CONTROL BASED ON NUMERICAL METHODS AND RECURSIVE BAYESIAN ESTIMATION IN A CONTINUOUS ALCOHOLIC FERMENTATION PROCESS

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Biotechnological processes represent a challenge in the control field, due to their high nonlinearity. In particular, continuous alcoholic fermentation from *Zymomonas mobilis* (*Z.m*) presents a significant challenge. This bioprocess has high ethanol performance, but it exhibits an oscillatory behavior in process variables due to the influence of inhibition dynamics (rate of ethanol concentration) over biomass, substrate, and product concentrations. In this work a new solution for control of biotechnological variables in the fermentation process is proposed, based on numerical methods and linear algebra. In addition, an improvement to a previously reported state estimator, based on particle filtering techniques, is used in the control loop. The feasibility estimator and its performance are demonstrated in the proposed control loop. This methodology makes it possible to develop a controller design through the use of dynamic analysis with a tested biomass estimator in *Z.m* and without the use of complex calculations.

Keywords: Bioprocess; Zymomonas mobilis; Non linear control; Numerical Methods; Particle Filtering; Bayesian Estimation

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INTRODUCTION

Growing attention has been devoted to the conversion of lignocellulosic material into fuel ethanol, considered the cleanest liquid fuel as an alternative to fossil fuels. Significant advances have been made towards ethanol fermentation technology. It is now understood that it is important to use biomass energy as a means of providing modern energy to the billions who lack it. It would complement solar, wind, and other intermittent energy sources in the renewable energy mix of the future. One of the most immediate and important applications of this kind of energy systems could be in the ethanol fermentation from biomass concentration. Biomass is one of our most important renewable energy resources, and its consumption for energy production has increased significantly in recent years.

There are several topics around the conversion process of biomass in energy: the substrate used for it, the fermentation scale, measurement equipment, and of course, the biomass itself. For the fuel ethanol industry there is a need for research on more

competitive ethanologenic microorganisms. Zymomonas mobilis (Z. m) has attracted attention as a promising bacterium regarding improving ethanol production (Daugulis *et al.* 1997). Variations in oil prices have prompted increasing focus on renewable carbohydrate-based feedstocks for fuels and chemicals. A review of the works of Rogers *et al.* (2007) addresses opportunities offered by Z. m for higher value products through its metabolic engineering and use of specific high activity enzymes.

The alcoholic fermentation of the Z. m has a few advantages when is compared to other microorganisms; it provides ethanol levels near to the ones achieved theoretically in the production from glucose, has a low biomass wastage, there are not any oxygen requirements, the fermentation can be produced at a low value of pH, and it shows a high specific productivity efficiency (around 98%) in ethanol production and a specific rate twice bigger (O'Mullan *et al.* 1991). This results in higher ethanol yields and higher specific productivities (see Kesava and Panda 1996; Kesava *et al.* 1996), this last feature being the main benefit. From the estimation and control perspective the knowledge about this microorganism will allow us to analyze, in the future, the possibility of using Z. m in culture medium and large scale fermentation.

Process control has played a rather limited role in the biochemical industry, as the economic incentive for improved process operation is often small in relationship to the costs associated with research and development. A reason for this has been the lack of online sensors for critical process variables. While this will remain an important issue for the foreseeable future, recent advancements in biochemical measurement technology make the development of advanced process control systems a realistic goal. These trends suggest that biochemical processes will emerge as an important application area for control engineers (Daoutidis and Henson 2002).

The continuous fermentation of *Zymomonas mobilis* involves the same estimation and control issues as bioprocesses in general. These microorganisms show a highly nonlinear and oscillatory kinetic behavior, and some states of the process are difficult or impossible to measure. For example, it is difficult to measure the biomass concentration and intermediate variables, which represent the ethanol production rate and are used to determine the inhibition effect. As an appropriate solution for this lack of information in bioprocess, some practitioners have been developing several state estimators based on different techniques. The observer or a state estimator choice depends inherently on the particular problem specifications. In general, when the prior knowledge about the plant or the model is incomplete, different approximation techniques may be used, looking for the state estimation from the input/output data information. In the literature, several proposals for the state estimation on bioprocess can be found, of which the most representative are: the works of Dochain (2002, 2003); Boillereaux and Flaus (2000); Leal (2001); Adilson and Rubens (2000); and finally Rallo *et al.* (2002).

Previous studies allow us to review the dynamic behavior of bacteria, to establish the features to use it as ethanol producer, taking advantage of the natural properties and with the aim to reach the optimum point of productivity. As a result, a state estimator has been proposed for the process of continuous alcoholic fermentation of *Zymomonas mobilis*, through non lineal filtering based on the recursive application of the Bayes rule and Monte Carlo techniques (Dochain, 2002; Quintero *et al.* 2008d). There are several variations of this method, and it is known with different names in the literature such as:

particle filters, recursive Bayesian filters, Monte Carlo recursive filters and simulation based filters (see Doucet 1998; Crisan and Doucet 2002; Briers 2006, and references therein).

An important aspect of this work is that details for the real data results are not published yet, but the estimator is used in this paper for closed loop purposes in simulation, and real data are used as desired trajectories for fermentation.

The use of a benchmark is common for a continuous fermentation process, and the most used models have two (2) state variables. State variables such as biomass and substrate in conjunction with growth rates equations give to the models high non-linearities. Also, the complex dynamics of bioreactors have been researched for the design of different non-linear stabilizing control techniques. The mentioned techniques have been applied to relatively simple continuous fermentation processes used to reach this aim, the linear case being used as a reference for non-linear methods. Bioreactors control has been studied by other authors, including the schemes based on adaptive control (Aguilar *et al.* 2001), optimum control, and neural networks based control (Onder *et al.* 1998). Nevertheless, the investigations in this field remain in a latent stage, and it seems that more effort is needed to find a control scheme well developed to be implemented in practical (large scale) bioreactors with high performance.

The present work fronts the challenge to handle a continuous fermentation process in which a microorganism with high kinetic complexities is involved. This approach is suited for a process that presents highly non-linear dynamics and the modeled system becomes a challenge for control purposes.

From a control perspective, for effective control and operation of a non-linear process, low dimensional linear models are highly desirable. It is not always possible to represent a non-linear process by a single linear model. Consequently, a multiple model approach has attracted increased attention in recent years, applied to a variety of areas (Murray-Smith and Johansen 1997). The previously mentioned approximations for the control problem solution are all valid, but we are looking for a more simple, understandable, feasible, and easy to generalize solution for the bioprocess field. Consequently, in this work the use of numerical methods is proposed, not only to simulate the fermentation process evolution, but also to find the control actions that allow the state variables to go from current state in discrete time "n" to the desired next state. As a result, a controller for substrate and product concentration are designed and calculated; later a complex controller that involves biomass recycle is derived. It is important to note that the complex calculations to get the control signal are not necessary with this technique; it makes this solution an easily implementable answer to the control challenge (Scaglia 2006a,b; Scaglia *et al.* 2007).

