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Model Based Soft-Sensor for On-Line Determination of Substrate

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Abstract

A software sensor for on-line determination of substrate was developed based on a model for fed-batch alcoholic fermentation process and on-line measured signals of ethanol, biomass, and feed flow. The ethanol and biomass signals were obtained using a colorimetric biosensor and an optical sensor developed in previous works that permitted determination of ethanol at a concentration of 0–40 g/L and biomass of 0–60 g/L. The volume in the fermentor could be continuously calculated using the total measured signal of the feed flow. The results obtained show that the model used is adequate for the proposed software sensor and determines continuously the substrate concentration with efficiency and security during the fermentation process.

Index Entries: Soft-sensor; substrate; alcohol fermentation; ethanol; biomass.

Introduction

The control of a fed-batch alcoholic fermentation process can be obtained by controlling the substrate concentration in the medium by manipulation of the feed flow. The fermentation process presents complicated kinetic mechanisms. In addition, there is the absence of accurate and reliable mathematical models as well as the difficulty of obtaining direct measurements of the process variables owing to a lack of appropriate on-line analyzers and sensors. Control systems are formed by a set of instruments and control mechanisms connected through electrical signals in the

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form of control loops, leading to increased efficiency and optimized process operation, reducing the costs of industrial production. A feasible way to control and monitor these processes is the use of software sensors. The software sensor uses a process model to estimate variables that neither can be directly measured nor are easily accessed through on-line available data. The limitation of currently used models is that they rarely use biologic state variable measurements *(1)*. These measurements are extremely important to providing good regulation of process performance when the physical and biochemical properties are constantly changing.

The process model can be obtained by different forms, and in bioprocesses mass balance equations can provide much information. However, in order to have efficient process models and software sensors, a previous adjustment of the model is necessary using on-line data collected from a plant under different operational conditions. This databank is important to guarantee that the model remains calibrated and represents the plant adequately. Some requisites are indispensable for the experimental implementation of models in software sensors: response speed to disturbances in the system and appropriate inference of primary variables of interest during key points of the process.

Court *(2)*, Eberhard *(3)*, and Tyagi et al. (4) have reported some applications of computers and software sensors for fermentation control in experimental research in data acquisition of bioreactors. Neural network models were used to interprete sensor signals in the control of an alcohol fed-batch fermentation *(5)* and in the detection of the individual components of a gas mixture and to measure the concentration of both gases *(6)*.

This article presents the design and implementation of a software sensor for the continuous determination of substrate concentration based on a simple model of a fed-batch fermentation process and the available signals of two other sensors—one for on-line biomass determination *(7)* and the other for on-line ethanol determination *(8)*—developed in previous works. The software sensor proposed provides a continuous signal that can be used in a control loop to manipulate the substrate feed flow in order to maintain almost constant substrate concentration and obtain an excellent level of productivity and yield during all of the process, as shown in experimental control strategy studies in previous works *(9)*.

Methods

Mathematical Model

A simple mathematical model is used for quantitative description of the process and consists of a set of equations relating inputs, outputs, and key parameters of the system. The model for an alcoholic fermentation fed-batch process developed by Mayer *(10)* and adapted with the Ghose and Tyagi *(11)* linear inhibition term by the product was used as the starting point for the development of a model-based substrate sensor with product (ethanol) and biomass on-line measurements.

To simplify study of the fed-batch alcoholic fermentation process and with the purpose of regulating the substrate concentration in the fermentation medium, the following assumptions were made: (1) the substrate concentration in the feed is constant; and (2) the volume change in the fermentor is a function of feed flow.

The following equations describe a general form of the model for the fed-batch process and total medium, having only substrate as feed flow, and include mass balances for substrate, product, biomass, and kinetic relations, respectively.

$$\frac{dV}{dt} = Fe \tag{1}$$

$$\frac{dS}{dt} = (Sa - S)\frac{Fe}{V} - X\left(\frac{\mu}{Yx/s} + \frac{\gamma}{Yp/s}\right) \tag{2}$$

$$\frac{dP}{dt} = \gamma X - \frac{Fe}{V} \tag{3}$$

$$\frac{dX}{dt} = X\left(\mu - \frac{Fe}{V}\right) \tag{4}$$

$$\mu = \mu_{max}\left(\frac{S}{Ksx + S}\right)\left(1 - \frac{P}{Kps}\right) \tag{5}$$

$$\gamma = \gamma_{max}\left(\frac{S}{Ksp + S}\right)\left(1 - \frac{P}{Kpp}\right) \tag{6}$$

