State observers for the online monitoring of a biorreactor: Industries 4.0 approach

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***Abstract*—Online monitoring of fermentation processes is a necessary task to determine concentrations of key biochemical compounds, demonstrate failures in process operations, and implement feedback controllers. However, obtaining the signals of all the important variables in a real process is a task that can be difficult and expensive due to the lack of suitable sensors or simply because some variables cannot be measured directly. From the above, a model-based approach, such as the state observer, can be a viable alternative to solve the estimation problem. This article discusses the real-time performance of a familiar sliding-mode observation strategy to reconstruct key variables in a batch bioreactor for fermentative ethanol production. For estimation purposes, the Hinshelwood model for ethanol production by *Saccharomyces cerevisiae* is used. The experimental results reported here show that the selected observer performs well since the structure used is robust to uncertainties and detection noise, properties that benefit the bioprocess estimation process.**

Keywords— Virtual sensor, Real-time implementation, Batch bioreactor, Ethanol fermentation, Sliding mode observer.

I. INTRODUCTION

Bioprocesses are currently an industry of great economic importance [1]. A bioprocess is a process that uses living cells or one of their components, such as enzymes, to develop products. The use of yeast to produce alcohol, for example, is a bioprocess.

The optimization of this is essential to obtain quality products, and minimize costs, operating times, and environmental pollution [2]. In this sense, online monitoring is essential to carry out control tasks, fault diagnosis, and the determination of the concentrations of the products of interest [2, 3]. However, this is a complicated, expensive, and sometimes very impractical task, this is due to the complexity of the reaction mixtures, which have to be carried out in controlled environments, with certain nutritional requirements for cell growth to exist and in consequence the production of the metabolites of interest (Products) [3, 4]. In addition, it is very common to find situations where the product to be measured requires prior treatments and/or specific techniques to carry out its quantification, which requires excessively long processing times [3]. It is worth mentioning that many times the instrumentation and available sensors do not always cover all the needs or at least the necessary ones [2]. The low availability of sensors in the market, their high costs, the presence of noise measurement, the operational policies of bioreactors, and their intrinsic nonlinear behavior are strong obstacles to bioreactor instrumentation [2, 3].

For all the above, virtual sensors (VS) are seen as a viable alternative to monitor key variables in bioprocesses. Furthermore, it has been shown that compared to expensive and relatively complex analytical techniques, SVs can provide reliable estimates online, are maintenance-free, and are less expensive [3].

Virtual sensors are mechanisms that allow variables to be calculated or estimated from the combination of online measurements provided by probes or sensors (hardware) and a computational algorithm (software) [3]. The objective is to use only the strictly necessary sensors and thus reduce costs for operation and maintenance [4]. Its operation and design is based on the principle that the dynamic evolution of all the variables is closely related to each of the process variables. Fig. 1 shows a general scheme of a virtual sensor.

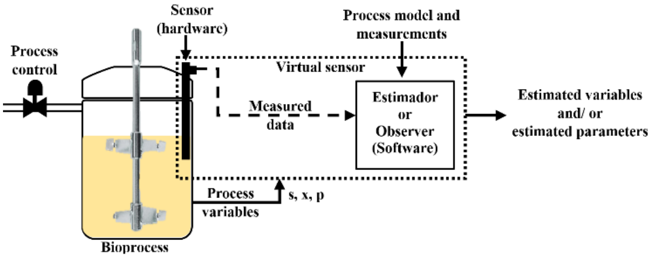

Fig 1. General scheme of the implementation of a virtual sensor in a bioprocess.

SVs can be classified into:

a) Techniques based on historical data: They use large amounts of data from the process considered in order to classify them and find correlations between their variables to infer behavior patterns. In this class of techniques, artificial neural networks, vector-supported machines, and regressive models stand out [1]. These techniques are very useful when there is no process model or the model is very complex, however, their greatest disadvantage is that they require a high computational cost and long training and adaptation times, in addition, the engineer or researcher in charge of designing them or implementing them must have a certain degree of experience in the system to be able to identify the possible correlations that exist between the data and the process variables.

b) Model-based techniques: They combine online measurements using physical sensors and an estimation algorithm based on a dynamic model that allows the phenomena that occur in a given process to be represented by means of differential equations [1]. The complexity of these models depends on the level of approximation to be achieved. On the one hand, the use of mathematical models facilitates the interpretation of the results, they do not require a specific architecture for their processing or training, they are easy to implement and have a lower computational cost, however, the efficiency of the algorithms depends directly on the quality of the models and measurements that are available [2]. Among the mechanisms for the development of virtual sensors are state observers, whose main objective is to estimate dynamic variables of a process from inputs, outputs, and a mathematical model. The idea is to reproduce the model with faster dynamics, which allows for predicting the behavior of the desired variables [1, 2].

