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CONTROL OF ONE STAGE BIO ETHANOL PRODUCTION BY RECOMBINANT STRAIN

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ABSTRACT

A new method for adaptive control of simultaneous saccharification and fermentation of starch to ethanol by the recombinant strain Saccharomyces cerevisiae YPB–G is proposed. The process monitoring is enriched by new software sensors of glucose consumption and production rates. The difference between their values is defined as a control marker which is used for switching from batch to fed-batch mode automatically and for determining the amplitude and duration of starch feeding pulses (control input). Simulation results have shown that the proposed control strategy stabilizes the process at an equilibrium state for the glucose concentration. In this way, the ethanol concentration in the reactor and the productivity of the process are increased.

Keywords: adaptive control, ethanol production, simultaneous saccharification and fermentation, fed-batch process.

Introduction

During the past years, the demand for the production of bio-fuels has increased rapidly, especially in the bio-ethanol case, which currently is produced mostly from sugar cane and starch containing raw materials. Traditionally, ethanol production from starchy materials is done in a sequential two-step process which includes two main stages: *i*) the enzymatic hydrolysis of starch to glucose (by means of the enzymes α-amylase and glucoamylase) and *ii*) the fermentation of glucose to ethanol (mostly by the action of yeast). A crucial drawback of the sequential (two-step) process is the slow hydrolysis rate (usually hours) due to the reduction of the enzymatic activity caused by an inhibitory effect when high sugar concentrations are present. A challenging perspective to overcome this problem and at the same time to increase the yield of the ethanol production process is to conduct the process in a one-step mode doing the simultaneous saccharification and fermentation of starch to ethanol (SSFSE) by means of recombinant strains (2, 16). In this way, the ethanol production process from starch is more efficient not only in terms of saving overall production time but also in terms of reducing equipment costs.

Nowadays, for SSFSE processes, *Saccharomyces cerevisiae* recombinant strains are mainly used (1, 2, 4, 14, 15). Recently, the genetically modified *S. cerevisiae* YPB – G strain which is able to convert directly starch to ethanol has been developed. This strain secretes a bifunctional fusion protein that contains both the *Bacillus subtilis* α- amylase and the *Aspergillus awamori* glucoamylase. Previous studies have demonstrated the potential use of the YPB – G strain in SSFSE processes for ethanol production (2, 4, 23).

In order to increase starch conversion efficiently, kinetic models such as those proposed in (6, 14, 16, 17, 20, 21), have to be used for process investigation and control. In (16), experimental data of SSFSE process using *S.cerevisiae* YPB–G strain were evaluated in order to develop a two-hierarchic level unstructured model. The first level modelled enzymatic hydrolysis of starch to glucose by bifunctional protein while the second level includes the utilization and bioconversion of glucose to ethanol by yeast. It is remarkable that no publications on control design of SSFSE processes were found in the open literature.

Recently, new methods for adaptive control of bioprocesses where one intermediate metabolite is produced in one reaction and then is used as substrate in other process reactions have been proposed in (7, 13). The SSFSE process could be accounted into such class of processes because the glucose is produced as intermediate product by starch and then consumed as substrate for biomass growth and ethanol production. The methods mentioned above are based on the so called General Dynamical Model Approach (3, 8-13, 18). Software sensors of intermediate metabolite production and consumption rates are designed [19] and included in the adaptive control law (7, 13).

In this paper, the methods proposed in (7, 13) are adapted and applied for the control of a SSFSE process using *S. cerevisiae* YPB – G recombinant strain. The procedure starts with the specification of the process reaction scheme and the derivation of the model for control. Estimators of glucose production and consumption rates are synthesized and applied for maintaining the glucose concentration in an equilibrium state. Applicability of the proposed adaptive control is investigated by simulations of the control scheme where an unstructured model proposed in (20) is used as the object for control.

Materials and Methods

Model for control

According to the General Dynamical Model Approach (3), the model for control is derived on the basis of a process reaction scheme. The mechanism of bio-ethanol production by *Saccharomyces cerevisiae* from starch is presented as follows:

$$S \xrightarrow{j_1} G$$

$$G \xrightarrow{j_2} X + Enz \qquad (1)$$

$$G \xrightarrow{j_3} E$$

where φ_1 represents the rate of enzymatic hydrolysis, that is, the conversion of starch into glucose. The glucose is consumed in the second reaction at a rate φ_2 for biomass growth and enzyme secretion and in the third reaction for ethanol production at a rate φ_3, as is presented in the unstructured model (16), (20).

