Memorias del 2023 Congreso Nacional de Control Automático
25-27 de Octubre, 2023. Acapulco, Guerrero, México.
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Controlled Semi-Batch Process to Enhance Glucose Concentration in an Enzymatic Hydrolysis Stirred Tank Reactor

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Abstract: In this work, it is explored a controlled semi-batch operation for a stirred tank reactor in which an enzymatic hydrolysis of cellulose is carried out to enhance the concentration of reducing sugar. A high concentration of reducing sugar impacts fermentation processes in a biorefinery. Cellulose and enzyme feedings are driven by controllers to maintain the highest cellulose concentration allowed by a physical constraint in the process; in consequence, more cellulose is fed than in a typical batch operation and a greater glucose concentration is obtained. The construction of controllers is systematic, and performance is illustrated by simulation.

Keywords: semi-batch, adaptive control, PI controller, enzymatic hydrolysis, stirred tank reactor.

1. INTRODUCTION

Enzymatic hydrolysis of agro-industrial residues is a key process in a biorefinery to obtain diverse products, ranging from alcoholic biofuels, as bioethanol and biobutanol, up to high value-added products, such as xylitol and lactic acid. This catalytic process is carried out in a stirred tank reactor, typically in batch operation; say, biomass and enzyme are loaded at an initial event, next the cellulosic material is converted to sugar at warm and little-acid conditions.

A main drawback of this process is that the amount of biomass loaded is low, which in turn makes a low product concentration in any downstream fermentation process. At the end, for example if the case is bioethanol, the bioethanol dehydration cost considerably increases with the decrease of the bioethanol concentration in the fermentation broth (Lara-Montaño, 2018, 2023). The load of biomass must be low because the mixture biomass-water becomes a slurry with the increase of the biomass content, and a slurry does not enable stirring and mixing; in other hand, it has been observed that the conversion rate is favoured with a low biomass concentration (da Silva et al., 2020).

There have been experimental attempts to increase the biomass load (da Silva et al., 2020): (i) enzymatic hydrolysis at high solids, and (ii) enzymatic hydrolysis in a semi-batch like operation. The first attempt means to load the reactor with a high biomass concentration and with the corresponding amount of enzyme; next, it is let the enzyme gradually breaks the biomass. This implies a long-time process with a reduced yield of fermentable sugar with respect to loaded cellulosic mass. In the other hand, the semi-batch like operation has been performed with the addition of little-amounts of biomass into the reactor at certain instants along the process. The process is initiated with a full reactor with a biomass load that enables stirring and mixing; the biomass little-amounts are determined empirically, as well as the addition instants. Many times, the addition is driven by the enablement of stirring and mixing. While the semi-batch process is longer than a typical batch one, the yield is not reduced significantly as with a high solids process; even the process time is shorter than the high solids batch process (Hernández-Beltrán and Hernández-Escoto, 2018).

Although semi-batch operation is promising to enhance fermentable sugar concentration, its design (i.e., which is the trajectory of biomass addition along the process) has been scarcely addressed. In a model-based framework, for an enzymatic hydrolysis of corn stover, Hodge et al. (2009) designed the trajectory of cellulose addition through an optimization problem aimed to track a desired cellulose concentration trajectory. The outcomes show how high concentration of glucose is obtained while a low cellulose concentration is maintained. Cavalcanti-Montaño et al. (2013) defined operational policies for the enzymatic hydrolysis of sugarcane bagasse through a maximization problem, in which the objective function was formed by the final concentration of fermentable sugar plus cellulose conversion. Resulting feeding trajectories of cellulose and enzyme resulted in broths with high concentration of fermentable sugar. Both works followed the Pontryagin minimum principle to solve the optimization problem, and the operational policies resulted in a cellulose feeding in such a way cellulose concentration was maintained as low as mixing is assumed as realizable; even in Hodge et al. (2009), the desired cellulose concentration trajectory was proposed as constant along the process. The operational policies were applied in experimental tests, in which the outcomes show the enhancement of glucose concentration in comparison with batch processes, but there is a considerable offset between expected glucose trajectories and current ones. It can be said that process control was missing to follow defined glucose trajectories, so adjustment of operational policies was not available.

