

## Optimizing the ethanol production from lignocellulosic-materials of sorghum by a sequential hydrolysis and fermentation methodology.

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**Abstract.** The production of ethanol from sorghum lignocellulosic-materials by a sequential methodology was investigated in the present work. The methodology involves first the enzymatic hydrolysis of the lignocellulosic materials and later the alcoholic fermentation of the hydrolysate with *Saccharomyces cerevisiae*. The kinetics of both stages are characterized in order to present a model for the sequential process. Later the kinetic equations were embedded on a mathematical model used to solve an optimal control problem aimed at maximizing the productivity of the reactors train by managing the flow of hydrolysate feeding the fermentor.

**Keywords:** Sorghum; Enzymatic hydrolysis; Fermentation; Cellulosic ethanol. Optimal control.

### 1 Introduction

The world economy dependency on oil, the oil price volatility, and a long-term forecasted decline in worldwide petroleum reserves motivates the research on alternative energy sources. Bioethanol is regarded as a creditable replacement fuel because fulfils most criteria for an inexhaustible and renewable fuel. Ethanol production from grains has been a very active research-field but the production of ethanol based on crop starches competes with food and fiber production and do not significantly diminish greenhouse gas emissions [1]. Therefore, the research focus has shifted towards producing ethanol from lignocellulosic materials such as agricultural/forestry residues and from dedicated energy crops. A huge amount of lignocellulosic residue is produced as byproduct of sweet sorghum [2, 3] and this lignocellulosic residue, the so-called bagasse, has nonfood applications. Therefore, this residue is a very cheap carbohydrates source. Like other lignocellulosic materials, it contains a considerable amount of carbohydrate polymers (cellulose and

hemicellulose) and lignin. These carbohydrates can be first hydrolyzed to fermentable sugars by alkalis or enzymes and then fermented to ethanol. However, the efficient utilization of lignocellulosic materials requires an initial pretreatment step in order to minimize lignin and hemicellulose barriers so that an effective enzymatic hydrolysis can take place [4]. After a pretreatment stage, the hydrolysis takes place to liberate fermentable sugars to be later utilized as the main carbon-source on a fermentation step. It is also worth noting that the hydrolysate is not only a sugars source; it is also rich in micronutrients [5] that allow fermenting the hydrolysate without the need of adding further nutrients.

Since ethanol is practically a raw commodity, production profits are usually very tight and therefore its production must be carried out at maximum efficiency levels. So, the aim of this study is to model a trend of two reactors involving an enzymatic hydrolysis reactor and a fermentative step with *Saccharomyces cerevisiae* that takes the hydrolysate as main carbon, nitrogen and micronutrients source. The kinetics of both stages first characterized are later integrated with macroscopic balances to yield a representation of the reactor train. This model is later used to solve a dynamic optimization problem aimed at computing the optimal flow of hydrolysate from the hydrolysis reactor to the fermentor with the aim of maximizing the train productivity.

## 2 Materials and methods

### 2.1 Raw material

*Sorghum* sp. stems and leaves kindly provided by Ing. Javier Beccaria Ibáñez (Synapsis S.A., San Carlos Centro, Argentina). The material was kept at  $-20^{\circ}\text{C}$  and, after unfreezing, triturated to obtain 0.5 cm particles. Later it was dried at  $50^{\circ}\text{C}$  during 24 h. After that, it was finely grinded by a laboratory mill (Dalvo, Argentina).

### 2.2 Hydrolysis process

The enzymes used in this study was Cellulase of *Myceliophthora thermophila* (FibreZyme G4, Dyadic's, USA). Hydrolysis was carried out at  $50^{\circ}\text{C}$  during 24 h using a rate grinded-sorghum to enzyme-solution of 35 mg/mL. Enzymes were suspended in citrate buffer (50 mM, pH = 5.0). After hydrolysis, the sugar rich supernatant were obtained by centrifugation (5000 x g, 10 min.) and sterilized ( $121^{\circ}\text{C}$ , 15 min.). After that was stored at  $4^{\circ}\text{C}$  until use.

