medium scale bioreactors used to produce enzymes and/or high-added values specialty metabolites. This has enabled a research line dedicated to develop generic and robust controllers based on the minimal modeling concept. In (Picó-Marco et al., 2005), a sliding mode controller applicable to the regulation of growth-linked fed-batch processes is presented. Just on-line measurement of biomass concentration and volume, as well as an upper-bound on the growth rate are needed. Other authors have incorporated an estimation of the controlled variable to the control algorithms, obtained from on-line measurement of biomass concentration (Smets et al., 2004; Gnoth et al., 2008; Smets et al., 2002; Dabros et al., 2010). Pioneering work in the field of growth rate observers was performed in (Bastin and Dochain, 1986).

In (De Battista et al., 2012), a different approach is proposed to design non-linear PI controllers which relies on

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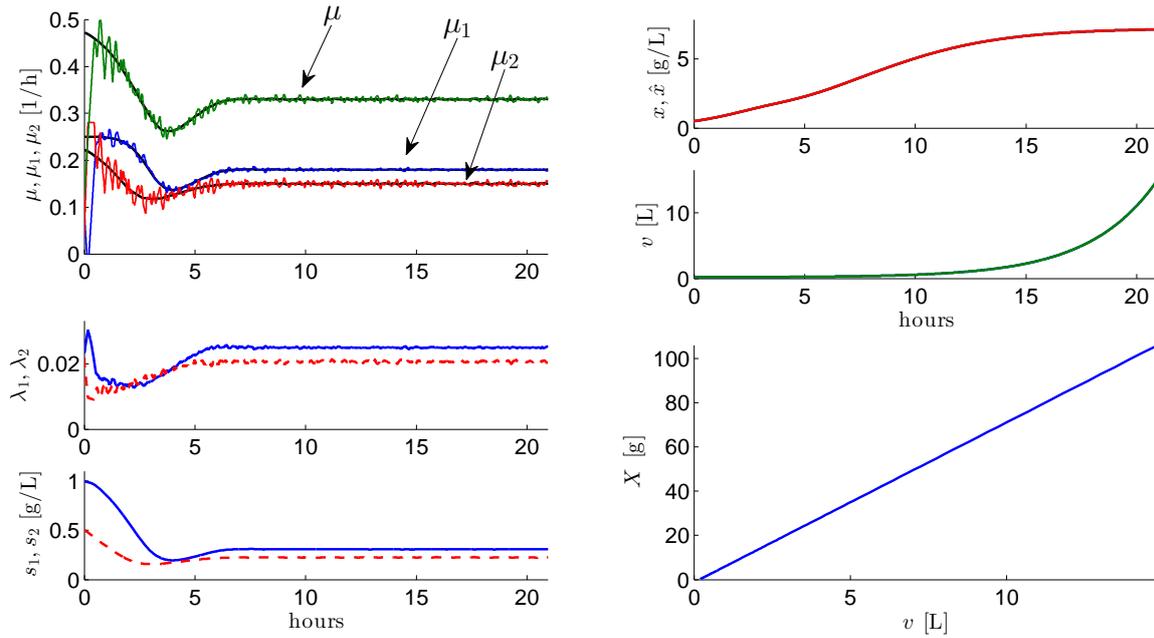


Fig. 1. (Scenario 1) Responses from low initial substrates concentration under nominal conditions, *i.e.* invariant gains λ_{r_i} , are known. The two plots at the bottom left show λ and substrate: blue-solid is λ_1, s_1 and red-dashed is λ_2, s_2

geometric properties of the process and specification structures. Ideas and concepts of invariant control and passivity are combined to achieve PI controllers that outperform previous developments where some of these ideas were exploited separately (Picó-Marco et al., 2005; De Battista et al., 2006). In (Pico-Marco and Navarro, 2008), an invariant and stabilizing controller is used to control dual-substrate fed-batch fermenters using only biomass measurement and growth rate estimation. Thereafter in (Nuñez et al., 2013), an algorithm for several kinetic rates estimation based on second-order sliding mode ideas was presented, providing smooth estimates, which are achieved in finite-time and without adding dynamics. In this paper, we use the mentioned previous work to estimate several substrate consumption rates and extend the nonlinear PI control (De Battista et al., 2012) for multi-substrate fed-batch fermentation in order to track desired consumption rates for each substrate.