The paper is organized as follows: In Section 2 the continuous fermentation process to obtain ethanol from *Zymomonas mobilis* and the bioprocess model are described. Section 3 presents a brief summary of the estimation tools and the algorithms structure used for state estimation. Later, in Section 4 the numerical methods and linear algebra methodology for controller design and the proposed controllers are presented. Then, in Section 5 the dynamic analysis shows the obtained results compared with experimental data trajectories. Finally, the conclusions are summarized.

STUDY CASE

Alcoholic Continuous Fermentation of *Zymomonas mobilis* and Dynamic Behavior

A promising ethanol producer is the bacteria Zymomonas mobilis (Z.m), which reaches ethanol yields close to the stoichiometrical value (0.51 g ethanol/g glucose). There is an industrial interest in the use of Z.m due to its capability to produce ethanol and sorbitol (Oliveira *et al.* 2005). The continuous alcoholic fermentation process of Z.m presents high ethanol performance, but it has oscillatory behavior of the state variables. From the control perspective, it represents a challenge, due to the difficulties to measure some of these states, with the aim to be used as feedback signals (Bravo *et al.* 2000; Rogers *et al.* 2007).

Modeling of Continuous Alcoholic Fermentation from Zymomonas mobilis

A basis model of the process was used and modified by several authors (Maher and Zeng 1995; Daugulis *et al.* 1997; McLellan *et al.* 1999; Echeverry *et al.* 2004). Elements of the model for this bioprocess have been represented by the differential and algebraic equations, as in the work of Quintero *et al.* (2008d).

With the aim of showing the complexity of the non-linear dynamics involved in a fermentation process, it is necessary to put all equations in form of state variables. In Quintero *et al.* (2008a) and working with the defined flows (Echeverry *et al.* 2004), the biomass term will be expressed as function of the mentioned D, which is the total dilution rate. The total dilution rate is $D_s + D_r$, in which D_r is the dilution rate associated with biomass recycle R and substrate dilution rate D_s . Then the following set of differential equations are considered, where the states variables are defined as follows (Quintero *et al.* 2008d, eq. 12): x_1 is the change in biomass concentration, x_2 the change in substrate concentration, x_3 the change in product concentration, x_4 the weighted average of the ethanol concentration rate, and x_5 an intermediate variable auxiliary for the inhibition effect determination.

$$\dot{x}_{1} = \left[\left(\frac{1}{2} \left(1 - \frac{e^{\lambda x_{4} - \delta} - e^{-\lambda x_{4} - \delta}}{e^{\lambda x_{4} - \delta} + e^{-\lambda x_{4} - \delta}} \right) \right) \left(\frac{\mu_{\max} x_{2} \left(1 - \left(\frac{x_{3}}{P_{ma}} \right)^{a} \right) \left(1 - \left(\frac{x_{3} - P_{ob}}{P_{mb} - P_{ob}} \right)^{b} \right)}{K_{s} + x_{2} + \frac{x_{2} \left(x_{2} - S_{i} \right)}{K_{i} - S_{i}}} \right) \right] x_{1} + \left[RD/4 + D_{s} \right] (R-1) x_{1}$$

$$(1)$$

$$\dot{x}_{2} = \left(-\frac{1}{Y_{p/s}}\right) \left(\left[Qp_{\max}\left(\frac{x_{2}}{K_{mp} + x_{2}}\right) \left(1 - \left(\frac{x_{3}}{P_{me}}\right)^{\alpha}\right) \right] x_{1} \right) + D * S_{in} - Dx_{2}$$

$$\tag{2}$$

$$\dot{x}_{3} = \left[Qp_{\max} \left(\frac{x_{2}}{K_{mp} + x_{2}} \right) \left(1 - \left(\frac{x_{3}}{P_{me}} \right)^{\alpha} \right) \right] x_{1} - Dx_{3}$$
(3)

$$\dot{x}_4 = \beta \left(x_5 - x_4 \right) \tag{4}$$

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$$\dot{x}_{5} = \beta \left[\left[Q p_{\max} \left(\frac{x_{2}}{K_{mp} + x_{2}} \right) \left(1 - \left(\frac{x_{3}}{P_{me}} \right)^{\alpha} \right) \right] x_{1} - D x_{3} - x_{5} \right]$$
(5)

The parameters (Daugulis *et al.* 1997; McLellan *et al.* 1999) used to simulate oscillatory behavior of the *Z. m* are presented in Table 1. These parameters have been used in previous studies on simulation. The solution proposed to simulate the real behavior of the continuous fermentation was performed through the use of Matlab, with the Euler and Runge Kutta numerical methods in self developed functions to be used as part of the filtering simulation. The sample time used for simulation was the considered most appropriate for real plants.

Parameters	Values
α	8.77
β	0.0366
δ	0.824
λ	21.05
а	0.3142
b	1.415
$Qp_{\rm max}$	2.613
$\mu_{ m max}$	0.41
$Y_{p/s}$	0.495
Pma	217 g/l
Pob	50 g/l
Pmb	108.0 g/l
Ks	0.5 g/l
Ki	200 g/l
Si	80.0 g/l
Pme	127 g/l
Kmp	0.5 g/l

Table 1. Parameters for Model in Oscillatory Behavior

In this process it is very important to reach an accurate estimation of the nonmeasurable system states, with the purpose to use them for posterior control purposes. On the other hand, due to its capacitance and dynamic behavior, the bioreactor is an inherent low pass filter to the random noise signals. A general process scheme is presented in Fig. 1, taking into account that the pH control loop is already stabilized, and the microorganisms, substrate, and product are variables for control purposes; also, the model used to develop the controller does not have the pH as a variable to dynamic behavior. Consistently with the notation used in previous work in *BioResources* (Quintero *et al.* 2008d) for reader understanding, the variables defined are X as biomass concentration, S substrate concentration on the input flow, P product concentration, and F quantities are the names of the flows 1 to 5. The total dilution rate D is given by $D_s + D_r$, in which Dr is the dilution rate associated with biomass recycle R and substrate dilution rate D_s .