SVs have already been applied in bioprocesses, we can find literature on the design and implementation of some state observers. However, a limiting factor when implementing in real time is the lack of adequate sensors [2-4]. It is common to find jobs where numerical simulations are carried out to evaluate the performance of the observers [5, 6]. The Luenberger observers and the extended Kalman filter are the most reported algorithms, which have been implemented in biohydrogen production processes, alcoholic fermentations, and anaerobic digesters where the main measurable variable is glucose concentration [7, 8], although we can also find literature on real-time implementations where the bioprocesses are already equipped with the necessary hardware and software for their implementation [9, 10]. For example, Petre et al. [11] developed an adaptive control law design based on nonlinear estimation algorithms for unknown inputs and kinetics. A common practice is to perform numerical simulations and validate the numerical results using experimental data. For example, Aviles et al. [8] designed an interval observer applied to a dark fermenter for biohydrogen production. The observer estimates the concentrations of glucose and biomass and the flux of hydrogen produced and compares its results with offline experimental data.

This work studies the performance of a state observer for real-time monitoring of substrate and ethanol concentrations in a batch fermenter of the *Saccharomyces cerevisiae* strain, based on biomass measurement. The experimental configuration and the characteristics of the bioprocess are briefly exposed. The Hinshelwood kinetic model is used for the description of ethanol production. The effectiveness of the observation strategy is evaluated using the absolute integral error performance index.

II. MATERIALS AND METHODS

A. Batch fermenter

Batch fermenters are characterized by being systems closed to mass transfer and convergence in finite time. This type of bioprocess is carried out in bioreactors where the necessary environmental conditions are guaranteed [2]. In general, batch fermentation begins when a nutrient solution is added with an aliquot of the microorganism that is going to carry out the fermentation process and stops at the end of the logarithmic phase for the primary metabolites, or before the death phase begins for secondary metabolites [2]. Monitoring of state variables and reaction speeds is important to guarantee product quality, avoid losses and reduce costs [3].

B. Experimental platform

The experimental configuration of the batch fermenter is shown in Fig. 2, the reactor consists of a 2 L beaker which works at a temperature of 30°C and stirring at 150 rpm, the reactor is instrumented with a turbidity probe Low cost. Data acquisition and processing are performed with the NI CRIO-9030 high-performance real-time controller.

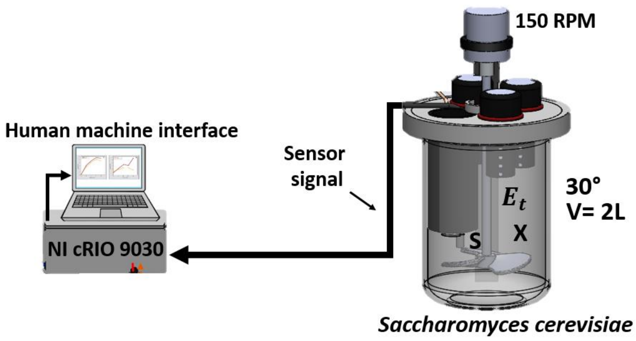

Figure 2. Scheme of the experimental setup in batch operation.

Fermentation was carried out with an inoculum of 0.1 g L-1 of *Saccharomyces cerevisiae*, with 1 L of sterile YM medium. The initial substrate concentration was adjusted to s0 = 46 g/L. The incubation temperature was 30°C and the agitation was 150 rpm [12]. The fermentation time was 24 h.

Total reducing sugars were estimated using the dinitrosalicylic acid (DNS) colorimetric method. Ethanol was determined by chromatography. The standard curve was obtained with HPLC-grade ethanol (Sigma-Aldrich). A Varian CP-9002 gas chromatograph with a flame ionization detector equipped with a ZB-FFAP column was used. Optical density measurements, the dry weight method, and the GT-TSW-30 probe were used to follow the course of cell growth. The optical densities of the samples were measured spectrophotometrically at 230 nm using a cell-free medium as blank [12].