The work is organized as follows. The next section presents the control problem. In section III the second-order sliding mode observer together with the invariant control and the PI correction terms are presented for the case of multi-substrate fermentation with non-monotonic kinetics. Section IV shows the observer and controller performance using simulation data with realistic noise and perturbations. Finally, in section V the main conclusions of the work are given.

2. PROBLEM FORMULATION

Consider biphasic biomass growth. The commonly used model to describe dual-substrate fed-batch fermentations accepts the following description in state-space (Bastin and Dochain, 1990; Dunn et al., 2003; Chang, 2003):

$$\mathcal{S} : \begin{cases} \dot{x} = f(\mu_1, \mu_2)x - (D_1 + D_2)x \\ \dot{s}_1 = -y_1\mu_1(s_1)x + D_1s_{1in} - (D_1 + D_2)s_1 \\ \dot{s}_2 = -y_2\mu_2(s_2)x + D_2s_{2in} - (D_1 + D_2)s_2 \\ \dot{v} = (D_1 + D_2)v = F_1 + F_2 \end{cases} \quad (1)$$

where $f(\cdot)$ is the specific growth rate, usually the sum or the product of its arguments. Additionally, the state variables are x biomass concentration, s_i concentration of substrate in the tank, and v volume. The specific consumption rates μ_i are unknown nonlinear function of substrates. In the following we will center the analysis in the case of non-monotonic Haldane kinetics, where the consumption rates have the following form:

$$\mu_i(s_i) = \mu_{mi} \frac{1 + 2\sqrt{k_{si}/k_{ii}}}{(k_{si}/s_i) + 1 + (s_i/k_{ii})}. \quad (2)$$

The parameters y_i are yield coefficients. The other two parameters s_{in} are the substrate concentrations in the corresponding feeding flow. Finally, the D_i dilution rates are equal to the ratios F_i/v . The substrates may play different roles (see Zinn et al. (2004)). For example in two common cases:

- (1) Both substrates are carbon sources and contribute both to growth and production.
- (2) One substrate is a carbon source mainly affecting growth and the other one a nitrogen source affecting production and product characteristics.

In either case there are mainly two goals from the process point of view:

- (1) It is desirable to keep a given specific growth rate $f = \mu_{ref}$, and hence consumption rates μ_1 and μ_2 corresponding to a desired physiological state at which the microorganism behaves optimally with respect to production, does not produce inhibiting products, etc.