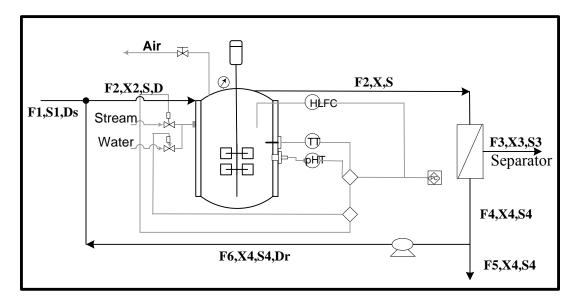


Figure 1. Continuous Fermentation Process scheme

Nonlinear filters

The critical variables are: biomass concentration and intermediate variables that represent the ethanol production rate and to determine the inhibition effect. The main reason for this is the absence of online sensors or the non-measurable nature of the inhibition variables respectively. In Quintero *et al.* (2008a) the non-linear filtering in simulation approach and generalities for the states estimation were presented. The improved state estimator presented in this part of the paper involves the development of a better uncertainty model that not only consists of noise components added to the space state model, but also contains a structured uncertainty model not yet reported in literature. Readers should note that the purpose of this paper is to report the combination in closed loop of both Bayesian estate estimator and numerical method based controllers. Figure 2 depicts the estimation and control scheme used for this work.

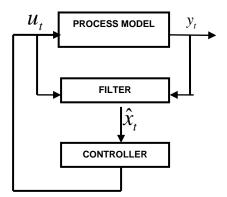


Figure 2. Controller and estimator scheme

Uncertainty model

Disturbance and uncertainty models were added to the basic model from eqs. (1) - (5), under considerations of modeling and measurement uncertainties of diverse nature. In this way, the model will be posed as a stochastic differential equation (SDE). The mathematical treatment of the SDEs has some similarities, but also slight differences, to the usual theory of ordinary differential equations (ODE). One needs to be aware of these similarities and differences when considering numerical methods to solve them. The discrete time models can be obtained for non-linear systems based on numerical solutions of stochastic differential equations. To find the exact solution of a stochastic nonlinear differential equations. Thus, only approximate sampled-data models will be obtained. The solution of the SDE's is not our main interest, but these are the samples taken from a model to calculate the approach of the probability density function of the states for estimation purposes through Monte Carlo techniques.

The discrete model used for the diffusion terms of both filter and process model was $\sigma_i(x_n, n) \in \Re^s$ from as a multidimensional Gaussian Noise dependent on a uniformly distributed intern variable z(n), updated each sample time, particle by particle. The uncertainty model is a stochastic process defined by the following components:

$$z(n) \sim p(z(n)|z(n-1)), z(n) \sim U(a,b)$$

$$\sigma_1(x_n, n) \sim p(x(n)|z(n|n-1), x(n-1))$$
(7)

$$\sigma_1(x_n, n) = p(x(n)|z(n|n-1), x(n-1))\Delta w_n$$
(8)

In eqs. (7) – (8) the symbol \sqcup means "sampled from" the conditional probability density function *p*. The function $\sigma_1(x_n, n) \in \Re^5$ corresponds to the term additional to the process model equations and can be written as

$$\sigma_{1}(x_{n},n) = \begin{bmatrix} \sigma_{11}(x_{n},n) & \sigma_{12}(x_{n},n) & \sigma_{13}(x_{n},n) & \sigma_{14}(x_{n},n) & \sigma_{15}(x_{n},n) \end{bmatrix}^{T}.$$
(9)

Also, $z(n) \in \Re^5$ and $\Delta w_n \in \Re^5$ is a white noise generated with different mean and variance vectors. The values for uniformly variable *a* and *b* are chosen by the designer. Being the states vector $x = [X \ S \ P \ Z \ I]^T$ one can approach the discrete model as:

$$\frac{\Delta X}{\Delta t} \approx \mu X + [RD/4 + Ds](R - 1)X + \sigma_{11}(x_t, t)$$

$$\frac{\Delta S}{\Delta t} \approx \left(-\frac{1}{Y_{p/s}}\right) (Q_p X) + DsSin - DsS + \sigma_{12}(x_t, t)$$

$$\frac{\Delta P}{\Delta t} \approx QpX - DsP + \sigma_{13}(x_t, t)$$

$$\frac{\Delta Z}{\Delta t} \approx \beta (I - Z) + \sigma_{14}(x_t, t)$$

$$\frac{\Delta I}{\Delta t} \approx \beta (QpX - DsP - I) + \sigma_{15}(x_t, t)$$
(10)

with $\Delta X = X_{n+1} - X_n$, $\Delta S = S_{n+1} - S_n$, $\Delta P = P_{n+1} - P_n$, $\Delta Z = Z_{n+1} - Z_n$, and $\Delta I = I_{n+1} - I_n$.

In the measurements model, the uncertainty term was selected as $\sigma_2(x_t, t) = 1$ combined with different Gaussian noise samples in the diffusion components. A simple white Gaussian Noise Δv_t was used, consistent with the Brownian motion discrete approximation, generated with statistics that vary dependent on the test performed. With the measured outputs vector considered $y = [S P]^T$ and $\Delta y = [\Delta S \Delta P]^T$

$$\Delta y_t = g(x_t, t)\Delta t + \sigma_2(x_t, t)\Delta v_t.$$
(11)

The use of sampled data models raises the question of the relationship between the discrete time description of the samples and the original continuous time model. It is tempting to simply sample quickly and then to replace derivatives in the continuous time model by divided differences in the sampled data model (Yuz and Goodwin 2004). This certainly leads to an approximate model. However, in practice, alternative models are necessary that describe the relationship between the discrete time actions taken on the system and the samples taken from its signals. Any sampled data model for a non-linear system will, in general, be an approximate description of the combination of two elements: the continuous time system itself, together with the sample and hold devices. An exact discrete time description of such a hybrid non-linear system is, in most cases, not known or impossible to compute (Yuz 2005). Thus, one needs to be clear about the potential accuracy achieved by any model. In fact, the accuracy of the approximate sampled-data plant model has proven to be a key issue in the context of control design; a controller designed to stabilize an approximate model may fail to stabilize the exact discrete-time model, no matter how small the sampling period is chosen.

This discrete approach can be justifiable due to the SDE's possible solution through the Euler Mayurama method, compatible with the solution proposed to control the system; the sample time chosen was enough not only to simulate the bioreactor dynamic behaviour, but also to calculate the control actions needed to carry the system from the current state to the desired one. Also, the calculations for biomass and inhibition variables can be done between that sample time (Quintero *et al.* 2008a). For estimation scheme, see Quintero *et al.* (2008e). The input signals to the filter (input substrate concentration, dilution rate, and microorganisms recycle term) and the output signals y(t)(outflow Substrate and product) corresponding to the model used as real plant, feed the filter block that makes the state estimation.