Two issues are pulled up from works mentioned above: (1) maintaining cellulose concentration as constant enhances final glucose concentration, (2) an operational policy resulting from an optimization approach will be still adjusted at its implementation in the real system. On these issues, this work addresses the hypothesis that by regulating cellulose concentration through a controller, enhancement of glucose concentration will be achieved, avoiding the need to address an optimal control problem.

In the next section, a representative system of enzymatic hydrolysis carried out in a stirred tank reactor is described; next, in Section 3, the process design problem is discussed and defined as a control problem. In Section 4, a model-based controller is constructed and implemented for the process, and PI controllers are also proposed to perform in an equivalent form to model-based controllers. Simulation outcomes are discussed in Section 5.

2. THE ENZYMATIC HYDROLYSIS OF CELLULOSE IN A SEMI-BATCH STIRRED TANK REACTOR

It is considered a stirred tank reactor in which glucose is obtained from cellulose through enzymatic hydrolysis (Figure 1). It is assumed that the reactor is equipped with a thermal system that maintains temperature at a required value (i.e., it is considered an isothermal process), and the stirrer drives a homogeneous mixture. A reactor top inlet port enables the initial load of a cellulose-enzyme-water mixture and the subsequent dosing of cellulose-water solution-like and enzyme. This equipment-process configuration is the simplest one in the framework of reducing sugar production from lignocellulosic mass by enzymatic hydrolysis, so it is considered a first methodological step to explore the semi-batch operation.

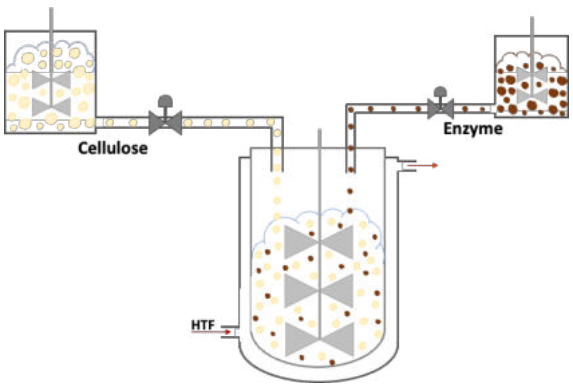

Fig. 1. Semi-batch stirred tank bioreactor for enzymatic hydrolysis of cellulosic mass

From a mass balance, the process is described by the following mathematical model:

$$\dot{C} = r_C(C,B,G,E) + \frac{F}{V}(C_I - C), \quad C(0) = C_0 \tag{1a}$$

$$\dot{B} = r_B(C,B,G,E) - \frac{F}{V}B, \quad B(0) = 0 \tag{1b}$$

$$\dot{G} = r_G(C,B,G,E) - \frac{F}{V}G, \quad G(0) = 0 \tag{1c}$$

$$\dot{E} = r_E(C,B,G,E) - \frac{F}{V}E + \frac{Q_E}{V}, \quad E(0) = E_0 \tag{1d}$$

$$\dot{V} = F, \quad V(0) = V_0 \tag{1e}$$

where C, B, G and E are positive and represent the concentration of cellulose, an intermediate substance called cellobiose, glucose and enzyme, respectively; V is the reactor content volume. F is the volumetric flow of the input stream of cellulose-water solution-like, in which C_I is the cellulose concentration in the influx; Q_E is the mass flow of enzyme dosing, neglecting its effect on volume since it is so much smaller than $F(t)$ and $V(t)$. r_m is the global reaction rate of the substance m ($m = C, B, G$ or E) (given below in Eqns. (2) with their foundation). So, in Eqns. (1a)-(1d), it can be observed that the first term corresponds to the concentration change rate of the substance driven by transformation process, and the second one, by the dilution caused by raw material addition.

For the global reaction rates, it is considered that the simplest kinetics mechanism for the enzymatic hydrolysis of cellulose is given by,

$$C + E \xrightarrow{v_{CB}} B + E \xrightarrow{v_{BG}} G + E, \qquad C + E \xrightarrow{v_{CG}} G + E$$

with a mass stoichiometric coefficient of one.