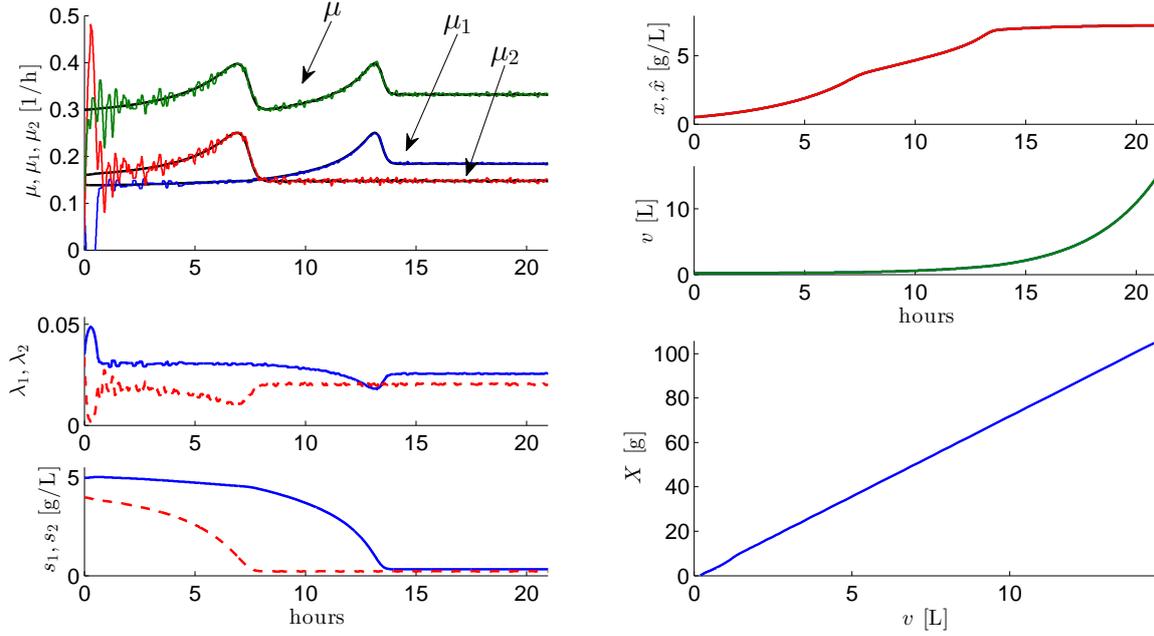


Fig. 2. (Scenario 2) Responses from very high initial substrates concentration under nominal conditions, *i.e.* invariant gains $\lambda_{r,i}$, are known. In the bottom left plots, of λ and substrate, blue-solid is λ_1, s_1 and red-dashed is λ_2, s_2

- (2) It has been reported in for example Kellerhals et al. (1999) and Xu et al. (2005) that in many instances the ratio s_1/s_2 affects the product characteristics, e.g. in PHB production the bioplastic physical properties.

Both goals could be achieved by regulating the consumption rates for each substrate using $F_{1,2}$. This constitutes the main problem addressed in this work.

Note that controller and observer design is subject to the following constraints:

- The only on-line measurable variables are volume and biomass and one of the substrates concentration.
- The control signals are nonnegative.
- The yield coefficients $y_{1,2}$ and the influent substrate concentration s_{in} are uncertain parameters that, moreover, may vary during the process.
- The specific consumption rates μ_i are not precisely known. We only assume they are Haldane-like non-monotonous functions, some initial estimation of the maximum consumption rates (an informed guess is enough), estimated upper bounds on their time derivative, and a rough idea of the region $(\mu_i(s_i), s_i)$ where the functions $\mu_i(s_i)$ live.

Thus, in the next section we extend a non linear PI controller for growth regulation (De Battista et al., 2012) to a multi-substrate fermentation and we combine it with the multiple rate high-order sliding mode observer developed in (Nuñez et al., 2013) in order to estimate the consumption rates of each substrate. Then we introduce the estimates into the controller to adapt the invariant gains, improving the robustness with respect to model uncertainties and perturbations.

3. NONLINEAR PI CONTROLLER AND SECOND ORDER SLIDING MODE OBSERVER

In this section, we present the nonlinear PI controller for growth regulation developed in (De Battista et al., 2012). Then we show how to tune the multi-rate observer developed in (Nuñez et al., 2013) for this particular case. Having estimates of the multiple consumption rates, allows us to extend the control to the multi-substrate case.

3.1 Nonlinear PI control

Consider the system (1). We start by applying an invariant control (Pico-Marco and Navarro, 2008), from where we obtain the invariant gains $\lambda_{r1,r2}$. The basic idea is to take a reference model of exponential growth (compatible with the control objectives) and make the generated goal manifold for our system (1) to be invariant. That is, if the system is driven to the manifold, it will stay on it. This is achieved with the invariant gains, which can be calculated from the reference substrates from (1),

$$s_{r1} = k_{s1} \frac{\mu_{r1}}{\mu_{m1} - \mu_{r1}} \quad (3)$$

$$s_{r2} = k_{s2} \frac{\mu_{r2}}{\mu_{m2} - \mu_{r2}} \quad (4)$$

as follows

$$\lambda_{r1} = \frac{s_{r1}y_2\mu_{r2} - s_{r2}y_1\mu_{r1} + s_{2in}y_1\mu_{r1}}{s_{2in}s_{1in} - s_{2in}s_{r1} - s_{1in}s_{r2}} \quad (5)$$

$$\lambda_{r2} = \frac{-s_{r1}y_2\mu_{r2} + s_{r2}y_1\mu_{r1} + s_{2in}y_2\mu_{r2}}{s_{2in}s_{1in} - s_{2in}s_{r1} - s_{1in}s_{r2}} \quad (6)$$