The model for control for the considered fed-batch process is presented as follows:

$$\frac{dS}{dt} = -\varphi_1 - \frac{F}{V}S + \frac{F}{V}S_{in} \qquad (2a)$$

$$\frac{dG}{dt} = k_1\varphi_1 - k_2\varphi_2 - k_3\varphi_3 - \frac{F}{V}G \qquad (2b)$$

$$\frac{dX}{dt} = \varphi_2 - \frac{F}{V}X \qquad (2c)$$

$$\frac{dE}{dt} = \varphi_3 - \frac{F}{V}E \qquad (2d)$$

$$\frac{dEnz}{dt} = k_4\varphi_2 - \frac{F}{V}Enz \qquad (2e)$$

$$\frac{dV}{dt} = F \qquad (2f)$$

where F is starch feed rate; V is reactor volume; S_{in} is starch concentration in the feed;

$$\varphi_1 = R_{Sus}S_{Sus} + R_{res}S_{res} \qquad (2g)$$

$$\varphi_2 = \mu X \qquad (2h)$$

$$\varphi_3 = \nu X \qquad (2i)$$

k_1- k_4 are yield coefficients., R_{Sus}, R_{res} - susceptible and resistant starch utilization rate respectively, μ and ν - specific growth and ethanol production rates respectively.

Since the model for control has described the dynamics of the main variables as well as the unstructured one, an identification of the parameters for model (2) is done using the batch phase of the process, applying an optimization procedure proposed in [9, 11, 12, 13]. The optimization criterion is the minimization of the mean square error between the state variables of unstructured model and model (2). The obtained optimal values of the parameters are: k_1=1.086, k_2=1.1151, k_3=2.0226, k_4=28.1748.

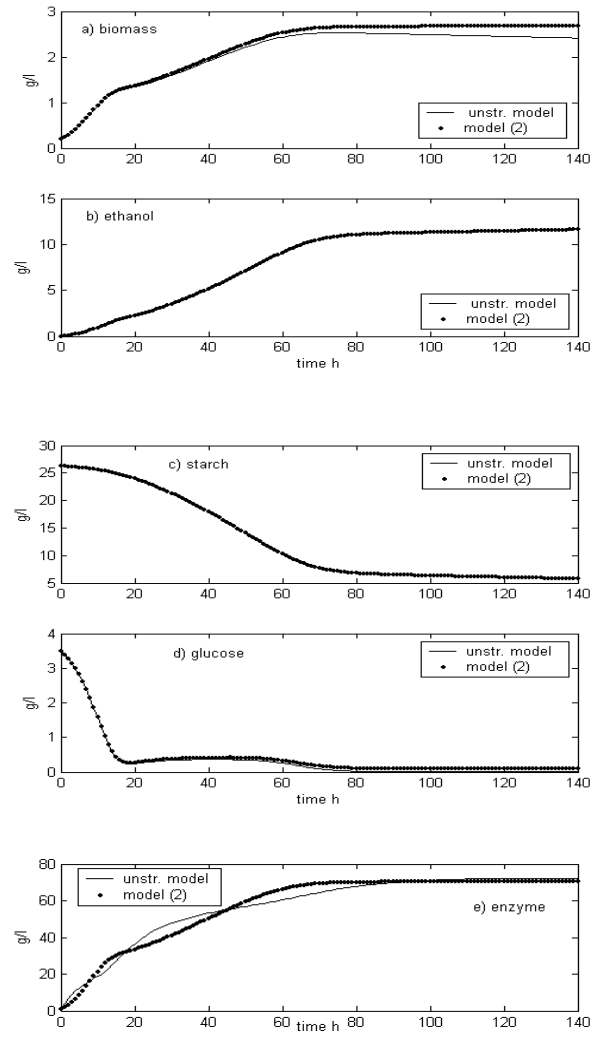

Fig.1. Unstructured Model vs. Model for Control.

