MONITORING AND CONTROL BASED ON A FIA-BIOSENSOR SYSTEM WITH AUTOMATIC CORRECTION

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Abstract: In this work, an enzymatic FIA-Biosensor system for lactose is used to on-line monitoring and control of a cultivation process to produce the enzyme lactase. Temperature effects on the output signal of the sensor are also studied. The whole system is modeled and, with this model, an automatic compensation is proposed to deal with temperature variations.

Keywords: Biotechnology; Compensation; Control applications; Bio control; Sampling systems; Dynamic modeling.

1. INTRODUCTION

During the last decades, the application of biotechnological processes has grown in several sectors, such as pharmaceuticals/medicine, agrochemicals, food technology, sustainable development etc. As operating parameters - quality of substrate and microorganisms - may change, an automatic control of the substrate supply is mandatory for the vast majority of the continuous and fed-batch processes, in order to keep high productivity levels. It will be more efficiently reached if appropriate measuring devices are available to provide on-line information about state variables. The development of specific biosensors interfaced with adequate sampling systems appeared as an important contribution to maximize the productivity of the bioprocesses. Biosensors are composed of a biological element connected to a transducer system, that can transform a biological signal into a measurable electrical signal. Several kinds of biosensors may be found in the literature, applied to a broad range of areas (Vega *et al.*,1998; Gill *et al.,* 1998; Adányi *et al.*, 1999; Heim *et al.*, 1999; Rasooly and Rasooly, 1999; Qin *et al*., 2000; D'Souza, 2001; Schroth *et al.*, 2001; Berney *et al.*, 2000).

Sampling systems (e.g., Flow Injection Analysis) are necessary for the on-line utilization of the biosensor, as its in-situ use is restricted by the fact that the sterilization procedures do not preserve the biological integrity of the biosensor. Integrated FIA-Biosensor systems have been extensively reported in the literature (Adányi *et al.*, 1999; Milardovic *et al.*, 1999;, Min *et al.*, 1999; Folly *et al.*, 1996; Weigel *et al.*, 1996), however only a very small number was effectively employed for automated control purposes (Ferreira *et al.*, 2001a; Hitzmann *et al.*, 2000).

The output signal of the FIA-Biosensor system is influenced by several parameters including the carrier rate and concentration, pH, temperature and composition of the sample. For that reason, a supervisory control, able to systematically analyze the data and detect instrument failures, should also be available (Olsson *et al.*, 1998, Linko, 1998, Lohn and Hitzmann, 1999). This work investigates the utilization of a lactose biosensor, integrated to a FIA system, for the on-line monitoring and control of a cultivation process. A dynamical model able to describe the whole *measurement system* is also formulated. This model allows the automated correction of the measurements for several parameters.

2. FIA-BIOSENSOR SYSTEM

The technology of the biosensor employed in this work was developed by Weigel (1995). It is

composed by two enzymes, β-galactosidase (β-gal) and glucose oxidase (GOD), which catalyze the following enzymatic reactions:

$$\text{Lactose} + H_2O \xrightarrow{\beta\text{-gal}} \text{D-glucose} + \text{D-Galactose} \quad (1)$$

$$\text{D-glucose} + O_2 \xrightarrow{GOD} \text{Gluconic Acid} + H_2O_2 \quad (2)$$

The oxygen consumption was measured and related to the lactose concentration in the sample. Fig. 1 shows the calibration curve to each microreactor. The responses are in volts as the amperometric electrode was coupled with a voltage converter (Wheatstone Bridge). The lowest concentration measured was 1 g/L.

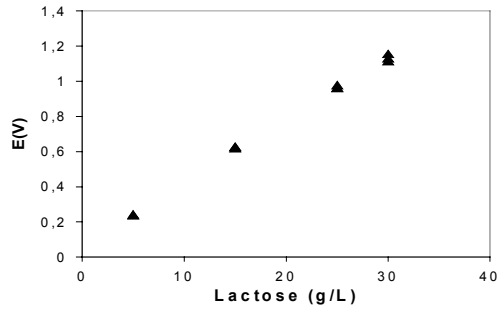

Fig. 1. Calibration curve for lactose measurements.

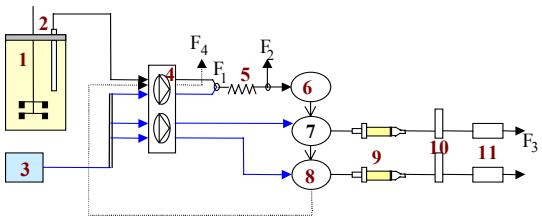

Fig. 2. FIA-Biosensor experimental set-up.