So, the global reaction rates of substances are given by:

$$r_C = -v_{CB} - v_{CG} \tag{2a}$$

$$r_B = +v_{CB} - v_{BG} \tag{2b}$$

$$r_G = +v_{BG} + v_{CG} \tag{2c}$$

$$r_E = 0 \tag{2d}$$

As it is noticeable in Eqn. (2d), it is assumed that the enzyme incorporates into the broth once it breaks cellulose, and that it does not suffer any spoilage effect (e.g., denaturalization), so its reaction rate is assumed as quasi-stationary.

The reaction rates v_{CB}, v_{BG} and v_{CG} of each mechanism step are described by a Michaelis-Menten type kinetic form,

$$v_{CB} = \frac{V_{CB}(E)C}{K_{CB}\left(1+\frac{B}{K_{ICB}}\right)+C} \tag{3a}$$

$$v_{BG} = \frac{V_{BG}(E)B}{K_{BG}\left(1+\frac{G}{K_{IBG}}\right)+B} \tag{3b}$$

$$v_{CG} = \frac{V_{CG}(E)C}{K_{CG}\left(1+\frac{G}{K_{ICG}}\right)+C} \tag{3c}$$

Corresponding to each mechanism step, V_{CB}, V_{BG} and V_{CG} are the maximum reaction rates depending on E; K_{CB}, K_{BG} and K_{CG} are Michaelis-Menten constants, and K_{ICB}, K_{IBG} and K_{ICG} are inhibition constants. The kinetics structures (3) are recalled from Gusakov and Sinitzyn (1985) with a simplification for this first study: only amorphous cellulose is considered,

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considering that cellulose in pretreated lignocellulosic material has changed its cristallynity degree with respect to raw lignocellulosic material. Mathematical modelling study in Gusakov and Sinitzyn (1985) is recalled because its comprehensive phenomenon addressing, which is encountered in recent works of modelling enzymatic hydrolysis of lignocellulosic biomass.

Finally, maximum reaction rates are given by the following polynomial relationships:

$$V_{CB} = a_{CB}E + b_{CB}E^2 + c_{CB}E^3 \quad (4a)$$

$$V_{BG} = a_{BG}E + b_{BG}E^2 + c_{BG}E^3 \quad (4b)$$

$$V_{CG} = a_{CG}E + b_{CG}E^2 + c_{CG}E^3 \quad (4c)$$

Along the semi-batch operation, there can be distinguished the following instants and periods:

(1) Initial time (t_0) in which raw material is loaded resulting in initial concentrations of cellulose (C_0) and enzyme (E_0), and an initial volume (V_0).

(2) Dosing period ($P_D = \{t \mid t \in [t_0,\ t_L)\}$), along which

$F(t) \geq 0$ as long as $V(t) \leq V^R$,

where V^R is the full reactor volume.

(3) Dosing stop (t_L; $t_L \geq t_0$), in which $F(t_L)$ is set to 0 steadily, typically because $V(t_L)$ has reached V^R.

(4) Cellulose run out period ($P_B = \{t \mid t \in [t_L,\ t_B)\}$), along which $F(t) = 0$.

(5) Process stop instant (t_B; $t_B \geq t_L$).

Typically, process performance is determined by the conversion of cellulose into glucose (X) at the process end. To calculate it, firstly it is necessary to consider the total mass of cellulose loaded (M_L),

$$M_L = M(t_L) = V_0C_0 + (V_L - V_0)C_I, \quad \text{where } V_L = V(t_L) \quad (5)$$

and the remaining cellulose when the process ends,

$$M_B = V_LC_B, \quad \text{where } C_B = C(t_B). \quad (6)$$

In addition, it can be thought on an initial cellulose concentration equivalent to a "high solids" batch process (C_0^B),

$$C_0^B = \frac{M_L}{V_L} \quad (7)$$

Then, conversion X is defined as:

$$X = \frac{M_L - M_B}{M_L} = \frac{G_B}{C_0^B}, \quad \text{where } G_B = G(t_B). \quad (8)$$

G_B is the glucose concentration at the process end (t_B).

To simulate the process, Table 1 shows the parameter values.