C. Kinetic model

Mathematical models help describe the changes and phenomena that occur inside the reactors. Mathematical models are key pieces to guarantee a good performance of the observation strategies [2-4]. These can be classified into structured and unstructured models. Structured models use a more detailed approach to cell metabolism, with the aim of better describing the dynamic behavior of the process. On the other hand, we have unstructured models, which completely ignore the variation of biomass composition in response to environmental changes. These types of models, although very simple in nature, have been applied with considerable success in many studies, as well as in industry, for observation, control, and optimization purposes [4]. The present work used the Hinshelwood model [13], for the description of bioethanol production in a batch bioreactor by the microorganism *Saccharomyces cerevisiae* as described in Equations (1)–(3).

Substrate Balance (s):

$$\dot{s} = -\left(\frac{1}{y_{\frac{x}{s}}}\dot{x}\right) - \left(\frac{1}{y_{\frac{p}{s}}}\dot{E}_t\right) \tag{1}$$

Biomass balance (x):

$$\dot{x} = \mu_{max}\left(\frac{s}{k_{sx}+s}\right)\left(1 - K_{px}E_t\right)x \tag{2}$$

Ethanol balance (E_t):

$$\dot{E}_t = Q_{max}\left(\frac{s}{k_{sp}+s}\right)\left(1 - K_{pp}E_t\right)x \tag{3}$$

here $(\mu_{max}\ and\ Q_{max})$ are the specific growth and production rates, $(k_{sx}\ and\ k_{sp})$ are the average rate constants, (K_{px}, k_{pp}) are the inhibition constants.

In general, the system of equations (1-3), can be represented by the following nonlinear system:

$$\dot{\boldsymbol{\lambda}} = \boldsymbol{f}(\boldsymbol{\lambda}, \boldsymbol{u});\ \ \boldsymbol{\lambda}(\boldsymbol{t_0}) = \boldsymbol{\lambda_0} \tag{4}$$

$$y = h(\lambda) + \delta$$

where $\lambda \in \Re^n$ is the vector of state variables; $u \in \Re^q$ is the vector of control inputs; $y \in \Re^p$ are the measurable inputs; δ is an additive bounded noise obtained during the measurement.

D. *Observability through an inferential diagram*

Inference diagrams are a technique used to study the observability of nonlinear systems. The technique starts from the principle that there are connections between its states, inputs, and outputs [2, 14]. The inference diagram is built considering the following points [15]:

1) Draw a bond, $\lambda_i \rightarrow \lambda_j$ if λ_j appears in the differential equation for λ_i. This implies that by monitoring λ_i it is possible to obtain information about λ_j.
2) Decompose the inference diagram into a unique set of maximal strongly connected components (SCC). SCCs are subgraphs selected such that there is a direct path from every node to every other node in the subgraph. Dotted lines enclose the SCCs. It is worth noting that each node in an SCC contains information about the other nodes. The so-called root SCCs do not have output links.
3) We chose at least one node of each root SCC, which would be the sensor node, to guarantee the observability of the whole system.

Fig. 3 shows the inference diagram of Equations (1-3), where the set of state variables $\boldsymbol{\lambda} = [\boldsymbol{s}, \boldsymbol{x}, \boldsymbol{E_t}]$ is represented by nodes on the graph, where the sensor node $y = [x]$ are marked in red and the set of target nodes $\theta = [s, E_t]$ is marked in green.

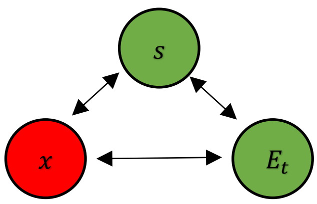

Fig. 3. Observability analysis (inference diagram) of Equation (1-3).

Postulate 1: *Equation (4) is observable for the output vector* $y = [0,1,0]^T \aleph$

E. *Sliding-Mode Observer*

For the design of this type of observer, a sliding variable is selected, which represents the difference between the measured variable (y) and the estimated $(\hat{y})$, so that it has a relative degree 1 concerning the designed injection signal. The discontinuous control signal acts on the first derivative with respect to the time of the sliding surface σ to maintain the trajectories of the system in the sliding set $y - \hat{y} = 0$ [16]. The discontinuous term is the one that allows the system to reject disturbances and parametric uncertainties [16], but it is also the one that produces chattering. In most cases, sliding-mode observers are obtained by injecting a nonlinear discontinuous term that depends on the output error within the observing system. The discontinuous injection must be designed so that the system trajectories are constrained to lie on some sliding surface in the error space. The resulting movement is called sliding mode [16].