The experimental set-up is shown in Fig. 2. A sample is withdrawn from the bioreactor(1) through a micro-filtration membrane(2), being diluted immediately after the pump(4). After passing by a mixer(5), part of the mixture is discarded and the remainder follows to a selection valve(6). The calibration solutions (glucose and lactose) are connected to it, allowing to make recalibrations during the cultivation. From the selection valve, the sample goes to the injection valves(7 and 8). In this valve, two positions are possible: **L**(load) or **I** (injection). On **L** position, only the sample passes through the injection loop and the buffer(3) is directly sent to the lines of the biosensors(10) and amplifiers(11). On **I** position, the buffer goes through the injection loop, carrying the sample to the biosensors.

3. "FIA-BIOSENSOR" SYSTEM TO ON-LINE MONITORING AND CONTROL

3.1 Materials and Methods

Cultivations were conducted in BIOSTAT B (B. Braun) bioreactors, in a 5 L vessel, with 600 rpm agitation and 8 L/min input air flow rate. Temperature and pH were respectively controlled at 37°C and 5.5. These operating conditions were determined by Rech (1998). *Kluyveromyces marxianus* CBS 6556 yeast was the microorganism used for the cultivations. The culture media was composed of reconstituted powdered cheese whey (7% w/v) and yeast extract (0.5% w/v). The feed stream of the fed-batch was composed of cheese whey (28% w/v) and yeast extract (2% w/v). Cells were measured by optical density and lactose is measured by the DNS Method (Chaplin and Kennedy, 1994). The CAFCA software (ANASYSCON) completely automated the sampling and analysis measurement.

3.2 Controlled Fed-batch Cultivation.

The FIA-biosensor system provided signals related to the lactose concentration in the bioreactor, which were used to control the fed-batch cultivation manipulating the substrate feed rate. Effective integration of biosensors in control schemes are still very rare in the literature. To the knowledge of the authors, only one previous work (Hitzmann *et al.*, 2000) effectively used biosensors in a closed control loop. In the cited work (Hitzmann *et al.*, 2000), glucose concentration was controlled during a fed-batch cultivation, using a glucose biosensor and also a Kalman Filter to estimate cell concentration and the parameter μ_{max} of the Monod model.

In the present contribution, the measurements from the FIA-biosensor system were used by a PI controller implemented in MATLAB (gain K_c=0.025 g h/L^2 , reset time τ_i=2.8 h) to control the lactose concentration at 2 g/L (set-point). This value was chosen because it is known (Rech, 1998) that, in this cultivation, high enzyme activity rates are reached at low substrate concentrations. The results for the controlled variable are shown in Fig. 3. It can be seen that the lactose concentration was lead to the set-point in an oscillating fashion. The following reasons may have contributed for this behavior: (1) the PI was tuned by trial and error based on simulations of a model (Secchi *et al.*, 2001), which did not provide a good representation for fed-batch operation (because the model was originally fitted for batch operation mode); (2) the system's intrinsic noise disturbed the controller.

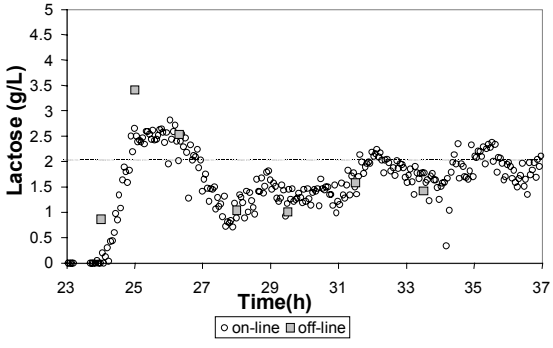

Fig. 3. Measurements of lactose concentration, on and off-line, during the control phase.

In one of the cultivations, at the beginning of a batch operation, the biosensor erroneously indicated that the lactose concentration was increasing, instead of decreasing. These results should not have been accounted for, as the temperature increased gradually during the first hours of the experiment. When the room temperature was kept constant and the system was recalibrated, the results coherently agreed with the off-line measurements. This fact clearly indicates that the temperature has a strong influence on the biosensor measurements. Hence, either the FIA-biosensor system should be thermally isolated or a compensation should be sought for the temperature effects. The second option is addressed here as presented in the sequel.