In **Fig. 1**, simulations of the model for control (2) are cross-validated with unstructured model data for the batch condition. As can be seen in the figures, the model (2) (points) describes the dynamics of the main process variables as well as the unstructured model (lines). However, some differences can be noticed in **Fig. 1a** due to the effect of the cell death constant, included in the unstructured model. It is important to remark that for the batch conditions at around 20-60 hours the process reaches an equilibrium state for the glucose concentration (**Fig. 1d**), which is characterized by a constant biomass growth rate (**Fig. 1a**), a constant ethanol production rate (**Fig. 1b**) and constant starch degradation rate (**Fig. 1c**). However, after 60 hours, this equilibrium state can not be maintained because of the low level of starch concentration in the reactor. Therefore, in order to keep the equilibrium condition and obtain high ethanol production rates for longer times, it is necessary to feed additional starch into the reactor, which means to operate

under fed batch conditions. For maintaining the process at that equilibrium state for glucose concentration, under fed batch conditions, it is necessary to estimate first the glucose production and consumption rates, which is done in the next section through the use of software sensors.

Software sensors of glucose production and consumption reaction rates

Software sensors design is done applying the method proposed in (13). It is assumed that starch and glucose concentrations are measured on-line by industrially available hardware sensors (5, 24, 25). The first step is on-line estimation of starch consumption rate φ_1 using on-line measurement of starch concentration. The software sensor of φ_1 is an observer-based estimator with structure:

$$\frac{d\hat{S}}{dt} = -\hat{\varphi}_1 - \frac{F}{V}S_m + \frac{F}{V}S_{in} + C_{1s}(S_m - \hat{S}) \tag{3a}$$

$$\frac{d\hat{\varphi}_1}{dt} = C_{2s}(S_m - \hat{S}) \tag{3b}$$

where C_{1s} and C_{2s} are estimator parameters, S_m=S+ε, ε is measurement noise.

The design parameters of estimator (3) are derived using an optimal tuning procedure, proposed in [10]. For the considered case, the following expressions are obtained:

$$C_{1sopt} = 2\xi\sqrt{\frac{m_{11s}}{2m_{21s}}} \quad C_{2sopt} = \frac{(C_{1sopt})^2}{4\xi^2} \tag{4}$$

where: m_{11s} is the upper bound of $d\varphi/dt$; m_{21s} is the upper bound of additive noise of starch; ξ= damping coefficient, a usual value is 0.99 (3).

Glucose production rate is estimated using the first term of the right hand side of equation (2b), where φ_1 is substituted by its estimates from (3b):

$$\hat{\Phi}_1 = k_1\hat{\varphi}_1 \tag{5}$$

The next step is to design of the glucose consumption rate estimator. The second and third terms of right hand side of the eq. (2b) are presented as an unknown time-varying parameter:

$$\Phi_2 = k_2\varphi_2 + k_3\varphi_3 \tag{6}$$

An estimator of Φ_2 can be derived as follows:

$$\frac{d\hat{G}}{dt} = \hat{\Phi}_1 - \hat{\Phi}_2 - \frac{F}{V}G_m + C_{1g}(G_m - \hat{G}) \tag{7a}$$

$$\frac{d\hat{\Phi}_2}{dt} = C_{2g}(G_m - \hat{G}) \tag{7b}$$

The design parameters of estimator (7) are derived using the tuning procedure proposed in [10]. For the considered case, the following expressions are obtained:

$$C_{1gopt} = 2\xi\sqrt{\frac{m_{11g}}{2m_{21g}}} \quad C_{gsopt} = \frac{(C_{1gopt})^2}{4\xi^2} \tag{8}$$

where: m_{11g} the upper bound of $d\Phi_2/dt$; m_{21g} is the upper bound of noise ε.

Simulations are carried out using the values of the design parameters C_{1s}, C_{2s}, C_{1g}, and C_{2g} calculated by eqs. (4) and (8) for estimators (3) and (7), respectively, where m_{11s}=0.35, and m_{21s}=1.3, m_{11g}=0.45, and m_{21g}=0.1. The white noise signals, ε, simulate measurement noises at standard deviation 5% of the mean S and G concentrations. Therefore, the optimal values of the design parameters are: $C_{1s\,opt}$ = 1.23, C_{2opt} = 0.386, $C_{1g\,opt}$ = 4.427, C_{2g} =5.