### 2.3 Microorganism and inoculum preparation

An industrial strain of *Saccharomyces cerevisiae* LFF-S04 available on the *Laboratorio de Fermentaciones* of *Facultad de Bioquímica y Ciencias Biológicas* (*Universidad Nacional del Litoral*) was used. The strain was maintained at  $4^{\circ}\text{C}$  on MEA medium: 2% malt extract (Britania, Argentina), 2% glucose (Cicarelli, Argentina), 0.1% tryptone (Britania, Argentina) and 1.5% agar-agar (Britania, Argentina); at pH = 6.0. In order to avoid a phase lag, the inocula were also developed in a hydrolyzed sorghum extract broth. These cultures were incubated in an orbital shaker (150 RPM), at  $28^{\circ}\text{C}$  during 24 h.

### 2.4 Fermentation process

The stored hydrolysis supernatants were inoculated with the *Saccharomyces cerevisiae* strain. The cultures (150 mL in 250 mL conic flasks) were incubated statically and in anaerobic conditions. Several samples were withdrawn from each culture to be later analyzed. These samples were stored at  $-20^{\circ}\text{C}$  after removing solid waste.

### 2.5 Analytical methods

Glucose concentration was determined in thawed samples applying an enzymatic colorimetric assay (Glicemia Enzimática, Wiener Lab, Argentina). Yeast cells count was performed with a Neubauer chamber by diluting samples, if necessary. The yeast cells counts (cells/mL) were converted to biomass concentration (g/L) by multiplying the count by  $8.32 \times 10^{-8}$ . Ethanol concentration was determined by micro-diffusion carried out in Conway chambers [6].

### 2.6 Numerical methods

Ordinary differential equations (ODE) and differential-algebraic equations (DAE) were solved by using the MATLAB ODE 45 solver (Mathworks, Natick, MA, USA) and parameters fitting were performed by using the *fminsearch* MATLAB function couples with the *ODE45* routine used to integrate ODE and DAE systems. Optimal control problems were solved by the GPOPS 5.2 toolbox [7] running on MATLAB 9.0.

## 3 Model development

### 3.1 Characterization of the hydrolysis kinetics

A suspension of 0.325 g of dry grinded sorghum and 5 mL of FibreZyme G4 suspension was prepared and incubated at  $50^{\circ}\text{C}$  during 24 h. Several 100  $\mu\text{L}$  samples were taken in order to follow the evolution of glucose concentration. The kinetics of the hydrolysis was fit according to the following ODE system.

$$\frac{dS_h}{dt} = r_{S_h} = -Y_h \mu_h \frac{S_h^2}{k_{S_h} + S_h} \quad (1)$$

$$\frac{dG_h}{dt} = r_{G_h} = \mu_h \frac{S_h^2}{k_{S_h} + S_h} \quad (2)$$

where  $r_{S_h}$  is the rate of consumption of lignocellulosic material,  $r_{S_h}$  is the glucose production rate,  $S_h$  is a variable representing a fictitious concentration of hydrolyzable lignocellulosic material,  $G_h$  is the glucose concentration,  $\mu_h$  the maximum specific hydrolysis rate,  $k_{S_h}$  is the saturation constant and  $Y_h$  is the observable yield of glucose on the lignocellulosic material. A least squares regression was performed by integrating the ODE system defined by Eqs. (1) and (2) and by comparing numerical predictions with experimental data about the concentration of glucose along the 24 h time-span.

The initial variable values were  $S_h(0) = 280.0 \text{ g/L}$  and  $G_h(0) = 0.0 \text{ g/L}$ . The value  $S_h(0)$  was derived from the observed yield  $Y_h$  above reported and from the end-concentration of glucose.

