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Performance Evaluation and Mathematical Modelling of Enzymatic Hydrolysis in 2G Bioethanol Production

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In this work, two mathematical models are proposed for a class of enzymatic hydrolysis of lignocellulosic material; one is for a quick evaluation of performance, and the other one is for behavior description. The parameters of the performance model, which is a first order linear differential equation, are used to determine the yield and the velocity of the process. On the other hand, to describe the behavior of the process, a Michaelis-Menten kinetics is recalled, where it is considered an inhibitory effect that becomes present with the reducing sugar formation. To construct and characterize model features, two different substrate-enzyme systems were performed in an automatized stirred tank reactor; along the experiments, samples were frequently taken out to obtain reducing sugar trajectories. The substrate of one of the systems was wheat straw pretreated with acid, and of the other one was the same wheat straw but pretreated with alkali; a commercial enzyme complex was used in both systems. The parameter identification was performed by minimizing squared errors between experimental trajectories and model predictions: the parameters identified for the performance model allowed a quantitative comparison between the two systems, and the descriptive model made evident the presence of an inhibitory effect along the reducing sugar formation.

1. Introduction

Bioethanol has been regarded as a gradual substitute of gasoline, and producing it from lignocellulosic material has gained interest since the last decade, because this kind of material is abundant worldwide and can be obtained from agricultural and forest industry residues (Hahn-Hägerdal et al., 2006). There are four main steps in the transformation chain to produce second generation bioethanol (2G bioethanol): delignification, saccharification, fermentation and ethanol purification; for each one, there exist different configurations given by the diversity of lignocellulosic material sources, chemical reagents and bio-reagents, and process equipments (Wyman, 1996). Within this 2G bioethanol framework, this study focuses on the saccharification of delignified lignocellulosic material via enzymatic hydrolysis carried out in a stirred tank bioreactor.

Typically, the R&D of enzymatic hydrolysis processes of lignocellulosic mass for certain substrate-enzyme-equipment system is based on how much sugar is obtained at the end of the experiments, and another question can be added about how fast the process evolves. Most of the studies exploring this kind of processes are experimental (e.g., Chen et al., 2013), and give answers to the first question; however, just a few address the second question (e.g., Shill et al., 2012), which is basically related to the construction of a dynamic model; consequently, model-based works are scarce. A justification of the shortage of dynamics model could lie in the challenges posed by the variety of reducing sugars involved, and the variability and uncertainties of the composition and of the availability-to-be-hydrolysed of the cellulose and hemicellulose in a lignocellulosic material after the first step of pretreatment. The multiplicity of corresponding enzyme complex systems also makes the kinetics description a problem with multiple viewpoints (Gusakov and Sinitsyn, 1985; Hosseini and Shah, 2011).

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On the other hand, in order to emphasize the diversity on this kind of systems, it is worth taking into account the several ways of delignifying lignocellulosic mass (e.g., by a dilute acid, by alkali medium, by autoydrolysis or explosion with steam), which provide different compositions of cellulose, hemicellulose and lignin to the material and different availability for these components of processing by a certain enzyme or enzyme complex (Wyman, 1996). By following certain pretreatment, batch-to-batch, variability in the material properties will likely occurs, which in turn causes variability in the hydrolysis process.
Therefore, establishing a comprehensive mechanistic kinetics model for the enzymatic hydrolysis of lignocellulosic mass is a current challenge, which seems that must be performed in a particular way for each specific system. Primarily focused on the engineering process, and considering the two questions posed above, in this work two different models are proposed for the enzymatic hydrolysis of wheat straw: one to characterize the performance of the process, and other that comprehensively considers main factors affecting the process. Wheat straw is considered a good representative lignocellulosic material.

2. Performance Evaluation and Descriptive Mathematical Modelling

Without the eagerness of ignoring that in this kind of processes different reducing sugars (such as glucose, xylose and arabinose) are involved, as a first methodological step, the set of reducing sugars is taken as an individual component, and as well the cellulose and hemicellulose are bundled into holocellulose; thus, holocellulose is considered as the single source of sugar.

2.1 Performance Evaluation

Typically the performance of a hydrolysis process is given in terms of the yield of certain reducing sugar or the yield of the set of reducing sugars ($Y_{P/S}$), as in this work:

$$Y_{P/S} = P_x/S_0 \tag{1}$$

This parameter relates the concentration of the sugar at the end of the process (P_x) with respect to the concentration of holocellulosic mass loaded into the reactor (S_0), regarding the composition of the lignocellulosic material. In the studies of this kind of processes, experiments are carried out over a long period of time (e.g., 72 h) to assure a maximum conversion of holocellulose to reducing sugars (Ruiz et al., 2012); although a total conversion is never achieved, the maximum conversion is always almost completed in a shorter time, as can be observed in Figure 1 where sugar trajectories of enzymatic hydrolysis experiments are illustrated. At first sight, the shape of experimental trajectories seem to be ones of a dynamic system of first order; therefore, for the purpose of obtaining an evaluation of the process performance not just in terms of the maximum amount of reducing sugars obtained but also in how fast the process is, a first order system is recalled:

$$\tau\dot{P} + P = P_f, \qquad P(0) = 0; \quad t_f = 4\tau \tag{2}$$

P is the concentration of reducing sugars, P_f is the maximum concentration of reducing sugars that can be obtained, and τ is a constant time. Once τ is identified, the settling time t_f of the P-trajectory can be estimated (2), which is directly related with the time where P_f is practically achieved. The identified P_f can even be interpreted as a filtered value of the experimental data at the end of the experiments. Finally, $Y_{P/S}$ (1) is calculated on the basis of the identified P_f (2). Thus, the performance of a particular system can be quickly characterized through the two parameters $Y_{P/S}$ and t_f.
The identification of the model paremeters (τ, P_f) can be easily performed by minimizing the sum of squared errors between the model predictions and the experimental data, using conventional software: e.g., through the Microsoft Excel® tool called “Solver” linked to the solution of the differential equation with the Euler method and experimental data.

2.2 Modelling for Process Description

For the purpose of describing the hydrolysis for a certain system substrate-enzyme in a mechanistic form, it is considered the simplest kinetics mechanism for cellulose enzymatic hydrolysis (S + E $\Rightarrow$ P + E; Ye and Berson, 2011), but it is assumed that holocellulosic mass (S) is the single component to be converted to a single sugar (P) by the enzyme (E). In addition, it is included an inhibition effect related to the increase of sugar concentration (Taherzadeh and Karimi, 2008). The model takes the following form:

$$\dot{S_a} = -r_S(S_a, P, E, K_C, K_S, K_I),\ \ S_a(0) = S_{a,0},\quad S_{a,0} = P_f, \tag{3a}$$

$$\dot{P} = +r_S(S_a, P, E, K_C, K_S, K_I),\ \ P(0) = P_0,\quad P_0 = 0 \tag{3b}$$

$$\dot{E} = 0,\ \ E(0) = E_0, \tag{3c}$$

$$r_S(\cdot) = K_C E \frac{S_a}{K_S + S_a + \frac{P}{K_I}},\quad \mu_{MAX} = K_C E \tag{3d}$$

S_a and P are the concentrations of available holocellulose and reducing sugars, respectively; E is the enzyme concentration that is constant throghout the process (3c), and r_S is the consumption rate of holocellulose whose function is of the Michaelis-Menten type (Pandey et al., 2005); K_C, K_S and K_I are the constants related to the catalyst action, the substrate affinity, and the inhibition effect, respectively. Thus, the modelling problem becomes identifying such constants.

The identification can be performed by minimizing the sum of squared errors between the model predictions and experimental data. In this case, since P is the only variable feasible to be measured, the minimization is based on the errors between the predicted-by-model P (3) and the experimental P.

Besides, a precision must be made about S_a, particularly on $S_{a,0}$: assuming that by unknown inhibition effects, the hololocellulose will not be totally converted, even in an infinite time, it is only considered the part of holocellulose that will actually be converted ($S_{a,0}$). $S_{a,0}$ is set through P_f (3a), which in turn is estimated through the performance model (2).

3. Experimental Methods

As it was mentioned, this work considers particularly the enzymatic hydrolysis of wheat straw carried out in an automatized stirred tank reactor. For testing the mathematical models and to set a comparison framework, two practically different substrates were explored. Although the lignocellulosic material is of the same nature, the difference between the substrates lies in that the wheat straw was pretreated by two different methods.

3.1 Substrate and enzyme complex

One lot of the wheat straw was pretreated with diluted sulphuric acid, and another one with alkaline-oxidative medium (a mixture of NaOH and H_2O_2); details of pretreatments are given in Sun et al. (2000) and in Silverstein (2014), respectively. After pretreaments, the composition of cellulose and hemicellulose are 47.8 %w/w and 3.2 %w/w, respectively, for the acid wheat straw (AWS), and 75.1 %w/w and 5.9 %w/w, respectively, for the alkaline wheat straw (KWS). The composition was analysed by the Goering and Van Soest method (Goering and van Soest, 1970). The enzyme complex was C-Tec® from Novozyme®, kindly provided by one of the research centers of the National Council for Science and Technology of Mexico (CIATEJ; Guadalajara, México). This enzyme complex was designed for lignocellulosic biomass processing.