Then we can use these gains as initial conditions for the adaptation algorithm (nonlinear PI) from (De Battista

et al., 2012), but extended to a multi-substrate fermentation in the following way (for $i = 1, 2$):

$$\mathcal{C} : \begin{cases} F_i = \lambda_i x v \\ \lambda_i = \lambda_{ai} \left(1 - \tanh \left(\frac{k}{\mu_{ri}} (\hat{\mu}_i - \mu_{ri}) \right) \right) \\ \dot{\lambda}_{ai} = -\phi \lambda_{ai}^2 x \frac{\hat{\mu}_i - \mu_{ri}}{\mu_{ri}}, \lambda_{ai}(t_0) = \lambda_{ri}, i = 1, 2. \end{cases} \quad (7)$$

Where k is the proportional gain of the controller and ϕ is the integral gain which determines the speed of adaptation of λ_i . Notice we need an estimation $\hat{\mu}_i$ of the consumption rates, in order achieve output error injection into the algorithm. Thus, we will use the previously mentioned observer for the substrates consumption rates estimation.

Assuming the growth rate of the microorganisms is related directly with the consumption rates of the substrates in an additive way

$$\mu = \mu_1 + \mu_2, \quad (8)$$

we will in fact estimate the specific growth rate and the consumption rate of the measured substrate. Then, we can simply subtract the estimated consumption rate from the growth rate in order to obtain the other consumption rate. Other relationships can be handled easily (e.g. multiplicative consumption rates).

3.2 Multiple rates observer

In order to estimate the specific growth rate and the consumption rate of the measured substrate we will use the observer developed in (Nuñez et al., 2013). First consider the following system which describes the measured variables z in a state-space model of a bioprocess stirred tank (Bastin and Dochain, 1990):

$$\begin{cases} \dot{z} = \mathbf{K}_p \mathbf{G}_p(\cdot) \alpha_p - D z + F \\ \dot{\alpha}_p = \mathbf{R} \rho(t), \end{cases} \quad (9)$$

where \mathbf{K}_p is a pseudo-stoichiometric matrix, D is the dilution rate, and F is the input flow rate. $\mathbf{G}_p(\cdot) \alpha_p$ represents the reaction rates which are linearly combined by the rows of \mathbf{K}_p . The specific reaction rates for each reactant are α_p and $\mathbf{G}_p(\cdot)$ is a diagonal matrix. Finally $\mathbf{R} = \text{diag}\{\rho_i\}$ arranges the bounds of the time derivatives of the rates α_p . Note, that $\rho(t)$ is an unknown vector of continuous functions where $\|\rho(t)\|_\infty \leq 1$.

Then the second order sliding mode observer \mathcal{O} converges to the specific reaction rates $\hat{\alpha} \equiv \alpha_p$ in finite-time (Nuñez et al., 2013).

$$\mathcal{O} : \begin{cases} \dot{\hat{z}} = \mathbf{K}_p \left(k_1 \mathbf{G}_p(\cdot) \mathbf{R} u + 2k_2 \mathbf{G}_o \mathbf{R} \text{ABS}(\sigma)^{1/2} \text{SIGN}(\sigma) \right) \\ \quad - D \hat{z} + F \\ \dot{u} = k_1 \text{SIGN}(\sigma) \\ \hat{\alpha} = \mathbf{R} u \end{cases} \quad (10)$$

with

$$\sigma = (\mathbf{K}_p \mathbf{G}_o \mathbf{R})^{-1} (z - \hat{z}) \quad (11)$$

where \mathbf{G}_o is a matrix related to the bounds on $\mathbf{G}_p(\cdot)$. The functions $\text{ABS}(\sigma) = \text{diag}\{|\sigma_i|\}$ and $\text{SIGN}(\sigma) = \text{col}(\text{sign}(\sigma_i))$ are matrix extensions of the respective scalar functions.

Comment on stability and convergence. For details on the stability analysis of the controller and convergence of the observer see respectively (De Battista et al., 2012; Nuñez et al., 2013). In combining the two strategies, the only precaution to take is that the observer should converge before the process state leaves the domain of attraction (if it is not global). Anyway, in the practical industrial operation, there is always a batch open-loop phase previous to switching the fed-batch phase on. The observer will converge during this phase.