Table 1. Value of model parameters for enzymatic hydrolysis below 100 g/L of cellulose and between 5 and 60 g/L of enzyme (Gusakov and Sinitzyn, 1985)

Parameter	Value	Parameter	Value	Parameter	Value
K_{CB}	13	K_{BG}	0.6	K_{CG}	15
K_{ICB}	0.8	K_{IBG}	0.32	K_{ICG}	11
a_{CB}	0.294	a_{BG}	0.0054	a_{CG}	0.144
b_{CB}	-0.003	b_{BG}	-5.4×10^{-6}	b_{CG}	-4.8×10^{-5}
c_{CB}	1.8×10^{-5}	c_{BG}	6.0×10^{-7}	c_{CG}	-4.2×10^{-5}

3. THE DESIGN PROBLEM OF THE SEMI-BATCH PROCESS

Typically, the enzymatic hydrolysis is carried out in batch operation (i.e., $F(t) = 0\ \forall\ t \in [t_0, t_B]$), and the process design challenge means setting up C_0 and E_0 in such a way that X be as high as possible, but C_0 must not surpass the critical value in which the reactor mixture becomes a slurry (C^*). So, the following optimization problem is typically addressed:

$$\max_{(C_0,E_0)} J = X \quad (9a)$$

s.t. Mathematical Model (1)-(4), $F(t) = 0$, $C_0 \leq C^*$. (9b)

The disadvantage of batch operation lies on the low final glucose concentration ($G_B < C^*$), which in turn produces broth with low ethanol concentration in a downstream fermentation, which interferes the economic feasibility of bioethanol production.

So, besides a high conversion, this work is aimed at a high glucose concentration. The hypothesis is that it can be achieved if $C(t)$ is maintained at an effective concentration ($\bar{C}$) below C^*, while cellulose is converted into glucose and cellulose is added ($F(t) \geq 0$). In an optimization framework, the problem becomes:

$$\max_{(F(t),Q_{E(t)})} J = G_B \quad (10a)$$

s.t. Mathematical Model (1)-(4), (10b)

$$F(t) \geq 0,\ 0 < C(t) \leq C^*. \quad (10c)$$

It is expected that G_B reaches a concentration close to C_0^B,

$C_0 \leq G_B \leq C_0^B < C_I$.

3.1 Control system for designing the semi-batch process

Let us consider a nominal cellulose concentration ($\bar{C}$) enabling homogeneous mixing ($\bar{C} \leq C^*$) and, in other hand, a nominal enzyme concentration ($\bar{E}$) that drives a high conversion of $\bar{C}$. The optimization problem (10) is substituted by to design a control system that, along the dosing period (P_D) of the semi-

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batch operation, maintains the cellulose concentration at $\bar{C}$, and the enzyme concentration at $\bar{E}$; i.e.,

$$F(t) = \sigma_C(\bar{C}, C(t), \dots), \quad Q_E(t) = \sigma_E(\bar{E}, E(t) \dots), \tag{11}$$

in such a way $C(t) \to \bar{C}$ and $E(t) \to \bar{E} \ \ \forall\, t \in P_D$.

4. CONTROL SYSTEM FOR DESIGN AND CONTROL OF THE SEMI-BATCH PROCESS

4.1 Controllers to define cellulose and enzyme addition trajectories.

Since $C(t)$ is required as constant at $\bar{C}$, and $E(t)$ as well, at $\bar{E}$, along the dosing period P_D; i.e., C and E are required to be in a steady-like state along P_D, then Eqns. (1a) and (1d) become as:

$$r_C(\bar{C}, B(t), G(t), \bar{E}) + \frac{F(t)}{V(t)}(C_I - \bar{C}) = 0, \tag{12a}$$

$$r_E(\bar{C}, B(t), G(t), \bar{E}) - \frac{F(t)}{V(t)}\bar{E} + \frac{Q_E(t)}{V(t)} = 0, \quad t \in P_D \tag{12b}$$

As B and G are time varying, $F(t)$ and $Q_E(t)$ must vary accordingly to accomplish (12):

$$F(t) = \frac{V(t)}{\bar{C} - C_I}\, r_C(\bar{C}, B, G, \bar{E}), \tag{13a}$$

$$Q_E(t) = F(t)\bar{E} - V(t) r_E(\bar{C}, B, G, \bar{E}), \quad t \in P_D \tag{13b}$$

Then, the system formed by Eqns. (1b, c, e) and (13) is a control system that determine the trajectory of $F(t)$ and $Q_E(t)$ in such a way $C(t) = \bar{C}$ and $E(t) = \bar{E}$ along P_D.