Essentially we minimized the sum of squared error by solving the ODE system on a time-span defined by  $t_0 = 0 \text{ h}$  and  $t_f = 24 \text{ h}$  to get the model predictions and to compute the sum of squared deviations at times the experimental data were available. An educated guess for the model parameters was used to initialize the minimization procedure. The objective function to minimize is:

$$SE = \sqrt{\frac{\sum_{i=1}^S \sum_{k=1}^n (e_{ki}^p - e_{ki}^e)^2}{n}} \quad (3)$$

In Eq. (3),  $i$  is an indicator of the state variables whose predictions are to be compared with experimental values and  $k$  is the indicator of the sample number. In this case we just measured the concentration of the glucose produced during the hydrolysis process, and so  $i = S$ . We used the *fminsearch* MATLAB function to minimize the squared error function. The least squares values for the fitted parameters are summarized in Table 1. Since  $SE = 49.77$ , the average deviation between the predicted and experimental concentrations of glucose is  $1.89 \text{ g/L}$ .

### 3.2 Kinetics of the fermentation

In order to characterize the kinetics of the fermentation with *Saccharomyces cerevisiae*, a batch fermentation was carried out with the purpose of collecting experimental data along the whole fermentation time-span. The experimental data concerning the concentration of biomass, glucose and ethanol were used to fit the kinetic and yield parameters of an ODE-system modeling the fermentation kinetics. After testing several kinetic models, to characterize the biomass growth the Monod equation (with the glucose as the limiting substrate) was chosen because of its simplicity and of the low number of parameters to fit. Since an inhibition phenomenon was detected on late stages of the fermentation, a term considering that high ethanol concentrations inhibit the growth was also included. So, the ODE system given by Eqs. (4), (5) and (6), where the specific growth rate is stated by eq. (7), represents the fermentation.

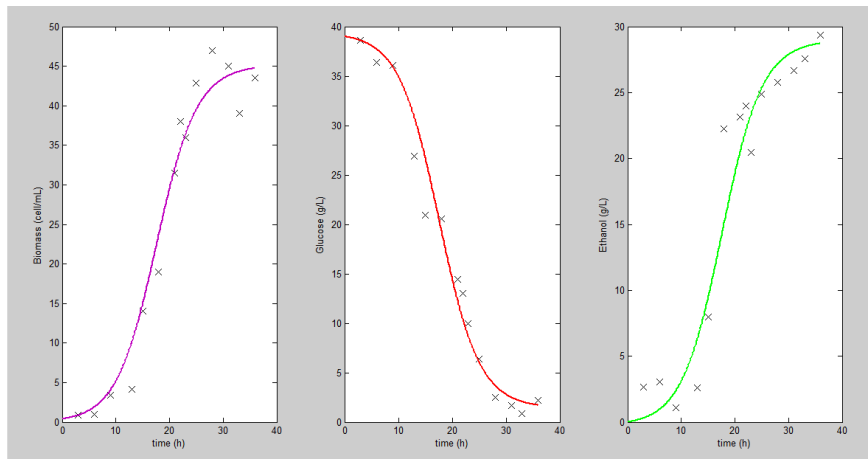
$$\frac{dX}{dt} = r_x = \mu X \quad (4)$$

$$\frac{dP}{dt} = r_p = a\mu X \quad (5)$$

$$\frac{dG_f}{dt} = r_{G_f} = -\mu \frac{X}{Y_f} \quad (6)$$

$$\mu = \mu_{\max} \frac{G_f}{k_G + G_f} \left( 1 - \frac{P}{k_p} \right) \quad (7)$$

Here  $r_X$ ,  $r_P$  and  $r_{Gp}$  are respectively the biomass growth rate, the ethanol production rate and the glucose consumption rate. Since the experiment was carried out on batch vessels, these rates correspond to the evolution of the concentrations of biomass ( $X$ ), ethanol ( $P$ ) and glucose ( $G_f$ ) over the time. Fitted parameters are also summarized on Table 1. Since  $SE = 319.0$ , the average deviation between the predicted and experimental concentrations of variable-states is 2.75 g/L. The comparison between experimental and predicted concentration values is depicted in Figure 1.