To maintain optimal conditions in the fermentor, and assuming that the substrate concentration in the medium is much higher than the saturation constants (*Ksx* and *Ksp* <<< *S*), one can simplify the inhibition terms as follows:

$$\mu \approx \mu_{max}\left(1 - \frac{P}{Kpx}\right) \tag{7}$$

$$\gamma \approx \gamma_{max}\left(1 - \frac{P}{Kpp}\right) \tag{8}$$

The substrate concentration in a fed-batch fermentor can be maintained nearly constant, as shown in previous works *(9)*, and in this case one can assume that $dS/dt \approx 0$, and from Eq. 1

$$S = Sa - \left(\frac{X\,\mu\,V}{Yx/s\,Fe}\right) + \left(\frac{X\,\gamma\,V}{Yp/s\,Fe}\right) \tag{9}$$

and substituting the kinetics relations and arranging the terms one obtains

$$S(t) = Sa - \left[\frac{V(t)}{Fe(t)}\right]X(t)\left\{\left[\frac{\mu_{max}}{Yx/s}\right]\left[1 - \frac{P(t)}{Kps}\right] - \left[\frac{\gamma_{max}}{Yp/s}\right]\left[1 - \frac{P(t)}{Kpp}\right]\right\} \tag{10}$$

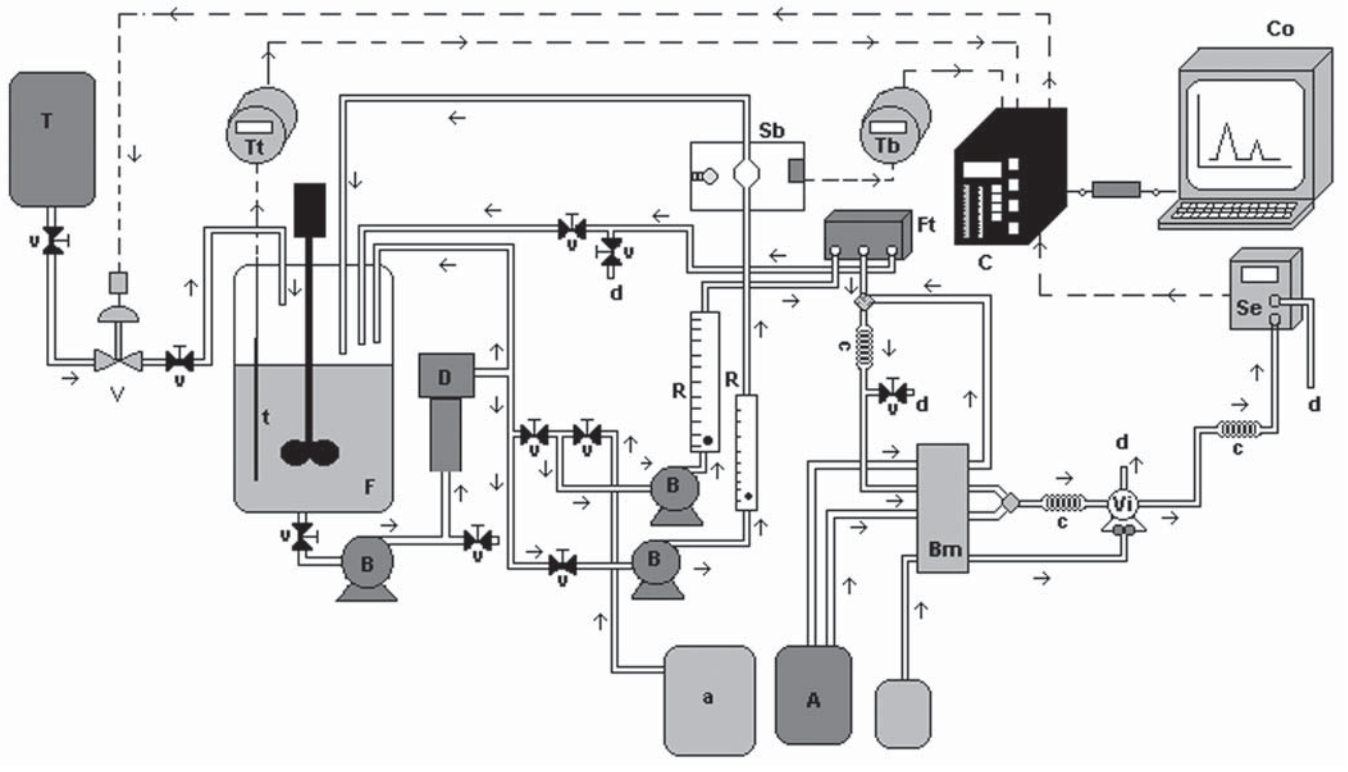

Fig. 1. Complete experimental setup for monitoring and control system for fermentor. T, feed tank; V, control valve; v, valves; F, fermentor; t, thermocouple; c, coils; A, dilutions tanks; Bm, multichannel pump; C, controller; Tt, temperature transmitter; Tb, biomass transmitter; Sb, biomass optical sensor; D, equipment to remove air bubbles; R, rotammeter; Ft, tangential filter; Co, computer; So, ethanol colorimetric sensor; Vi, injection valve; d, waste; E+R, reagents-enzymes tanks; B, pumps.

Using a new variable defined for the second term of Eq. 10 as a function of the on-line sensor signal of ethanol concentration, one obtains:

$$Pc(t) = \left[\left(\frac{\mu_{max}}{Yx/s} \right) - \left(\frac{\gamma_{max}}{Yp/s} \right) - \left(\frac{\mu_{max}}{Kps\ Yx/s} - \frac{\gamma_{max}}{Yp/s\ Kpp} \right) P(t) \right] \tag{11}$$

And using a new variable defined for biomass concentration as a function of the on-line measured biomass sensor, one obtains:

$$Xc(t) = X(t)\ Pc(t) \tag{12}$$

Finally, combining Eqs. 1, 10, 11, and 12, one obtains:

$$S(t) = Sa - \left(\frac{V_o + Fe\ (t)}{Fe} \right) Xc(t) \tag{13}$$

in which $Xc(t)$ is the combined function of the on-line measured variables; $X(t)$ is the biomass optical sensor signal, $P(t)$ is the ethanol colorimetric sensor signal, $Fe(t)$ is the feed flow signal–related control valve opening by calibration curve, and $V(t) = V_o + \int Fe\ dt$ is the volume signal calculated by the total block in the controller.

Integrated Control System for Fed-Batch Process

The complete experimental setup used as an integrated system for monitoring and control of the process variables is presented in Fig. 1. This inferred signal of substrate concentration can be continuously calculated on-line using essentially the model and combined signals of biomass and ethanol transmitters and the total of the feed flow measured signal. Implementation of this model-based sensor was carried out in a programmable digital multiloop controller combined with programmed modules in a supervisory software installed in a Pentium 200 microcomputer. The setup included on-line measurements of ethanol and biomass by sensors-transmitters adapted to appropriate sampling lines of the fed-batch fermentor, filtration equipment, feed and detecting solution tanks, pumps, and all hydraulic and electric accessories needed. Adapted equipment was used for removal of bubbles formed during the fermentation in function of the CO_2 produced in order to avoid interference in the biomass sensor signal.

The configuration of the controller was programmed to couple the signals of ethanol and biomass transmitters and infer the proposed model-based substrate sensor signal using specific calculation blocks. The controller was interfaced using an RS-232-485 interface with the microcomputer, where the supervisory software system was installed. The supervisory system software AimaxWin version 3.1 was configured, and historical trends for both direct and inferred process variables for $P(t)$, $X(t)$, $Pc(t)$, $Xc(t)$, and $V(t)$ could be incorporated directly into the previously designed screens and data sheets and tables.