As previously clarified, the structure of the estimator used is the one developed by (Quintero *et al.* 2007, 2008a, 2008d). This was illustrated in Fig. 2. It was improved mainly in some operative factors and uncertainty models based in real data and not only tested in simulation, but also compared and fed with real data from fermentation reported by Raposso *et al.* (2005). The dynamic behaviors and the delays on the inhibition variables can be easily observed; this way it can be seen that they make this process highly non-linear. Details for the real data results are not already published, but the estimator is used in this paper for closed loop purposes in simulation, and real data are

used as desired trajectories for fermentation. The sampling time values selected were 0.05 and 0.1 hours. The sampling strategy was used for estimation purposes. In next section we will observe that the success of the use of the probability distributions for estimation was an advantage for control calculations.

CONTROL STRATEGY BASED ON NUMERICAL METHODS

Most control designs are based on the use of a design model. In sections labeled as Modeling and Non Linear Filters we presented the deterministic and stochastic models built looking for the solution of both modeling and estimation problems over Z. m fermentation process respectively.

An ordinary differential equations set that describes the Z. m behavior was fixed in the previous section, but we are concerned that the relationship between models and the reality they represent is subtle and complex. A mathematical model provides a map from inputs to responses, and the quality of a model depends on how closely its responses match those of the true plant. Since no single fixed model can respond exactly like the true plant, we need, at the very least, a set of maps. However, the modeling problem is much deeper; in other words, the universe of mathematical models from which a model set is chosen is distinct from the universe of physical systems. Therefore, a model that includes the true physical plant can never be constructed. It is necessary for the engineer to take a leap of faith regarding the applicability of a particular design based on a mathematical model. To be practical, a design technique must help make this leap small by accounting for the inevitable inadequacy of models. A good model should be simple enough to facilitate design, yet complex enough to give the engineer confidence that designs based on the model will work in the true plant (Zhou *et al.* 1995).

This work describes a stochastic model through the addition of uncertainty terms needed to consider the filtering as solution to the lack of information and biomass estimation. Now, the interest is to look for a proposal that solves the control issue and also could be used in complementary fashion with the estimation tool developed. To reach this aim, the model under consideration of Certainty Equivalence Principle is used to suppose the states estimated by particle filter can be used as certain information for control purposes. Certainty Equivalence Principle states that the optimal control law for a stochastic control problem has the same structure as the optimal control law for the associated deterministic (certainty equivalent) problem. The only difference is that in the stochastic control law the (unknown) true state variables are replaced by their estimated values. The validity of this principle leads to a conclusion that the designs of the estimator and the controller can be optimized separately. The controllers are design based on the deterministic model presented and the stochastic model mentioned in this paragraph.

Compared to linear control theory, it should be noted that separation property is weaker than the certainty equivalence used here (Bar Shalom and Tse 1976). Certainty equivalence implies separation, but not vice versa. The existence of the separation property was raised first by Kalman and Koepcke (1958), who asked the question: "Does separate optimization of statistical prediction and control-system performance yield a system, which is optimal in the overall sense?" This question received a positive answer a few years later for discrete time linear systems with unbounded controls, quadratic cost, and white Gaussian noise and disturbance (LQG) by Gunckel and Franklin (1963). It was also established that in discrete time linear systems with bounded controls and with strictly classical information pattern (available history of all previous measurements and controls), the conditional probability distribution of the estimated state is independent of the optimal control formulation A proof for the separation property in continuous time systems was given by Wonham (1968 a,b), who extended the validity also for problems with non-quadratic cost. The validity of the Certainty Equivalence Principle was proven first for LQG systems with unbounded controls, quadratic cost, and white Gaussian noise and disturbance, assuming classical information pattern. The proof was later extended to some other linear cases with different statistics.

Control Strategy Basics

The use of numerical methods in the simulation of the system is based mainly on the possibility of determining the states of the system at instant n+1 from the state, the control action, and other variables at instant n. Let us consider the following differential equation,

$$\dot{y} = f(y, u, t); \quad y(0) = y_0$$
 (12)

where *y* represents the output of the system to be controlled, *u* the control action, and *t* the time. The values of y(t) at discrete time t = nTo, where T_0 is the sampling period, and $n \in \{0,1,2,3,\cdots\}$ will be denoted as y_n . Thus, when wishing to compute y_{n+1} by knowing y_n , equation (11) should be integrated over the time interval $nTo \le t \le (n+1)To$ as follows,

$$y_{n+1} = y_n + \int_{nT_0}^{(n+1)T_0} f(y, u, t) dt$$
(13)

There are several numerical integration methods to calculate y_{n+1} . For instance, the Euler, and trapezoidal methods could be used (Eqs. (14) and (15) respectively).

$$y_{n+1} \cong y_n + T_0 f\left(y_n, u_n, t_n\right) \tag{14}$$

$$y_{n+1} \cong y_n + \frac{T_0}{2} \left\{ f\left(y_n, u_n, t_n\right) + f\left(y_{n+1}, u_{n+1}, t_{n+1}\right) \right\}$$
(15)

where y_{n+1} on the right-side member of Eq.(15) is not known and, therefore, can be estimated by Eq. (14). So, y_{n+1} can be substituted by the desired trajectory and then the control action to make the output system evolve from the current value (y_n) to the desired one can be calculated. This work proposes applying this approximation to the dynamic model of a continuous fermentation and thus obtaining the control action that enables system variables to follow a pre-established trajectory during its experimental time (Scaglia 2006a; Scaglia *et al.* 2007, 2008).

Controller 1

Let us consider the following set of difference equations obtained by discretization of eqs. (1) - (5), where *X*, *S*, *P*, *Z*, and *I* represent the states of the bioreactor defined previously, and also, *S* and *P* represent the assumed measurements of the system to be controlled, *Ds* and *Sin* are the control action, and *t*, the time. The values of the variables at discrete time t = nTo, where *To* is the sampling period, and $n \in \{0,1,2,3,\cdots\}$ will be denoted as S_n and P_n . Same notation will be used for X_n, Z_n and I_n . Thus, wishing to compute S_{n+1} and P_{n+1} by knowing S_n and P_n , equation (1) - (5) should be integrated over the time interval $nTo \le t \le (n+1)To$ as cited on theoretical framework; for instance, the Euler approach will be used to calculate $X_{n+1}, S_{n+1}, P_{n+1}, Z_{n+1}, I_{n+1}$. Also, through a set of algebraic considerations with the flows defined in Echeverry *et al.* (2004), from eqs. (1-5) it follows that:

$$\frac{X_{n+1} - X_n}{To} = \mu_n X_n + [RD/4 + Ds_n](R-1)X_n$$

$$\frac{S_{n+1} - S_n}{To} = \left(-\frac{1}{Y_{p/s}}\right) (Qp_n X_n) + Ds_n Sin_n - Ds_n S_n$$
(16)
$$\frac{P_{n+1} - P_n}{To} = Qp_n X_n - Ds_n P_n$$

$$\frac{Z_{n+1} - Z_n}{To} = \beta (I_n - Z_n)$$

$$\frac{I_{n+1} - I_n}{To} = \beta (Qp_n X_n - Ds_n P_n - I_n)$$

This consideration will be used with the aim to design a controller and to consider a complete dynamic analysis. The quantities $X_{n+1}, S_{n+1}, P_{n+1}, Z_{n+1}, I_{n+1}$ on the left side member are not known and, therefore, can be estimated by the set of Eqs. (16).