Observer Implementation In order to use this observer in our particular case, we take the first and second equations from (1), which are the dynamics of the measured variables, hereafter $z = [x, s_1]^\top$, and we rearrange them, obtaining:

$$\dot{z} = \begin{pmatrix} 1 & 0 \\ 0 & -y_1 \end{pmatrix} \begin{pmatrix} x & 0 \\ 0 & x \end{pmatrix} \begin{pmatrix} \mu \\ \mu_1 \end{pmatrix} - (D_1 + D_2) z + \begin{pmatrix} 0 \\ D_1 s_{1in} \end{pmatrix} \quad (12)$$

where $\alpha_p = [\mu, \mu_1]^\top$. Note that in this factorization $\mathbf{G}_p = x \mathbf{I}_2$ and

$$\mathbf{K}_p = \begin{pmatrix} 1 & 0 \\ 0 & -y_1 \end{pmatrix}. \quad (13)$$

In order to tune the observer, upper (\mathbf{G}_1) and lower (\mathbf{G}_2) bounds for \mathbf{G}_p need to be obtained to calculate $\mathbf{G}_o = \frac{\mathbf{G}_2 + \mathbf{G}_1}{2}$, $\Delta \mathbf{G} = \frac{\mathbf{G}_2 - \mathbf{G}_1}{2}$ and $\delta = \|\mathbf{G}_o^{-1} \Delta \mathbf{G}\|_\infty$. The presupposed excursion of the biomass x gives us conservative bounds $\mathbf{G}_1 = 0.2 \mathbf{I}_2$ and $\mathbf{G}_2 = 15 \mathbf{I}_2$. From where we get $\mathbf{G}_o = 7.6 \mathbf{I}_2$ and $\delta = 0.9737$. With this we can calculate the suitable gains k_1 and k_2 (Table 1) to ensure finite-time convergence, by solving the associated GEVP problem (details omitted for brevity, see Nuñez et al. (2013)).

4. SIMULATIONS

In order to test the behaviour of the controller and observer described in this paper, simulations in three realistic scenarios have been performed. The model from (Chang, 2003) has been used with the following parameters and test conditions.

Table 1. Parameters and test scenarios

Process parameters and test scenarios			
μ_{m1} [1/h]	0.47	μ_{m2} [1/h]	0.5
k_{s1} [g/L]	0.5	k_{s2} [g/L]	0.55
k_{i1} [g/L]	2	k_{i1} [g/L]	2.1
y_1^a	2.1	y_2	2
s_{1in} [g/L]	15	s_{2in} [g/L]	15
$V(t_0)$ [L]	0.2	V_f [L]	15
$s_1(t_0)$ [g/L]	[1, 5, 1]	$s_2(t_0)$ [g/L]	[0.5, 4, 0.5]
$x(t_0)$ [g/L]	0.5		
Controller and observer parameters			
λ_{ri}	[1, 1, 0.7] λ_i	μ_r [1/h]	0.33
μ_{r1} [1/h]	0.18	μ_{r1} [1/h]	0.15
k	3	ϕ	30
k_1	2.9592	k_2	2.3038
ρ_1	0.25	ρ_2	0.2

^a Parameter y_1 in Scenario 3 grows at 3% per hour since $t = 10h$.

The controller and observer parameters used in the simulations are also listed in Table 1.

Note that by the assumption (8), the reference value for the specific growth rate is $\mu_r = 0.33$.