Table 1
Experimental Operational Conditions and Parameters of Model

Kinetics parameters	Operational conditions
μ_{max} = 0.05 h^{-1}	V_o = 3 L
γ_{max} = 0.20 h^{-1}	P_o = 0 g/L
Yx/s = 0.18 g/g	S_o = 0 g/L
Yp/s = 0.51 g/g	t_f = 4.5 h
Ksx = 0.05 g/L	V_f = 7 L
Ksp = 0.5 g/L	X_o = 54.1 g/L (test 1) and 58.9 g/L (test 2)
Kpx = 66.57 g/L	Sa = 187 g/L (test 1) and 142 g/L (test 2)
Kpp = 78.72 g/L	Temperature = 31–34°C

Operational Conditions and Kinetic Parameters of Model

A set of alcoholic fermentations experiments was conducted in order to verify the performance of the model-based substrate sensor. Diluted molasses was used in the experiments as feed substrate and bread yeast, *Saccharomyces cerevisiae*, as inoculum. The operational conditions and kinetic parameters used are given in Table 1 for two different experiments (tests 1 and 2), and values of ethanol and biomass concentrations were also determined off-line using gas chromatography (CG) and dry cell weight standard *(7)* methods, respectively.

Results

The on-line sensors–measured values for ethanol and biomass were compared with values determined in paired analysis using off-line standard and analytical methods, shown in Fig. 2, and coincident results confirm the reliability of the two sensors. The off-line analytical methods used were GC for ethanol and dry cell weight standard for analysis of cell concentrations. The dry cell weight method was carried out using small volumes of the analyzed solutions that were centrifuged for 10 min at 800*g* and washed three times with water (for sample cells in molasses), and the cell mass obtained was transferred to a vessel weighed previously and dried at 95°C for 48 h. After this time the dry cell mass was weighed, and the sample concentration was expressed as grams of cells/liter of solution.

Figure 3 shows the experimental results of tests 1 and 2 obtained for the substrate concentration calculated using the software sensor and the filtered sensor measurements for ethanol and biomass. The differences between the two experiments are the initial biomass concentration and concentration of substrate in the feed, as shown in Table 1.

Discussion

A viable model-based software substrate sensor was developed, and the model proposed for its implementation was shown to adapt well for

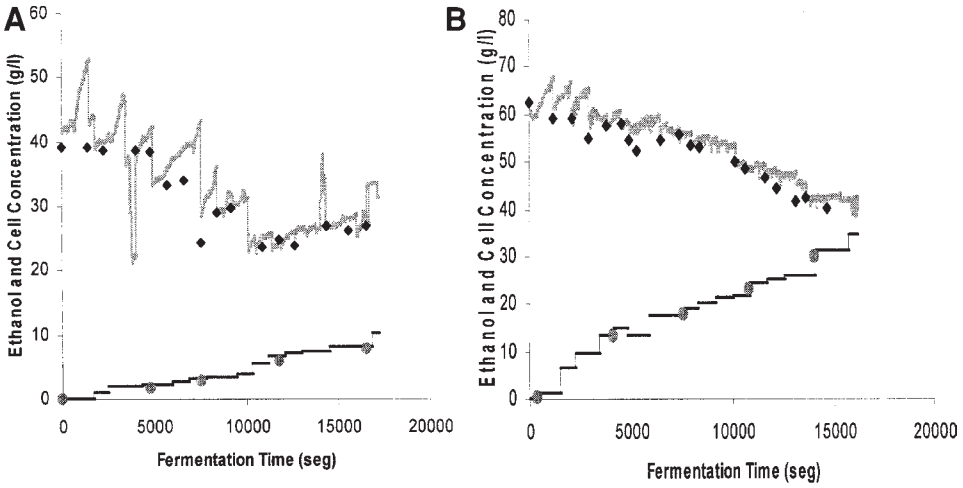

Fig. 2. Sensors on-line measurements (—— ethanol; —— biomass) compared to off-line analytical results (●, ethanol; ◆ biomass) for test 1 **(A)** and test 2 **(B)**.