The resulting trajectories $F(t)$ and $Q_E(t)$ can be implemented in a real system, expecting trajectory $G(t)$ follows the resulting one from Eqns. (1) with these feeding trajectories.

4.2 Linear PI controllers to preliminary on-line glucose concentration enhancement

Implementation of trajectories in a closed-loop framework requires on-line measurement of all state variables and overall depends on the accuracy of the process model. Although this drawback is alleviated with the addition of PI-type actions, resulting in a geometric controller (e.g., Alvarez, 1996).

Towards a feedback framework, in this work, also the feasibility of conventional PI linear controllers is explored:

$$F(t) = K_P^C(\bar{C} - C(t)) + \int_{t_0}^{t_L} K_I^C(\bar{C} - C(\tau))d\tau \tag{14a}$$

$$Q_E(t) = K_P^E(\bar{E} - E(t)) + \int_{t_0}^{t_L} K_I^E(\bar{E} - E(\tau))d\tau \tag{14b}$$

where K_P^C and K_P^E are proportional gains, and K_I^C and K_I^E are integral gains.

In this way, while the controllers (13) will provide a nominal process trajectory, the controllers (14) are expected to track that nominal trajectory. It is worthy to highlight that the controllers (14) do not depend on process model accuracy, but they need on-line measurement of C and E. This is a preliminary work, so real system implications will be addressed in future works.

5. RESULTS

5.1 The batch process as a starting point and reference

To set a starting point and a reference, it is determined the best scenario to carry out an enzymatic hydrolysis in batch operation, i.e., it is solved the optimization problem given by Eq. (9).

This followed the construction of a response surface of glucose concentration and glucose yield for a final process time of 72 h, which is a time typically experimented with. The ranges of initial concentration for cellulose and enzyme are according to the validity of process model parameters (Table 1); in all of the runs, initial conditions for B and G were set equal to zero, and $V = 1\ L$. Figure 3 depicts the outcomes.

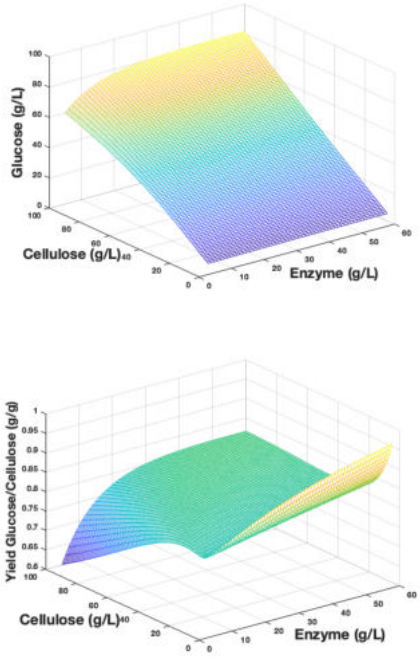

Figure 2. Response surfaces of glucose concentration and yield with respect to initial conditions, for an enzymatic hydrolysis carried out in a 72-h batch process.

It can be observed that as low the initial cellulose concentration as greater the glucose yield, but glucose concentration is low. It seems that there is an almost flat region in yield at medium cellulose concentration (e.g., 50 g/L), and enzyme concentration greater than 20 g/L. In other hand, in response surface of glucose, it can be observed that the glucose with respect to enzyme increase is almost flat after an enzyme concentration of 20 g/L.

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Then, the following conditions are set as an effective batch process:

$C_0^B = 50\frac{g}{L}, E_0^B = 23\frac{g}{L}$, resulting in $G^B(t_B = 72\ h) = 40\ g/L$.

5.2 The semi-batch process designed by model-based controller

In order to test the model-based controllers (13), and so to determine feeding trajectories, the following initial conditions were considered:

$C_0 = C_0^B,\ \ B_0 = 0,\ \ G_0 = 0,\ \ E_0 = C_0^B,\ \ V_0 = 0.1\ L$

with a maximum reactor volume $V^R = 1\ L$, and $t_B = 240\ h$.

As cellulose concentration in the dosing stream, according to the cellulose-water solution-like that can be prepared in practice, the following different values were considered:

$C_I = \{100, 150, 200\}\ g/L$.