The use of numerical methods in the simulation of the system is based mainly on the possibility of determining the state of the system at instant n+1 from the state, the control action, and other variables at instant n. So, $X_{n+1}, S_{n+1}, P_{n+1}, Z_{n+1}, I_{n+1}$ can be substituted by the desired trajectory and then the control action to make the output system evolve from the current value to the desired one can be calculated. This work proposes applying this approximation to the dynamic model of a continuous fermentation and, thus, obtaining the control action that enables system variables to follow a pre-established trajectory during its experimental time. The term D is the total dilution rate, which means $D_s + D_r$, where D_r is the dilution rate associated with the biomass recycle R. It is important to remark that into this controller proposal, R and D_r will remain constant, but it will be considered to analyze the dynamic effect over control actions S_{in} and D_s . Working on biomass differences equation from (16) one obtains:

$$X_{n+1} = X_n + \mu_n ToX_n + [ToX_n RD/4 + ToX_n Ds_n](R-1)$$

$$X_{n+1} = X_n + \mu_n ToX_n + Ds_n \left[ToX_n R^2/4 + \frac{3}{4} ToX_n R - ToX_n \right] + Dr \left[ToX_n R^2/4 - ToX_n R/4 \right]$$
(17)

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And this way we can obtain an expression for the control action Ds_n ,

$$Ds_{n}\left[ToX_{n}R^{2}/4 + \frac{3}{4}ToX_{n}R - ToX_{n}\right] = X_{n+1} - X_{n} - \mu_{n}ToX_{n} - Dr\left[ToX_{n}R^{2}/4 - ToX_{n}R/4\right]$$
(18)

Rewriting the set of equations (16) - (18), and working to put the system on matricial presentation, $\begin{bmatrix} & 2 & & 1 \end{bmatrix}$

$$\begin{bmatrix} X_{n+1} \\ S_{n+1} \\ R_{n+1} \\ I_{n+1} \\ I_{n+1} \end{bmatrix} = \begin{bmatrix} ToX_n R^2 / 4 + \frac{3}{4} ToX_n R - ToX_n \end{bmatrix} 0 \\ -ToS_n & To \\ -ToP_n & 0 \\ -To\betaP_n & 0 \end{bmatrix} \begin{bmatrix} Ds_n \\ Ds_n Sin_n \end{bmatrix} + \begin{bmatrix} X_n + \mu_n ToX_n + Dr \lfloor ToX_n R^2 / 4 - ToX_n R / 4 \rfloor \\ S_n - \left(\frac{1}{Y_{p/s}}\right)(Qp_n X_n To) \\ P_n + ToQp_n X_n \\ Z_n + To [\beta(I_n - Z_n)] \\ I_n + To [\beta(Qp_n X_n - I_n)] \end{bmatrix}$$
(19)

And developing the previous expression to reach the desired form and rewriting the system to the form Ax = b, in which x is the control actions vector,

$$\begin{bmatrix} ToX_{n}R^{2}/4 + \frac{3}{4}ToX_{n}R - ToX_{n} \end{bmatrix} 0 \\ -ToS_{n} & To \\ -ToP_{n} & 0 \\ 0 & 0 \\ -To\betaP_{n} & 0 \\ \hline A \end{bmatrix} \begin{bmatrix} DS_{n} \\ DS_{n}Sin_{n} \end{bmatrix} = \begin{bmatrix} X_{n+1} - X_{n}(1 + To\mu_{n}) - Dr \left[ToX_{n}R^{2}/4 - ToX_{n}R/4 \right] \\ S_{n+1} - S_{n} + \left(\frac{1}{Y_{p/s}} \right) (Qp_{n}X_{n}To) \\ P_{n+1} - P_{n} - ToQp_{n}X_{n} \\ Z_{n+1} - Z_{n} - To \left[\beta(I_{n} - Z_{n}) \right] \\ I_{n+1} - I_{n} - To \left[\beta(Qp_{n}X_{n} - I_{n}) \right] \\ \hline b \end{bmatrix}$$
(20)

Re-ordering the rows 4-5

$$\begin{bmatrix} ToX_{n}R^{2}/4 + \frac{3}{4}ToX_{n}R - ToX_{n} \end{bmatrix} = \begin{bmatrix} X_{n+1} - X_{n}(1 + To\mu_{n}) - Dr \begin{bmatrix} ToX_{n}R^{2}/4 - ToX_{n}R/4 \end{bmatrix} \\ -ToS_{n} & To \\ -ToP_{n} & 0 \\ 0 & 0 \\ -To\betaP_{n} & 0 \end{bmatrix} \begin{bmatrix} Ds_{n} \\ Ds_{n}Sin_{n} \end{bmatrix} = \begin{bmatrix} X_{n+1} - S_{n} + \left(\frac{1}{Y_{p}/s}\right)(Qp_{n}X_{n}To) \\ P_{n+1} - P_{n} - ToQp_{n}X_{n} \\ I_{n+1} - I_{n} - To \left[\beta(Qp_{n}X_{n} - I_{n})\right] \\ Z_{n+1} - Z_{n} - To \left[\beta(I_{n} - Z_{n})\right] \end{bmatrix}$$
(21)

Working on the matrix, the following expression will be obtained:

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$$\begin{bmatrix} ToX_nR^2/4 + \frac{3}{4}ToX_nR - ToX_n \end{bmatrix} = \begin{bmatrix} X_{n+1} - X_n(1 + To\mu_n) - Dr[ToX_nR^2/4 - ToX_nR/4] \\ -ToS_n & To \\ -ToP_n & 0 \\ -To\betaP_n & 0 \end{bmatrix} \begin{bmatrix} Ds_n \\ Ds_nSin_n \end{bmatrix} = \begin{bmatrix} X_{n+1} - X_n(1 + To\mu_n) - Dr[ToX_nR^2/4 - ToX_nR/4] \\ S_{n+1} - S_n + \left(\frac{1}{Y_{p/s}}\right)(Qp_nX_nTo) \\ P_{n+1} - P_n - ToQp_nX_n \\ I_{n+1} - I_n - To[\beta(Qp_nX_n - I_n)] \end{bmatrix}$$
(22)