4. MODELING THE FIA-BIOSENSOR SYSTEM IN THE PRESENCE OF TEMPERATURE EFFECTS

Fig. 4 shows the behavior of the output signal at different temperatures. The data plotted are the media and standard deviation of three experiments, to each temperature and concentration.

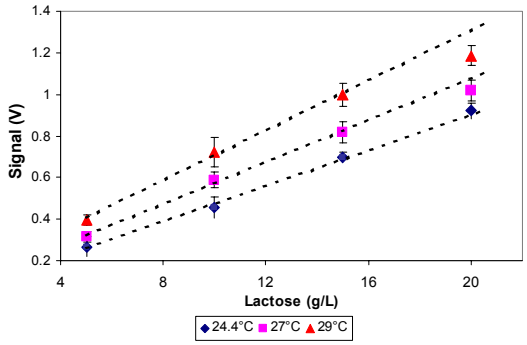

Fig. 4. Temperature influence on biosensor measurements.

It can be depicted from the figure that the temperature significantly affects the signal, imposing a nonlinear behavior which is stronger at highest temperatures and concentrations. If this effect is not considered, the sensor measurement will not be precise nor reliable. A compensation for the temperature effect was studied previously by the authors of this paper, using empirical correlations based on nonlinear regression and neural networks (Ferreira *et al.*, 2001b). In the present contribution, physical and chemical phenomena are considered in the development of the model allowing to include temperature effects. It must be emphasized that this approach is innovative, being not found in the present literature.

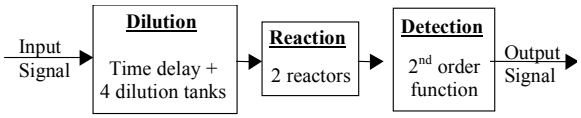

Fig. 5. Representative scheme of the model developed for the 'FIA-Biosensor' system.

The system was modeled taking the following elements into account (see Fig. 5): (i) dilution of the sample; (ii) enzymatic reaction in the biosensor; and, (iii) detection by the oxygen electrode.

4.1 Representation of the input signal

Even though the working characteristics of the injection valve make the input signals have a pulse shape, as the duration of the injection (0,0194 min) is very short, the input signal could be represented by an impulse function.

4.2 Modeling of the dilution line

The dilution line, that carries the sample to the biosensor, induces the concentration gradient responsible for the characteristic shape of the FIA's peaks (Fig. 8): the delay in the response of the sensor and the axial diffusion. In order to impart these characteristics to the model of the dilution line, n tanks were considered in series with a pure time delay element, as illustrated by Fig. 6.

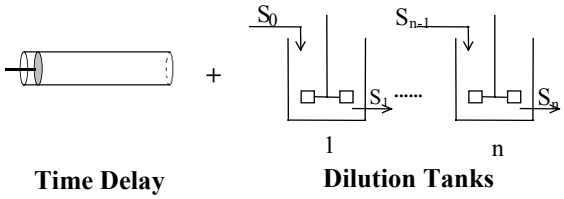

Fig. 6. Schematic representation of the dilution line, where n is the number of tanks.

The transfer function relating the output lactose concentration (S_n) to the input lactose concentration (S_0), in the dilution line, is:

$$\frac{S_n(s)}{S_0(s)} = e^{-t_d s} / (\tau s + 1)^n \qquad (3)$$

where s is the complex variable in the Laplace domain.

This approach allows to develop an analytical model to the dilution line by the Inverse Laplace Transform. According to the experimental data, the time delay for temperatures 27 and 29°C is 50 sec. For 24.4°C, this value is smaller, 45 sec. The dilution line is represented by 4 tanks in series, with a time constant of 0.117 min.

4.3 Modeling the enzymatic microreactor

As the biosensor is an enzymatic reactor, models describing the hydrolysis of lactose by immobilized enzymes were reviewed. In Ladero *et al.* (2001b) several enzymatic kinetic models were studied and a model based on the Michaelis-Menten equation, with competitive inhibition by galactose, proved to be most suitable. This model is described by the scheme and equations below:

$$E + S \rightarrow ES \rightarrow E + P$$
$$E + P \rightarrow EP$$

$$r_S = -kC_E S \Big/ \left(K_m \left(1 + \frac{P}{K_i} \right) + S \right) \qquad (4)$$

where:
S [g/L]: lactose concentration ; P [g/L]: product that inhibits the main reaction (glucose and/or galactose); C_E: concentration of the immobilized enzyme (mg of enzyme/g of support); k, K_m and K_i parameters of the Michaelis-Menten Model, including inhibition.