The following dynamical system is an asymptotic observer of Equation (4) [2, 5]:

$$\dot{\hat{\lambda}} = f(\hat{\lambda}, u) + \kappa(y - \hat{y}) + \sigma sign(y - \hat{y}) \tag{5}$$

where the next expression defines the sign function:

$$sign(y - \hat{y}) = \begin{cases} 1\ if\ y - \hat{y} > 0 \\ 0\ if\ y - \hat{y} = 0 \\ -1\ if\ y - \hat{y} < 0 \end{cases}$$

$\sigma = [\sigma_1, \sigma_2, \sigma_3]^T$ is the observer gain vector sliding mode and $\kappa = [\kappa_1, \kappa_2, \kappa_3]^T$ is the gains of the proportional part.

This observer structure is related to identification and observation problems by including an uncertainty estimator and a state observer. The observer proportional part has stabilizing effects on the observer performance; high proportional gains ensure that the estimation error will decrease. To guarantee the stabilizing properties, the proportional gains must be in function of a positive solution of the Riccati algebraic equation [5]. The sliding part of the observer serves to compensate for uncertain nonlinear terms and provides asymptotic convergence. When sufficiently large sliding gains are chosen, the instability effect of the bounded nonlinear element can be decreased. This behavior occurs because, once on the sliding surface, the trajectories of the system remain on that surface, so the sliding condition is taken and the surface and the invariant are configured. This implies that some disturbances or dynamic uncertainties can be compensated for by keeping the surface as an invariant set. For more information check the stability properties and the convergence test in [5].

III. Results and Discussion

Fig. 4 shows the dynamic evolution in the ethanol production process with an initial substrate concentration of 46 g/L. The ethanol concentration was 14.52 g/L and a biomass concentration of 4.1 g/L at the end of fermentation.

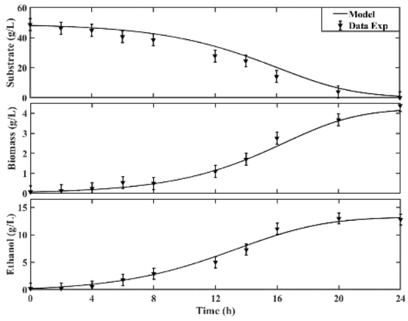

Fig 3. Dynamic behavior of the proposed model, experimentally validated.

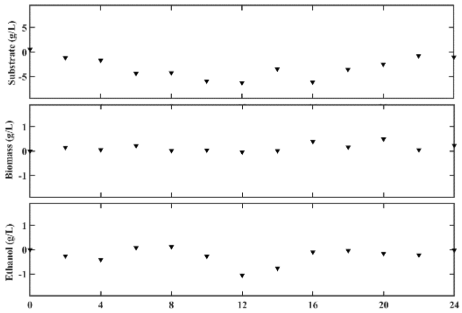

Fig 4. The residual value for substrate, biomass, and ethanol.

The results presented in Fig. 3 reveal that the proposed kinetic model was able to predict the concentrations of ethanol, substrate, and biomass (lines), obtained from experimental data (symbols). The observed trend indicates that the fermenting microorganism metabolizes the substrate to produce ethanol. This observation confirms the corresponding progressive increase in ethanol and biomass concentrations as cells metabolize the substrate to induce growth and subsequently produce ethanol [17]. The magnitude of a typical residual provides a general idea of the precision of our estimates fig 4.

The parameters of Equations (1)–(3) were calculated as a first approximation using the initial reaction rate method, taking into account the experimental data, followed by the Levenberg-Marquardt least squares minimization algorithm [18], in Table I we show the optimal values of the kinetic parameters. To generate the results reported in this paper, the ODEs were integrated using the ode15s function in MATLAB 2016a®.

TABLE I. KINETIC PARAMETERS FOR THE MATHEMATICAL MODEL

Symbol	Value	Units	Definition
μ_{max}	0.4 ± 0.2	$1/h$	Maximum Specific Growth Rate
Q_{max}	5 ± 2	$1/h$	Maximum substrate consumption rate
k_{sx}	30 ± 2	g/L	half speed constant
k_{sp}	$90 \pm .001$	g/L	half speed constant
$y_{x/s}$	0.085 ± 0.1		yield
$y_{p/s}$	0.2413 ± 0.1		yield
k_{px}	50 ± 5	L/gh	Setting parameter
k_{pp}	30 ± 5	L/gh	Setting parameter
n	$1.5 \pm .1$		Setting parameter

A common way to assess the fit of the model is to use statistical indicators such as the coefficient of determination (R^2, E). From this criterion, it was concluded that the model accurately portrayed the experimental data, evaluated by applying R^2 (average) = 0.9488 and E (average) = 0.9509 for the two initial conditions. Moreover, in all cases, R^2 and E were close to unity, indicating that the model produced a good fit.