Fig. 3 illustrates the performance of the model-based controller (13) for the different cellulose concentrations in the dosing stream, and the comparison with respect to the batch process (in which $V_0 = V^R$). In the box Glucose, it can be observed that $G(t_B)$ for any semi-batch case is considerably greater than the one of batch process; indeed, the greater C_I, the greater $G(t_B)$. However, the greater C_I, the greater the time in which V reaches V^R (box Volume); even with $C_I = 200\ g/L$, the reactor is not filled up and the loaded cellulose is not run out (box Cellulose). In the box Cellulose Feedflow, the cellulose dosing trajectories are depicted, which in a certain way, shows the velocity in which cellulose is being converted to cellulose. In the box Enzyme Feedflow, enzyme dosing trajectories are depicted; as expected, their forms are similar to those of cellulose dosing because the enzyme is being proportionally dosed with cellulose dosing.

The disadvantage of the semi-batch process lies in the longer time that is required in comparison with the one of a batch process; however, the amount of cellulose processed is still greater (box Cellulose Load).

5.3 The semi-batch process through PI controllers

In order to test the PI controllers (14), the same conditions as in Section 5.2 were considered. In this first work, the PI controllers were tested with high gains.

Fig. 4 depicts the performance of the PI controllers (14) for the case of $C_I = 150\ g/L$, and it is compared with the one of the model-based controllers (13). It can be observed that there is no mismatch between both controllers' performance.

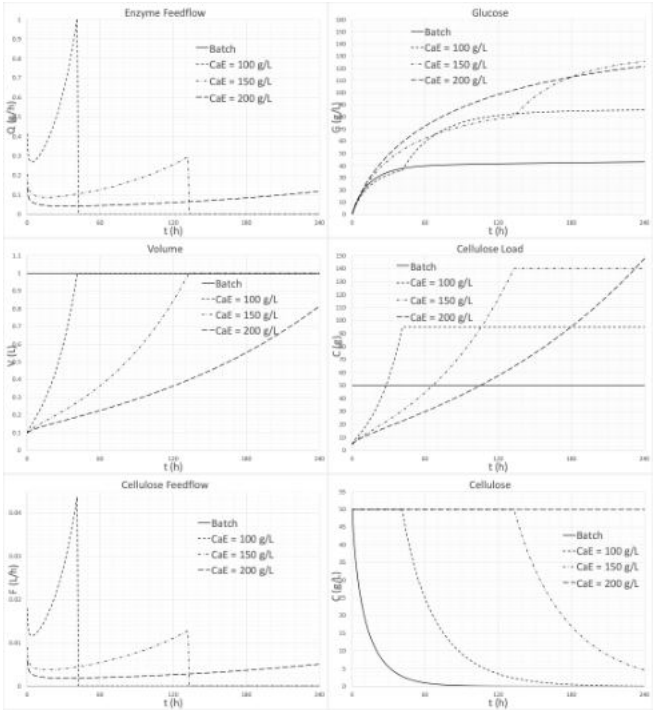

Figure 3. Performance of model-based controllers (13) to carry out semi-batch processes with different cellulose concentration of the feeding stream.

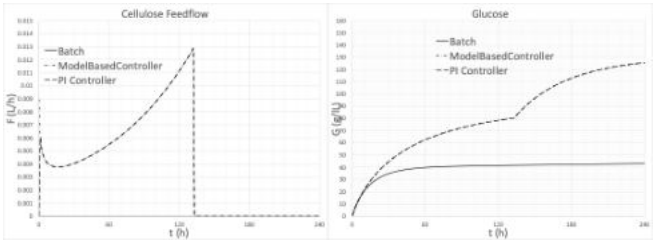

Figure 4. Linear PI controller performance in comparison with model-based controller for an enzymatic hydrolysis with $C_I = 150\ g/L$.

6. CONCLUSIONS

It is shown that a semi-batch operation for the enzymatic hydrolysis of cellulose in a stirred tank reactor enhances the final glucose concentration since the total amount load of cellulose is considerably greater than in a batch operation. The dosing policies of cellulose and enzyme can be determined through model-based controllers and even with linear PI controllers. This work is a first one towards the case of lignocellulosic mass where inhibitory aspects are present.

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