From Eq. (22) it can be seen that:

The columns of matrix A are linearly independent. There is a greater number of equations than unknown variables. The rank of the matrix A is 2, which is the same as the number of columns of A. To accomplish the objective to find the appropriate control actions, it is necessary to solve a system of linear equations for each sampling period. Eq. (21) can be expressed as a system with the form,

$$A\begin{bmatrix}x\\xy\end{bmatrix} = b \tag{23}$$

The expressions for the control variables will be available through the solution of the equation

$$\begin{bmatrix} x \\ xy \end{bmatrix} = A^{+}b = \begin{bmatrix} f \\ g \end{bmatrix}; x \begin{bmatrix} 1 \\ y \end{bmatrix} = \begin{bmatrix} f \\ g \end{bmatrix} = f \begin{bmatrix} 1 \\ g/f \end{bmatrix}$$
(24)

In eq. (24) A^+ is the Moore-Penrose pseudo inverse of matrix A. In this way, an easy approach to the control of Substrate and Product concentration, through the control variables *Ds* and *Sin* is presented. To accomplish this, it is necessary to solve a system of linear equations for each sampling period.

The optimal solution, by least squares, is (Strang 1982)

$$\begin{bmatrix} Ds_n \\ Ds_nSin_n \\ x \end{bmatrix} = \begin{bmatrix} \begin{bmatrix} ToX_nR^2/4 + \frac{3}{4}ToX_nR - ToX_n \end{bmatrix} & 0 \\ -ToS_n & To \\ -ToP_n & 0 \\ -ToP_n & 0 \\ A \end{bmatrix} + \begin{bmatrix} X_{n+1} - X_n(1 + To\mu_n) - Dr \begin{bmatrix} ToX_nR^2/4 - ToX_nR/4 \end{bmatrix} \\ S_{n+1} - S_n + \left(\frac{1}{Y_{p/s}}\right)(Qp_nX_nTo) \\ P_{n+1} - P_n - ToQp_nX_n \\ I_{n+1} - I_n - To \begin{bmatrix} \beta(Qp_nX_n - I_n) \end{bmatrix} \end{bmatrix}.$$
(25)

The control actions D_s and S_{in} , can be reached through the solution expressed by Eq. (25) each sample time; and then, to the right tuning of the control actions, a dynamic analysis must be done (Quintero *et al.* 2008b). It will be necessary to prove the controllers performance in the case of the worst disturbances, and analyze the control actions effect over the fermentation process performance (Quintero *et al.* 2008c). It is important to remark that the recycle variable will be taken into account in fixed values such as zero or a convenient value for dynamic analysis purposes. Section 5 of results will address these tests.

Controller Improvement

The controller improvement will be done with respect to the use of recycle term R, with the aim to handle the microorganism's concentration. The first equations are the same as considered previously into the controller design, then, the recycle variable starts to be taken into account as the control vector to pose the problem. This way, as cited in the theoretical framework, the Euler approach will be used to calculate all state variables $X_{n+1}, S_{n+1}, P_{n+1}, Z_{n+1}, I_{n+1}$. Rewriting the previous set of equations (16),

$$X_{n+1} = X_n (1 + To\mu_n) + \frac{ToX_n R_n^2 D_n}{4} + ToX_n R_n Ds_n - \frac{ToX_n R_n D_n}{4} - ToX_n Ds_n$$

$$S_{n+1} = S_n + To \left[\left(-\frac{1}{Y_{p/s}} \right) (Qp_n X_n) + D_n Sin_n - D_n S_n \right]$$

$$P_{n+1} = P_n + To (Qp_n X_n - D_n P_n)$$

$$Z_{n+1} = Z_n + To \left[\beta (I_n - Z_n) \right]$$

$$I_{n+1} = I_n + To \left[\beta (Qp_n X_n - D_n P_n - I_n) \right]$$
(26)

Working to express the values of the states in time n+1 and put the system on matricial presentation,

$$\begin{bmatrix} X_{n+1} \\ S_{n+1} \\ P_{n+1} \\ I_{n+1} \end{bmatrix} = \begin{bmatrix} X_n (1+To\mu_n) + \frac{ToX_n R_n^2 D_n}{4} + ToX_n R_n Ds_n - \frac{ToX_n R_n D_n}{4} - ToX_n Ds_n \\ S_n - To \left(\frac{1}{Y_{p/s}}\right) (Qp_n X_n) + ToD_n Sin_n - ToD_n S_n \\ P_n + ToQp_n X_n - ToD_n P_n \\ Z_n + To \left[\beta (I_n - Z_n)\right] \\ I_n + (1 - To\beta) + To\beta Qp_n X_n - To\beta D_n P_n \end{bmatrix}$$
(27)

and developing the previous expression to reach the desired form,

Rewriting the system to the matricial form Ax = b, in which x is the control actions vector,

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Re-ordering the rows 4-5,

$$\begin{bmatrix} \frac{ToX_n}{4} & ToX_n & -\frac{ToX_n}{4} & -ToX_n & 0 & 0\\ 0 & 0 & 0 & 0 & To & -ToS_n\\ 0 & 0 & 0 & 0 & 0 & -ToP_n\\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} R_n^2 D_n\\ R_n Ds_n\\ Ds_n\\ D_n^{Sin}\\ D_n \end{bmatrix} = \begin{bmatrix} X_{n+1} - X_n (1 + To\mu_n)\\ S_{n+1} - \left(S_n - To\left(\frac{1}{Y_{p/s}}\right)(Qp_n X_n)\right)\\ P_{n+1} - \left(P_n + ToQp_n X_n\right)\\ I_{n+1} - \left(I_n + (1 - To\beta) + To\betaQp_n X_n\right)\\ Z_{n+1} - \left(Z_n + To\left[\beta(I_n - Z_n)\right]\right) \end{bmatrix}$$
(30)