In **Fig. 2a** and **Fig. 2b**, a simulation verification of estimators (3) and (7) is shown respectively. In **Fig. 2**, a good tracking of $\hat{\Phi}_1$ and $\hat{\Phi}_1$ can be observed, following the trends of the "true" values obtained from unstructured model.

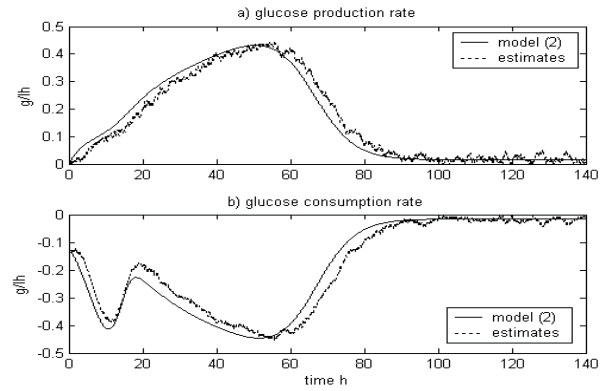

Fig. 2. Simulation verifications of glucose production and consumptions rates

Adaptive control design

The adaptive control scheme proposed here for the SSFSE process is shown in **Fig. 3**, where the manipulated variable is starch feed rate (F) and the controlled variable is glucose concentration (G). It is important to remark that although glucose is the "explicit" controlled variable, the real purpose of the adaptive control scheme proposed in **Fig. 3**, is to obtain a high ethanol concentration (and at the same time a high productivity value), by maintaining a proper value for the glucose concentration.

For increasing the productivity of the process operating under fed batch conditions, the main purpose of the control strategy proposed in this work, is to stabilize the glucose concentration in the equilibrium state observed during batch conditions as long as possible. In this way, the process control comes down to stabilize the glucose concentration using the starch feeding as manipulated variable. Software sensors of glucose production and consumption rates are used for recognition of this equilibrium state. The difference between software sensor's measurements is defined as a marker Δ for recognizing the equilibrium state:

$$\Delta = \hat{\Phi}_1 - \hat{\Phi}_2 \tag{9}$$

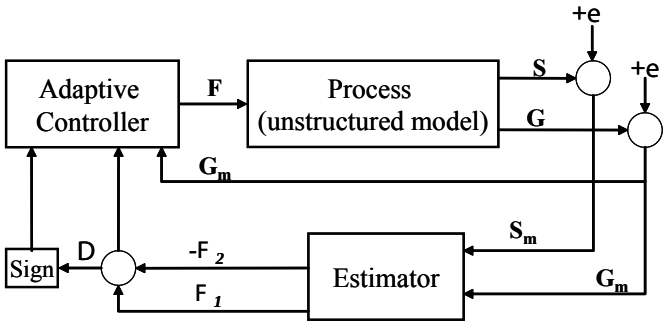

Fig. 3. Adaptive Control Scheme.

When the sign of the marker Δ is positive, the glucose production is higher than the glucose consumption. The negative sign shows the opposite situation. The main purpose is to observe the sign of the marker and to stimulate the glucose production by starch feeding when the consumption is higher. Therefore, the starch has to be added when the marker is negative only. The amplitude of the starch feed impulses could be calculated by the dynamical equation of glucose concentration eq. (7a) (without the last term), assuming zero dynamics of the glucose concentration:

$$F = (\hat{\Phi}_1 - \hat{\Phi}_2)V / G_m \tag{10}$$

Investigations of the control scheme (**Fig. 3**) are realized by simulations. The unstructured model proposed in [16, 20] is used as the object for control. Simulations of starch and glucose concentrations are corrupted by additive noise ε. These white noise signals, ε, simulate measurement noises at standard deviation 5% of the mean *S* and *G* concentrations. The 'estimator' block develops two tasks: **i**) it calculates the $\hat{\Phi}_1$, $\hat{\Phi}_1$ values and *ii*), it estimates the sign of the marker Δ, which is used for calculation of the law (10).