**Fig. 1.** Model predictions (line) vs. experimental data (cross symbols) for the batch biomass growth, the glucose consumption and ethanol production

According to the end concentrations seen in Figure 1 a yield higher than the theoretical yield of ethanol from glucose (0.51) is achieved. This phenomena was previously seen in other work [8] and is due to the other fermentable substrates, in addition to glucose, that are present in the hydrolysate.

**Table 1:** Least squares yields and kinetic parameters for the model of the hydrolysis and the fermentation

<b>Hydrolysis parameters</b>		
$\mu_h = 0.0383 \text{ h}^{-1}$	$Y_h = 6.55 \text{ g } S_h / \text{g } G_h$	$k_{Sh} = 0.01 \text{ g } G_h$
<b>Fermentation parameters</b>		
$\mu_{\max} = 0.259 \text{ h}^{-1}$	$Y_f = 1.126 \times 10^6 \text{ cells/g } S$	$k_G = 10.0 \text{ g } G$
$k_p = 51.4 \text{ g } P$	$a = 0.655 \text{ g } P / \text{g } X$	-

### 3.2 Modeling the reactors train

We developed the model of the reactor trend by embedding kinetic rates and the yield coefficients into macroscopic balances. The procedure is quite standard [9] and

basically consists on the introduction of kinetic rates linked by yield parameters on the balances equations corresponding to the reactor type used on each stage. It is assumed that the hydrolysis occur in a perfectly mixed stirred tank of shrinking volume and that the dynamics of the fermentor corresponds to the fed-batch mode. Due to the filtering of waste solids present on the hydrolisate, a filtering factor  $c = 0.95$  was introduced on the flow to the fermentor. So, the dynamics of the of the trend are defined by eqs. (9) to (15) to be next presented.

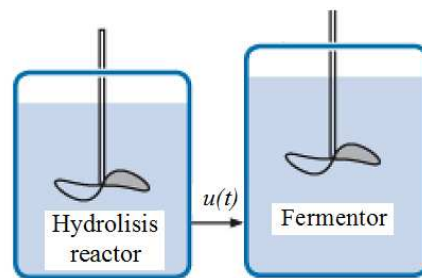


Fig. 2. Representation of the reactor train

#### 4 Optimal control of the hydrolizate flow

Optimal control is a subject where it is desired to determine the inputs to a dynamical system that optimize (i.e., minimize or maximize) a specified performance index while satisfying any constraints on the motion of the system. Because of the complexity of most applications, optimal control problems are most often solved numerically. While users benefit from COTS software, such programs require time to learn and even after overcoming the learning curve associated to using these programs, they often remain as 'black box'. Instead, we used GPOPS [7] software running over Matlab. GPOPS utilizes multilevel structures that enable compact specification of the lower and upper bounds on all variables and constraints as well as separated definitions of the performance index and the DAE system in MATLAB.

In our case the controlled variable is the flow of hydrolizate subject to the loss factor from the hydrolysis reactor to the fermentor. The objective function (8) defines the productivity criterion as the quotient between the quantity of produced ethanol and the time spent to obtain this quantity. This objective aims at avoiding large fermentation times for just obtaining marginal improvements on the quantity of obtained ethanol. So, we are defining a free terminal time optimal control problem. Eqs (9)-(10) represent the dynamics of the stirred-tank hydrolysis batch-reactor. Eqs. (11)-(12) set the volume dynamics for both reactors. Eqs. (13) to (15) are equations for respectively describing the dynamics of the concentration of biomass, ethanol and glucose in the fermentor according the fed-batch mode. Kinetics  $r_{Sh}$ ,  $r_{Gh}$ ,  $r_X$ ,  $r_P$ , and  $r_{Gf}$  are given by eqs. (1)-(2) and (4) to (7). Inequalities (16), (17) and (18) assure the feasibility of the optimal flow policy. In summary, the optimal control problem is defined by the objective function (8), the differential equations (9) to (15), the control constraint (16) and the state constraint (17)-(18) as follows:

Maximize

$$J = \frac{P(t_f)V(t_f)}{t_f} \quad (8)$$

Subject to

1. Dynamics of the train

$$\frac{dS_h}{dt} = r_{Sh} \quad (9)$$

$$\frac{dG_h}{dt} = r_{Gh} \quad (11)$$

$$\frac{dV_h}{dt} = -u \quad (12)$$

$$\frac{dV_f}{dt} = cu \quad (13)$$

$$\frac{dX}{dt} = r_X - u \frac{X}{V_f} \quad (14)$$

$$\frac{dP}{dt} = r_P - u \frac{P}{V_f} \quad (15)$$

$$\frac{\partial G_f}{\partial t} = -\frac{r_X}{Y_f} + u \frac{G_h}{V_f} - u \frac{G_f}{V_f} \quad (16)$$

2. Constraints on the control actions

$$0 \leq u \leq u_{\max} \quad (17)$$

3. Constraints on the states

$$0 \leq V_h \quad (18)$$

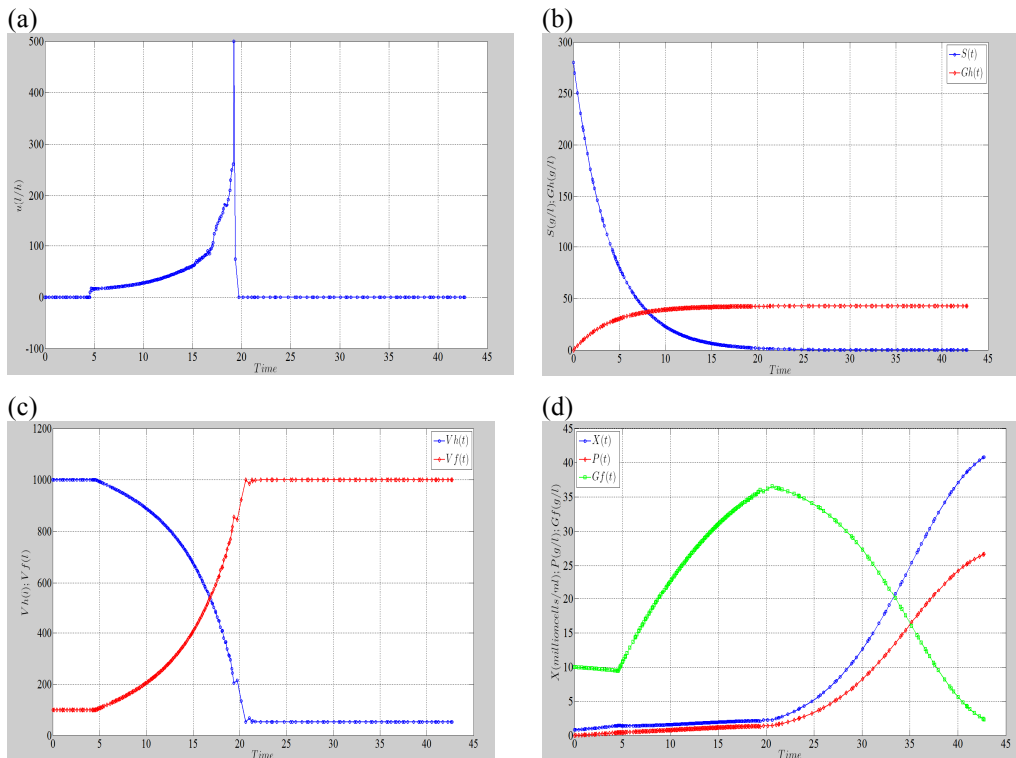
$$V_h \leq V_{f-\max} \quad (19)$$

The optimal control problem was coded on *GPOPS 5.0* [7] and solved in a 2.0 GHz 16 GRAM PC. We solved several instances by varying the initial states and the control and states constraints. Kinetic and yield parameters are those reported on Table 1. In all cases, the optimal flow policy consists of just an “injection” but the injection “shape” and start time is subject to changes depending on these parameters.