Ladero *et al.* (2000) defined temperature dependent parameters according to Arrhenius equation and estimated their values using experimental hydrolysis data. The case studied in this paper is similar to the one of Ladero *et al.*(2000), with the difference that, beyond β-galactosidase, glucose oxidase is also present. However, in the development of the present model it was considered that glucose was rapidly consumed. Hence, only the lactose hydrolysis by the β-galactosidase enzyme is taken into consideration.

Because of the characteristics of FIA, the sample injected to the system reaches the biosensor diluted approximately by a factor of 36 (D=36 min^{-1}). Consequently, the amount of galactose produced by the 1st reaction is very small (<< K_i) and competitive inhibitory effects were discarded from the model. Each reactor was modeled as a CSTR, according to equation 5.

$$\frac{dS_R}{dt} = D_R (S_n - S_R) - kC_E S_R / K_m + S_R \qquad (5)$$

where S_R and S_n are the lactose concentrations in the reactor and in the feed, respectively; D_R is the dilution rate of the reactor.

After linearization and application of Laplace Transform to the previous equation, a transfer function is obtained for each reactor (eq.6).

$$\frac{S_R'(s)}{S_n'(s)} = \frac{D_R}{\left(s + D_R + \frac{kC_E K_m}{(K_m + S_e)^2} \right)} \qquad (6)$$

where S_e is the lactose concentration at initial steady state.

The reaction was represented by two microreactors in series, each one having a volume of 0.05 mL. The concentration of the immobilized enzyme is 37.17 mg of enzyme/g of support. At the beggining, only the buffer (carrier) reaches the sensing element, then S_e was considered equal to zero. The kinetic parameters were estimated for each studied temperature(see.Table 1) and are represented by the following equations.

$$\ln k = \frac{-4384.3}{T} + 19.636 \qquad (7)$$

$$K_m = -3.378T + 1032.94 \qquad (8)$$

$$\ln K_i = \frac{-4521{,}7}{T} + 19.885 \qquad (9)$$

Table 1. Parameters to the reactor kinetic model.

T (°C)	24.4	27	29
k [min^{-1}]	135.5	150	170
K_m [g/L]	28	18.8	12.5
K_i [g/L]	108.91	123.44	137.35

4.4 Modeling of the oxygen electrode

An YSI oxygen electrode was used to measure the oxygen consumption. It was mentioned in the manufacturer's manual that steady state should be reached in 30 seconds and that 90 % of the response should be completed in 10 seconds. A transfer function describing the dynamics of the electrode was built based on these data, as shown in Fig. 7. The term 1/B describes the gain and converts the concentration input to a signal, in volts. The resulting function G can be modeled by transfer function in parallel, as shown in Fig. 7, where G_1 and G_2 exhibit slow and fast dynamics, respectively.

$$G = G_1 + G_2 = (8s+1) \big/ (20s^2 + 12s + 1) \qquad (10)$$

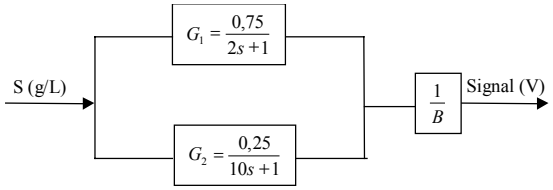

Fig. 7. Transfer functions for oxygen electrode.

4.5 Model Simulation

The final transfer function model can be derived from the product of the transfer functions of all components of the FIA-biosensor system:

$$G_{total} = G_{dilution}\, G_{reactor}\, G_{electrode} \qquad (11)$$

G_{total} is an 8th order transfer function. Fig. 8 shows the simulation of the model for the temperature of 24.4°C.

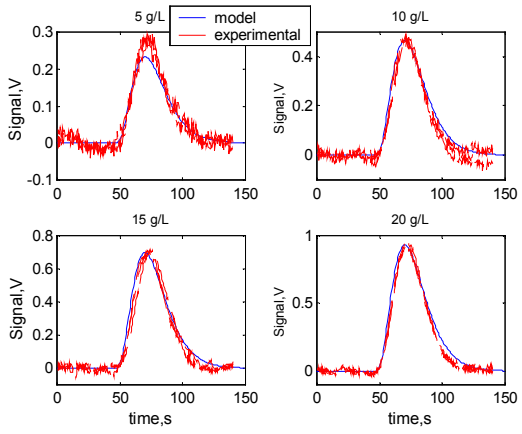

Fig. 8. Results of model simulation to T = 24.4°C.