Results and Discussion

The simulation results are shown in **Fig. 4** and **Fig. 5**. In **Fig. 4a** and **Fig. 4b**, the control outputs are presented, in **Fig. 4c** and **Fig. 4d** – the control marker Δ and the input – starch feed rate, are shown respectively. The process starts in batch phase and without using the marker (and therefore without control input calculation) until glucose reaches an apparent equilibrium state (around 20 hours). After that, the calculation of the marker starts, but the control is switched on only when the glucose production rate starts to decrease, which occurs around 50 h of fermentation as can be seen in **Fig. 2a**. As it is shown in **Fig. 4d**, the real starch feeding impulses appear with delay because of the estimator error shown in **Fig. 2a** and **Fig. 2b**. The control input shown in **Fig. 4d** keeps the glucose concentration close to the equilibrium state for more than 100 h. After that, glucose concentration increases as can be seen in **Fig. 4b**. In **Fig. 5a**, a decrease of biomass concentration is observed because of the dilution effect due to the fed-batch mode of cultivation. At the same time, the concentration of ethanol, shown in **Fig. 5b**, increases reaching the highest and constant value around 250 h of fermentation, which coincides with the time at which maximum productivity is reached (**Fig. 5c**). Until this time, the reactor volume is still in an acceptable range (**Fig. 5d**).

Conclusions

In this paper, an adaptive control strategy for the fed-batch SSFSE process is proposed. The process is monitored by means of new software sensors for glucose consumption and production rates. The difference between the estimated values for the consumption and production rates is considered as a control marker, which is used i) for switching from batch to fed-batch phase automatically, and ii) for determining the duration and amplitude of the impulses on the starch feed rate.

The proposed control adaptive algorithm maintains the glucose concentration at an equilibrium state during almost 100 h by feeding starch to the process only when the glucose consumption rate is higher than its production rate. This guarantees that the ethanol concentration, and therefore the productivity, increases constantly as long as the equilibrium state for glucose is maintained. The process may be ended when the ethanol productivity reaches a maximum and the working volume is still in an acceptable range.

The control algorithm is derived on the basis of industrial availability of sensors for glucose and starch concentrations. Under this assumption, it can be stated that the adaptive control scheme proposed for the SSFSE process is suitable for industrial applications.

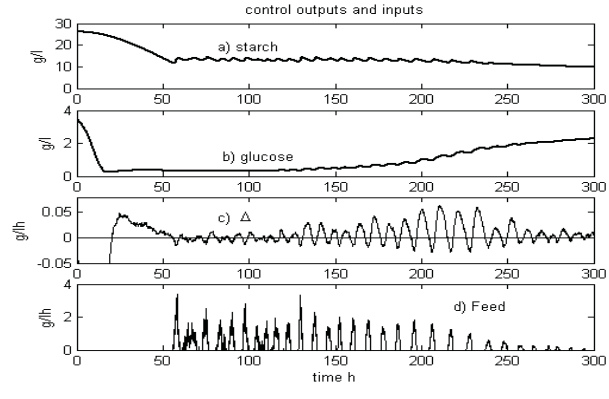

Fig. 4. Adaptive Control Results: Control Outputs and Inputs

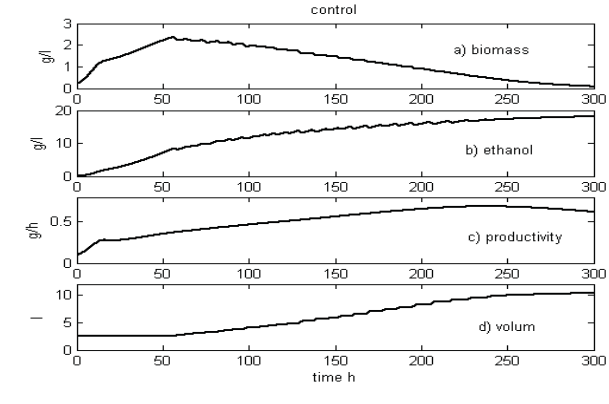

Fig. 5. Adaptive Control Results: Main process variables

Acknowledgments

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