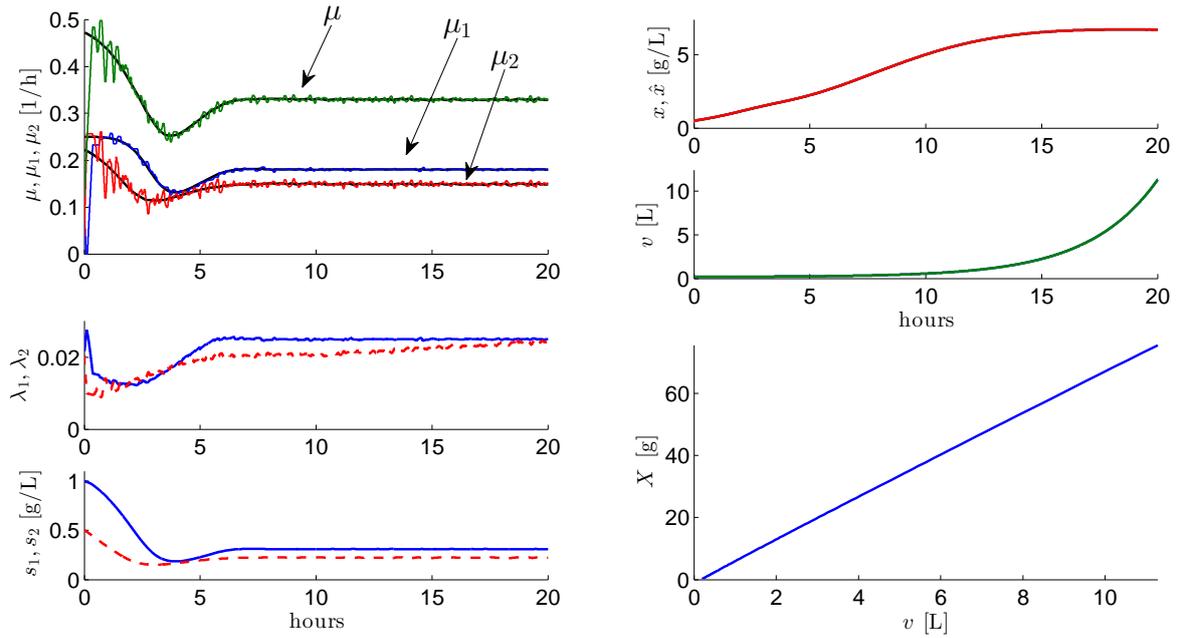


Fig. 3. (Scenario 3) Responses from low initial substrates concentration under uncertain conditions. In the bottom left plots, of λ and substrate, blue-solid is λ_1, s_1 and red-dashed is λ_2, s_2

Scenario 1. Low initial substrate concentration. In this first scenario, the aim is to show the performance of the controller and observer from low initial concentration of substrates and under nominal conditions. The proper invariant gains λ_{r_i} are known (or calculated from the model parameters). The results are presented in Figure 1.

Scenario 2. High initial substrate concentration. The second scenario shows the convergence property of the proposed controller from a high initial substrate concentration. The initial condition is selected beyond the maxima of the Haldane functions of both substrates. This is an inherently unstable region.

In fact, the control has the opposite effect to what is expected, thus producing a positive feed-back. To avoid wash-out, λ_1 and λ_2 are bounded, so that the substrate concentration inevitably falls below the maxima of the kinetic rate functions. Once in this region, the controller stabilizes the process around the goal trajectory. Notice that setting the bounds on λ_1 and λ_2 requires having a rough idea of the region $\mu_i(s_i), s_i$ where the functions $\mu_i(s_i)$ live, and a lower bound in the yield coefficients. But it does not require a precise model of the reaction kinetics.

Scenario 3. Robustness against parameter uncertainty. In this third scenario we evaluate the robustness of the proposed control and observer with respect to parameter uncertainty. One of the invariant gains, λ_{r1} is underestimated by a 30%. Further, after $t = 10h$, the yield coefficient y_{s1} is increased at the rate of 2% per hour. The evolution of the process variables is shown in Figure 3. See that, in the presence of the uncertain and time varying

parameter, the control law is still tracking the desired $\mu_{r1,2}$ by adapting the parameter λ_1 .

5. CONCLUSIONS

In this work we extended the previous proposed nonlinear proportional-integral control to multi-substrate fed-batch processes for multiple kinetic rates regulation. This was possible due to recent development of an algorithm for several kinetic rates estimation based on second-order sliding mode ideas, which allows us to obtain the consumption rates for each substrate together with the specific growth rate of the microorganism measuring only biomass and one of the substrates.

Robustness properties of both strategies are inherited. The controller provides robustness to model uncertainties and disturbances, which is one of its main attractive features. The observer provides noise rejection, and finite-time convergence of the consumption rates estimates. The latter allows to independently design the observer and the controller.

Performance of the system was shown by simulation of realistic scenarios, with noise, parameters uncertainty, and high initial substrate concentration, where an increase in concentration of substrates produces a decrease in growth rate, and therefore the fermentation is in an intrinsically unstable region.

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