In Figure 3 we depicted the optimal flow and the evolution of states for a train with initial states and constraint values presented in Table 2. The objective function value is 624.6 g P/h, and 26683 g P were produced in 42.7 h hours according such policy.

**Table 2:** Initial states and constraints

Initial states	Control and states constraints
$S_h(0) = 280 \text{ g/l}$	$u_{\max} = 500 \text{ l/h}$
$G_h(0) = 0 \text{ g/l}$	$V_{f-\max} = 1000 \text{ l/h}$
$V_h(0) = 1000 \text{ l}$	
$V_f(0) = 100 \text{ l}$	
$X(0) = 0.8 \text{ g/l}$	
$P(0) = 0 \text{ g/l}$	
$G_f(0) = 10 \text{ g/l}$	



**Fig. 3.** An optimal flow profile from the hydrolysis reactor to the fermentor: (a) flow profile; (b) states on the hydrolysis fermentor; (c) evolution of volumes of the hydrolysis reactor and the fermentor; (d) states on the fermentor.

## 5 Conclusions

A model based on experimental data for optimizing a train of a hydrolysis reactor and a fermentor aimed at producing ethanol was presented in this work. The kinetics of the hydrolytic production of glucose from Sorghum bagaze with FibreZyme G4



and the kinetic of ethanol production with *Saccharomyces cerevisiae* from the generated glucose were characterized. The cellular growth is considered to be limited by the availability of the glucose and inhibited by a high ethanol concentration. Kinetic equations were introduced into macroscopic balances equations for modeling a train consisting on a hydrolysis reactor and a fermentor. This allows to define an optimal control problem aimed at the optimization of the flow of hydrolysate to the fermentor. The dynamic model involves a lumped representation of the culture on both reactors. In this way, an example of this optimal control problem has been shown. The problem, that involves seven ODE, a control constraint and a state constraint and was efficiently solved by using GPOPS 5.2. As a consequence, the optimal flow profile was computed. It is worth noting that GPOPS is relatively user-friendly and allows avoiding the development of ad-hoc routines based on calculus of variations. It is, therefore, useful for people without a deep knowledge about optimization of dynamic systems.

## References

1. Anderson, I. Ethanol from sweet sorghum. [www.energy.iastate.edu/renewable/biomass/cs-anaerobic2.html](http://www.energy.iastate.edu/renewable/biomass/cs-anaerobic2.html) (2005).
2. Sipos, B., Reczey, J., Somorai, Z., Kadar, Z., Dienes, D., Reczey, K. Sweet sorghum as feedstock for ethanol production: enzymatic hydrolysis of steam-pretreated bagasse. *Applied Biochemistry and Biotechnology*, 153, 151 – 162 (2009).
3. Wu, X.R., Staggenborg, S., Prophet, J.L., Rooney, W.L., Yu, J.M., Wang, D.H. Features of sweet sorghum juice and their performance in ethanol fermentation. *Industrial Crops and Products*, 31, 164–170 (2010).
4. Sun Y. and Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83, 1 – 11 (2002).
5. Almodares, A. and Hadi, M.R. Production of Bioethanol from sweet sorghum: A review. *African Journal of Agricultural Research*, 4, 772 – 780 (2010).
6. Conway, E. J. and Byrne, A. An absorption apparatus for the micro-determination of certain volatile substances. *Biochemical Journal*, 27, 419 – 429 (1933).
7. Rao, A.V., Benson D., Darby C., Francolin C., Patterson M., Sanders I., and G.T. Huntington. *User's Manual for GPOPS; Version 5.2* (2012).
8. Borzani, W. and Jurkiewicz, C. H. Variation of the ethanol yield during very rapid batch fermentation of sugar-cane blackstrap molasses. *Brazilian Journal of Chemical Engineering*, 15, 225 – 233 (1998).
9. Dondo R. and D. Marqués. Mass and Energy Balances as State-space Models for Aerobic Batch Fermentations. *Latin American Applied Research*, 32, 195-204 (2002).