Table II. Statistical correlation coefficients for quantifying the effectiveness of the model in describing the experimental observations related to discontinuous fermentation.

TABLE II. STATISTICAL CORRELATION COEFFICIENTS FOR QUANTIFYING THE EFFECTIVENESS OF THE MODEL IN DESCRIBING THE EXPERIMENTAL OBSERVATIONS RELATED TO DISCONTINUOUS FERMENTATION

Variable	R^2	E
Substrate	0.9652	0.9659
Biomass	0.9462	0.9483
Ethanol	0.9352	0.9385

A. *Implementation of the State Observer in Real-Time*

The results of the implementation in real-time are shown below. In this work, a TS-300B turbidity sensor was used to measure biomass density. The sensor is composed of an infrared light-emitting diode on one side and a phototransistor to detect the intensity of light passing through the open channel to the opposite slide [2, 19]. The output signal (Vout) is read directly by NI cRIO 9030.

Fig. 5 shows the experimental prototype where the estimation algorithm was executed in real-time. The observation structure (5) was programmed in LabVIEW Real-Time with a sample time of 30 ms. The observer gains were obtained empirically, the vector $\sigma = [0.5,10,20]^T$ and $\kappa = [160,6\ ,10]^T$.

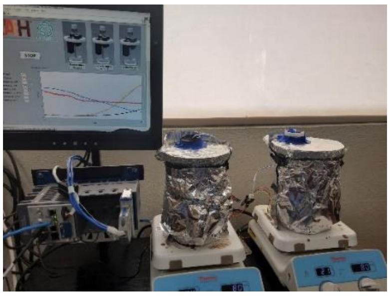

Figure 5. Implementation of observation strategies in the prototype plant.

The trajectories of each state observer (solid lines) were extracted from the NI cRIO 9030 and imported into MATLAB. In addition, a comparison with the experimental data was performed. Fig. 6 shows the performance of each state observer

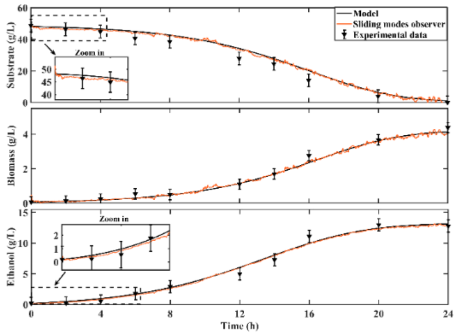

Fig 6. Real-time implementation of the observation structures and their comparison with the off-line experimental data.

The observation strategy was evaluated by using the performance index IAE (integral absolute error), Table III. Performance indices allow us to evaluate the behavior of the observer based on experimental data, for which an error function (e) defined as the difference between the measured variable (y_{exp}) and the estimated variable $(\hat{y})$ is used [2, 20]. In addition, a comparison is made between the IAE of the observer vs. the IAE of the model, where the error function for the model was defined as $e = y_{exp} - y$.

$$IAE = \int_{0}^{\infty} |(e)(t)| dt$$

The corresponding values of the performance index IAE are depicted in Table III.

TABLE III. REAL-TIME ESTIMATION ERROR

	s	x	Et
IAE		y_{exp} vs $\hat{y}$	
	81.45	2.56	7.61
IAE		y_{exp} vs $\hat{y}$	
	55.33	2.35	5.43

Fig. 7 shows the performance indices of the observer (blue line) and those of the model (red line), it is observed that the observer has a better performance obtaining smaller ISE values compared to the values obtained with the model.

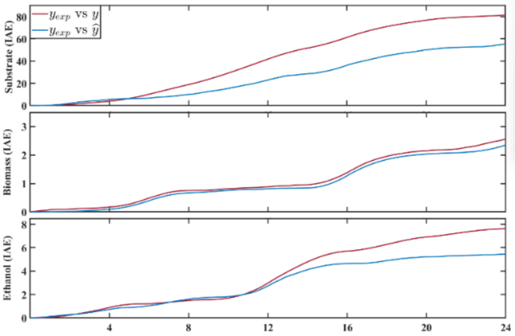

Fig 7. Comparison of observer performance index (blue line) vs. model performance index (red line).

IV. CONCLUSIONS

State observers are a viable alternative for the online monitoring of bioprocesses, they can provide reliable estimates online, require no maintenance, and are less expensive. It is important to emphasize that the modeling of bioprocesses is not something so trivial and the designer must carry out the validation of his model since the success of the observation strategy will largely depend on the quality of the model. The sliding mode observation strategy has been shown to perform satisfactorily for batch fermentation processes.

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