The results of the simulation to the other temperatures are similar to ones shown in Fig. 8. However, a minor deviation in the model prediction at the end of the FIA cycle appears due to the increase of the non-linearity at high temperatures and concentrations.

4.6 Innovative approach of temperature compensation

The analytical solution of the model can be derived by the application of the inverse Laplace transform of eq. 11, which explicitly relates the input signal (S_0), to the temperature and the output signal (S). The literal analytical solution is an equation composed of eight terms (Ferreira, 2002). The analytical solutionis described in the Appendix. This approach allows the implementation of an automatic temperature compensation as schematized in Fig. 9. The output signal of the FIA-Biosensor system, represented by **S**, is analyzed (by the height of the peak, in this study). If a disturbance is detected in the temperature (which must be measured on-line), the module of compensation is fired; on the opposite case, only the calibration curve is used. The term $f(t,p(T),\tau)$ represents the exponential terms in the equation of the Appendix. According to the methodology explained, the experimental values were supplied as inputs (**S**) to the module of compensation and the results obtained ($\mathbf{S_0}$) can be seen in Table 2.

Table 2. Results of temperature compensation.

S_0 (g/L)	24.4°C	27 °C	29°C
5	5.81	5.59	5.92
10	9.87	10.4	10.8
15	15.1	14.5	14.9
20	20.0	18.0	17.8

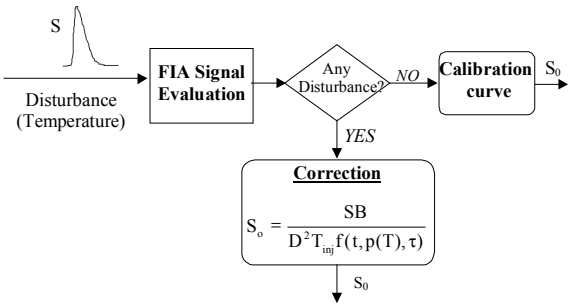

Fig. 9. Implementation proposal to compensate on-line temperature effects.

The following aspects of the proposed phenomenological model should be emphasized: (i) the use of the module of compensation can be extended to any variable considered in the model; for example carrier rate, time delay or injection time. Hence, any disturbance in these variables can be compensated; and, (ii) the dynamic behavior of the FIA-Biosensor system is completely modeled, what is important from the control point of view.

5. CONCLUSION

The following experimental characteristics of the biosensors were evidenced in this investigation:

- Short time for a complete analysis (about 2 minutes);
- Stability of the biosensor after almost 7000 measurements.

These features indicate biosensors as suitable devices for on-line monitoring of bioprocesses. Even so, based on the experimental experience acquired, the authors suggest for the case of industrial applications: (1) to follow the viability of the membrane that covers the oxygen electrode; (2) to measure the disturbances of the system and compensate them using models of the system; (3) to proceed frequent and automated recalibrations of the system (to avoid systematic errors caused by unmeasured disturbances); and, (4) to provide – in addition to recalibration – a failure detection scheme for the sensor, mainly when the system is being used for control. On-line and precise measurements make the implementation of the control substrate viable, allowing better conditions for the microorganisms to produce lactase. A first attempt to control this process is presented here. The initial results show that it is possible to use the biosensor for that purpose. However, additional studies should be endeavored to improve the performance of the controller.

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APPENDIX

Analytical solution equation of the phenomenological model.

$$S(t)=\frac{{D_R}^2\cdot S_0\cdot T_{inj.}}{B}\left\{\left[\frac{\frac{1}{6}(t-t_d)^3}{\tau^2(p\tau-1)^2}-\frac{(t-t_d)^2}{\tau(p\tau-1)^3}+\frac{3\tau}{(p\tau-1)^4}-\frac{4\tau}{(p\tau-1)^5}\right]e^{\frac{-(t-t_d)}{\tau}}\right\}+\left\{\frac{(t-t_d)}{(p\tau-1)^4}+\frac{4\tau}{(p\tau-1)^5}\right\}e^{-p(t-t_d)} \quad \text{(A1)}$$

where: $p=D_R+\frac{kK_m}{(K_m+S_e)^2}$; t, time; t_d, time delay; τ, time constant of dilution tanks; S_0, sample concentration of lactose; D, dilution rate.