Through elimination of the 5th row that is linearly dependent and working on the system, the following expression can be obtained:

$$\begin{bmatrix} \frac{ToX_n}{4} & ToX_n & -\frac{ToX_n}{4} & -ToX_n & 0 & 0\\ 0 & 0 & 0 & 0 & To & -ToS_n\\ 0 & 0 & 0 & 0 & 0 & -ToP_n\\ 0 & 0 & 0 & 0 & 0 & -To\betaP_n \end{bmatrix} \begin{bmatrix} R_n^2 D_n\\ R_n D_n\\ D_s_n\\ D_n \end{bmatrix} = \begin{bmatrix} X_{n+1} - X_n (1 + To\mu_n)\\ S_{n+1} - \left(S_n - To\left(\frac{1}{Y_{p/s}}\right)(Qp_n X_n)\right)\\ P_{n+1} - (P_n + ToQp_n X_n)\\ I_{n+1} - (I_n + (1 - To\beta) + To\betaQp_n X_n) \end{bmatrix}$$
(31)

From Eq. (30) it can be seen that: The Control actions R, D_s and S_{in} are coupled in the proposed solution. The columns of matrix A are linearly independent. There is a greater number of equations than unknown variables. The optimal solution by least squares, can be seen Strang (1982) is

$$\begin{bmatrix} R_n^2 D_n \\ R_n Ds_n \\ R_n D_n \\ Ds_n \\ D_n Sin_n \\ D_n \end{bmatrix} = \begin{bmatrix} \frac{ToX_n}{4} & ToX_n & -\frac{ToX_n}{4} & -ToX_n & 0 & 0 \\ 0 & 0 & 0 & 0 & To & -ToS_n \\ 0 & 0 & 0 & 0 & 0 & 0 & -ToP_n \\ 0 & 0 & 0 & 0 & 0 & 0 & -ToP_n \end{bmatrix}^+ \begin{bmatrix} X_{n+1} - X_n (1 + To\mu_n) \\ S_{n+1} - \left(S_n - To\left(\frac{1}{Y_{p/s}}\right)(Qp_n X_n)\right) \\ P_{n+1} - (P_n + ToQp_n X_n) \\ I_{n+1} - (I_n + (1 - To\beta) + To\betaQp_n X_n) \end{bmatrix}.$$
(32)

This way, an easy approach to the control of Substrate and Product concentrations through the control variables R, D_s and S_{in} was presented. The control actions R, D_s , and S_{in} for each sample time can be reached through the solution expressed by Eq (32), and then a dynamic analysis must be done for the proper adjustment and right tuning of the control actions. The effects over the fermentation process performance can be analyzed in the same way. Figure 5 depicts the results obtained.

RESULTS AND DYNAMIC ANALYSIS

This section will show the performance results of the controllers designed in previous section. The test scenario will be the fermentation process of *Zymomonas mobilis* during 150 hours time.

The controller performance will be tested using the set of parameters calculated to reproduce the natural oscillatory behavior of the bacteria. It is important to remark that with the aim of reproducing the experimental behavior of *Z.m*, the parameters set calculated by Daugulis *et al.* (1997) and Raposso *et al.* (2005) were used. This set of parameters allows us to consider the fermentation process in real conditions to make it reach the desired productivity that has been shown (Daugulis *et al.* 1997; McLellan *et al.* 1999; Raposso *et al.* 2005; Rogers *et al.* 2007), which is very important from the feasibility perspective for possible online implementation. The simulation results are presented as follows: the controllers for substrate and product have a good performance based on the criteria of smoothness in control loops, and the variables follow the desired trajectory. The control actions used to reach these conditions are between the physical limitations, defined by the flows and the speed of real actuators; control actions are limited by 0.03 < Ds < 0.2 and 10 < Sin < 250 (Quintero *et al.* 2008c).

The set point was selected according to the real behaviour of bacteria and by following the purpose of maximizing its productivity. Another important remark is that the initial conditions used to simulate the *Z.m* oscillatory open loop behaviour correspond to real data. To test the controller performance against disturbances, a scenario composed by a set of extreme additive disturbances in input flows was generated. It is cited in the literature that a frequent source of disturbance in this kind of systems is an augmenting or decreasing of input flow (or in batch systems, parameters with variations decreasing from batch to batch). The controller performance in simulation with both estimator and controller with a fixed recycling of 10% can be seen in Fig 3.

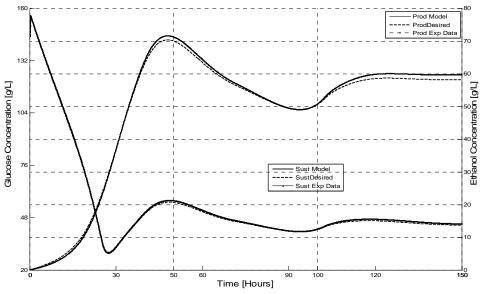


Figure. 3. Simulation controller and estimator performance. The solid line (Prod. – Subst. Model) represent the simulation results, the dotted line (Prod. - Subst. Desired) is the desired trajectory and the Prod. - Subst. Exp. data represent the real data in which the desired trajectory is based.

In order to be close to the real behavior of a controller implemented online, an experimental trajectory was selected (Raposso *et al.* 2005) and as in the study case, these experimental data were interpolated to obtain a continuous reference trajectory for substrate and product. The reference real data used for trajectory was the experimental results reported in (Raposso *et al.* 2005). For further information the reader should review that work.

Following the purpose of maximizing the productivity of culture, the trajectory defined by real culture behavior was smoothed. Another important remark is that the initial conditions used to simulate the Z. m oscillatory open loop behavior correspond to a real culture. The controller follows the pre-defined trajectory very well, and it corrects the inherent oscillations of the real fermentation; this it means that, under controlled conditions, the culture media will be more reliable to reach the maximum Z. mproductivity of ethanol. Also, the estimations made by a particle filter depend on the range of acceptable information that is used for reach a good controller performance. As criteria to the controller performance evaluation, the value of 5% around the desired trajectory was selected. Furthermore, the simulation was developed under disturbances chosen as highly dangerous for stability of culture, such as increasing or decreasing of one of the control variables. This was performed with the aim of simulating the scenario of a shutdown of actuators system or mistakes in pre-feeding process. Figures 4 and 5 depict the estimator performance in simulation with the controller following a smoothed trajectory generated with the use of real data. Figure 4 depicts the deviation of desired trajectory against disturbances and different controller constants. Note that in Fig. 5 the solid lines are within the plotted diamonds and circles, which means that the simulator performance in simulation was satisfactory.

Our purpose is to design an appropriate controller for the response system, such that the error system is asymptotically stable. These choices ensure that the error states converge to zero as time $t \rightarrow \infty$, and therefore the synchronization between estimator and controller is achieved. The proof for controller stability is analogous to that of Scaglia *et al.* (2009), and its presentation in a bioprocess will be found in a publication under preparation, which is focused on controllers.