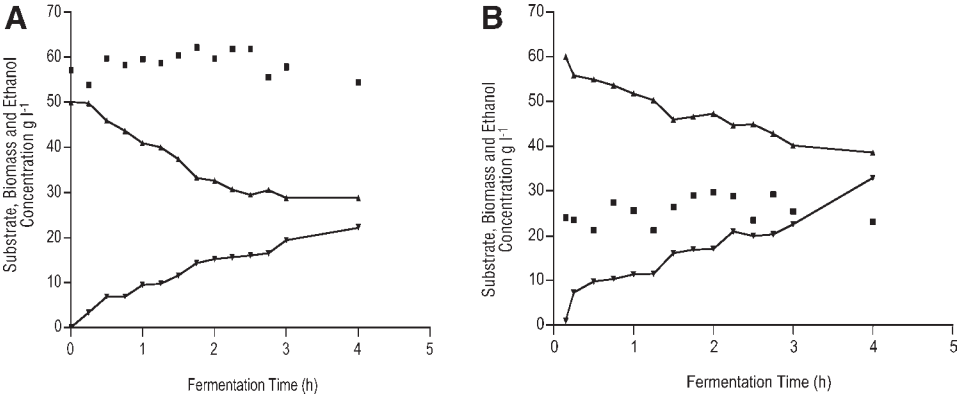

Fig. 3. Sensor filtered signals and inferred model–based substrate concentration for test 1 **(A)** and test 2 **(B)**: (▲) biomass; (▼) ethanol; (■) substrate).

fed-batch alcoholic fermentation. With on-line determinations of ethanol, biomass, and feed flow, it was possible to determine continuously the substrate concentration present in the fermentation medium during the process using this software sensor. The results of the experiments showed that the model presenting a simple, inexpensive, and robust method for substrate determination in fermentation medium, is highly efficient and reliable. In the future, this model also will allow control strategies to be implemented in order to improve the yield and efficiency of the process.

Nomenclature

ART	=	total reducing sugars (g/L)
Fe	=	feed flow to fermentor (L/h)
Kpp	=	inhibition constant of ethanol specific rate (g/L)
Kpx	=	inhibition constant of cell growth rate (g/L)
Ksp	=	saturation constant for specific ethanol production (g/L)
Ksx	=	saturation constant of substrate-microorganism (g/L)
P	=	ethanol concentration in fermentor (g/L)
S	=	substrate concentration (g/L)
Sa	=	substrate concentration in feed (g/L)
t	=	time (h)
V	=	volume of fermentor (L)
X	=	biomass concentration (g/L)
Yp/x	=	product yield coefficient based on biomass (g/g)
Yp/s	=	product yield coefficient based on substrate (g/g)
Yx/s	=	cell yield coefficient based on substrate (g /g)
μ	=	cell growth specific rate (h^{-1})
γ	=	ethanol production specific rate (g/[g·h])

Subscripts

ART	=	total reducing sugars (g/L)
o	=	initial
f	=	final
max	=	maximum value

References

1. Warnes, M. R. (1996), *Process Biochem.* **31,** 147–155.
2. Court, J. R (1988), *Progress in Industrial Microbiology*, vol. 25, Bushell, M. E., ed., Elsevier, Amsterdam, The Netherlands, p. 145.
3. Ebehard, O. V. (1992), *Biotechnol. Bioeng.* **40,** 572–582.
4. Tyagi, R. D., Du, Y. G., and Sreekrishnan, T. R (1993), *Process Biochem.* **28,** 259–267.
5. Ferreira, L. S., De Souza, M. B., and Folly, R.O.M. (2001), *Sens. Actuators B Chem.* **75(3),** 166–171.
6. Miguel Martín, A., Santos, J. P., and Agapito, J. A. (2001), *Sens. Actuators B Chem.* **77(1–2),** 468–471.
7. Salgado, A. M., Folly, R. O. M, and Valdman, B. (2001), *Sens. Actuators B Chem.* **75(1–2),** 24–28.
8. Salgado, A. M., Folly, R. O. M., Valdman, B., and Valero, F (2000), *Biotechnol. Lett.* **22,** 327–330.
9. Folly, R. O. M., Ramirez, N. I., and Valdman, B. (1994), in *Proceedings of the 5th International Symposium on Process System Engineering (PES/94)*, pp. 717–722.
10. Mayer, A. F. (1986), MS Thesis, Department of Engineering, Química EQ-UFRJ, Rio de Janeiro, Brazil.
11. Ghose, R. and Tyagi, B.(1984), *Process Biochem.* **19(4),** 136–141.