Previous work studied the performance of both state estimator (Quintero *et al. 2008a*) and controller (Quintero *et al.* 2008b) separately; those results and their improvement allowed us to develop a first approach to the closed loop of the estimator and simple controller in simulation (Quintero *et al.* 2008c). Consequently, we have presented the conjunction of simple and complex controllers with the improved particle filter estimators. This paper is the result of the estimation technique applied in both simulation and real data, with the control technique previously applied with success not only to bioreactors, but also to trajectory tracking of mobile robots (Scaglia 2006; Scaglia *et al.* 2006, 2007). Both elements are in conjunction a remarkable scientific and technological goal. The importance of those results is that they provide a new perspective and an improvement for the optimization of the ethanol production facilities and process. Currently, biofuels such as ethanol are in the scope of many researchers; with the aim to develop cleaner, safe, reliable, and highly productive ways to obtain it, while looking for the optimality and the saving of costs and energy.

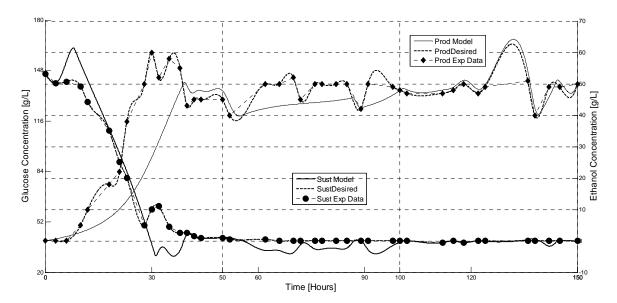


Figure. 4 Controller and estimator performance by the use of real data trajectory. In solid lines (Prod. – Subst. Model) is presented the simulation results, the dotted line (Prod. – Subst. Desired) is the desired trajectory, and the dotted diamond line (Prod. – Subst.) Exp. data represents the real data in which the desired trajectory is based.

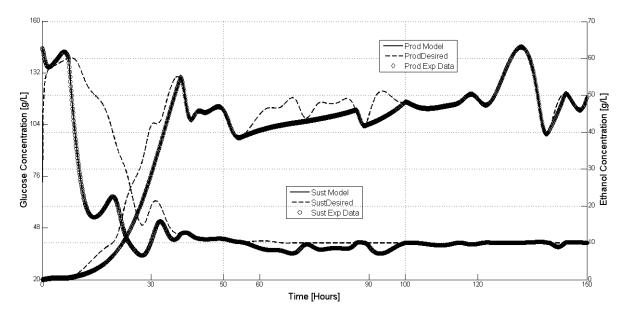


Figure 5. Improved controller and estimator performance by the use of real data trajectory. The solid lines (Prod. – Subst. Model) represent the simulation results, the dotted line (Prod. – Subst. Desired) is the desired trajectory, and the diamond-circle line (Prod-Sust) Exp data represents the real data upon which the desired trajectory is based.

CONCLUSIONS

- 1. In this work a controller based on numerical methods was built, and the performance of a recursive Bayesian state estimator was evaluated in closed loop. The application was illustrated in a continuous alcoholic fermentation process from *Zymomonas mobilis* bacteria. It was observed that the use of particle filtering as estimator of biomass and inhibition variables was acceptable, feasible, and of viable implementation. The use of the estimation tool makes it possible to solve the problem of the lack of online biomass estimation, and other important variables into a continuous process, due to its reliability and admissible computational cost to the real problem sample times. Its performance was satisfactory in the control loop.
- 2. The above control structures can be designed and implemented without great difficulty, because standard algebraic-numerical techniques are used. Simulation and experimental results of the developed controller designed for a *Z.m* continuous fermentation have been also addressed. Through the analysis of these experiments, it can be concluded that the trajectory error between the desired and the real trajectory of the fermentation is within the criteria of 5% around the desired value. It can be concluded that the proposed methodology is quite simple for selecting the parameters of the controller in order to achieve a good performance of the system. This methodology for the controller design can be applied to other types of systems.
- 3. The required precision of the proposed numerical method for the system approximation is smaller than the one needed to simulate the behavior of the system. Thus, the approach is used to find the best way to go from one state to the next one, according to the availability of the system model.
- 4. Real data trajectories showed that the controller is feasible and can be easily implemented with control actions bounded to the needed specifications of the real process. The term of recycle (R) added in this controller represents the likelihood of using biomass recycle as a control variable for continuous fermentation, and it improves the dynamic behavior.

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APPENDIX A

Filtering general algorithm detail, Sequential Importance Sampling SIS (Arulampalam et al. 2002):

$$\begin{bmatrix} \left\{ x_{k}^{i}, W_{k}^{i} \right\}_{i=1}^{N_{p}} \end{bmatrix} = SIS \begin{bmatrix} \left\{ x_{k-1}^{i}, W_{k-1}^{i} \right\}_{i=1}^{N_{p}}, y_{k} \end{bmatrix}$$

For $i = 1: Np$
 $x_{k}^{i} \Box q \left(x_{k/} x_{k-1}^{i}, y_{k} \right)$
 $W_{k}^{i} \propto W_{k-1}^{i} \frac{p(y_{k} / x_{k}^{i}) p(x_{k}^{i} / x_{k-1}^{i})}{q(x_{k}^{i} / x_{k-1}^{i}, y_{k})}$
end

Sequential importance sampling with resampling step *SIR* (Doucet *et al.* 2000): $\left[\left\{x_{k}^{j^{*}}, W_{k}^{j}, i^{j}\right\}_{i=1}^{N_{p}}\right] = RESAMPLE\left[\left\{x_{k}^{i}, W_{k}^{i}\right\}_{i=1}^{N_{p}}\right]$

$$\left[\left\{ x_k^{j^*}, W_k^j, i^j \right\}_{j=1}^{Np} \right] = RESAMPLE \left[\text{Initialize counter} \\ c_1 = 0 \\ For \ i = 2 : Np \\ \text{Construye la CDF} \\ c_i = c_{i-1} + W_k^i \\ end \\ \text{Starts at the beginning of CDF} \\ i = 1 \\ \text{Generates the start point} \\ u_1 \square U \left[0, \frac{1}{Np} \right] \\ For \ j = 1 : Np \\ \text{Go through CDF} \\ u_j = u_i + \frac{(j-1)}{Np} \\ \text{While } u_j > c_i \\ i = i + 1 \\ end \ While \\ \text{Asigns the sample} \\ x_k^{i^*} = x_k^i \\ \text{Asigns the weight} \\ W_k^j = \frac{1}{Np} \\ \text{Asigns parents} \\ i^j = i \\ end \\ \end{array} \right]$$

The resampling methods used were the residual, deterministic, and multinomial.

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