3.2 Experimental conditions of hydrolysis

Experiments were carried out in an automatized, jacketed, stirred tank reactor (Labfors-5® by Infors-HT®); throughout every process, pH, temperature and volume were kept constant. The reaction volume was 2 L. The conditions for every experiment of AWS were pH = 4.5, T = 45 ºC, and enzyme load of 0.3 mL/g-wheat straw, and the ones of KWS experiments were pH = 4.0, T = 50 ºC and enzyme load of 0.5 mL/g-wheat straw. Along every experiment, samples of 1.5 mL were taken frequently; these were analysed by the Miller method (Miller, 1959) to obtain the reducing sugars concentration. For each substrate, the diversity on the experiments corresponds to the hydrolysis with three different loads of wheat straw: (i) 1 %w/v, (ii) 3 %w/v, and (iii) 5 %w/v. The process conditions were determined by evaluating the yield obtained in diverse conditions, which were set down through a multilevel full factorial experiment design. The numerous experiments were carried out in 1.5 mL tubes put into an Eppendorf-thermomixer®. The experiment time was set in 1 hr. The space of conditions explored was: 4 to 6 for pH, 40 to 60 ºC for temperature, and 0.1 to 0.5 mL/g-wheat straw for enzyme load. It can be observed that conditions for each substrate are different, but they correspond to the conditions in which the greater amount of sugar is obtained from each substrate. Further discussion about the outcomes from microreaction system is beyond the purpose of this work.

4. Results

From the experiments mentioned above, for each substrate there resulted three trajectories of reducing sugars concentration, that also are put in terms of yield to get another sight of the process performance. For each substrate, Figure 1 illustrates the trajectory of reducing sugars concentration corresponding to the substrate load of 1 %w/v, as well as the yield trajectory. At a glance, the KWS is better hydrolysed than the AWS in the sense that the greater yield is observed with the KWS, and the shorter settling time is also observed with the KWS. Similar results are observed in the other wheat straw loads.

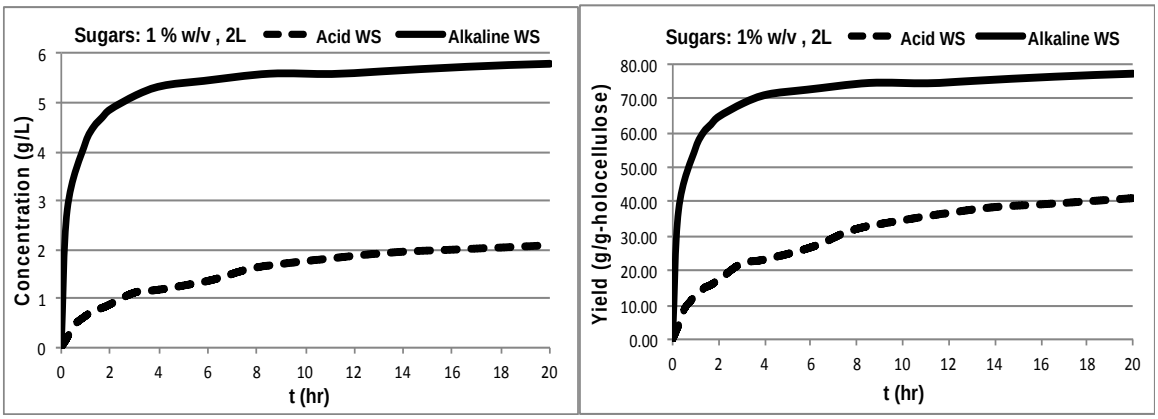

Figure 1. Experimental trajectories of reducing sugars concentration and yield.

In Table 1, for both substrates, the performance parameters are given for each substrate load, and Figure 2 illustrates the trajectory of reducing sugars concentration given by the performance model (2); it is observed that the model approximately draws the experimental trajectory. Comparing performances, every KWS experiment provided a greater $Y_{P/S}$ and a less t_f than the corresponding ones of AWS. Therefore, it can be said that the alkaline-oxidative pretreatment will drive a better enzymatic hydrolysis. A comparison between hydrolysis of a same substrate but of different straw loads can be made. For the KWS, the greater the straw load the less the yield but the faster the process; on the other hand, the AWS hydrolysis is not affected considerably by the straw load but the process becomes faster with a load increase. It is worth to say that the reactor cannot be loaded with a straw concentration greater than 10 %w/v because its density is very small. The increase in the speed of the process by the increase of the straw load is an opposite effect than one can expect of this process.

Table 1. Performance parameters for the different hydrolysis processes

Acid WS	τ (h)	P_F (g/L)	Alkaline WS	τ (h)	P_F (g/L)
1%	0.516491552	1.930448297	1%	0.184071927	5.432658943
3%	0.156871205	6.374656035	1.44%	0.146062838	6.8463684
5%	0.10191189	9.812397733	5%	0.042509496	23.52415541

With respect to the mechanistic model (3) for process description, for each substrate and for the substrate load of 5 %w/v, Figure 3 illustrates the model and experimental trajectories of reducing sugars concentration, as well as the model trajectory of available holocellulose concentration. It can be observed that the predicted-by-model sugar concentration trajectory fits the corresponding experimental trajectory.

The model parameters are given in Table 2, whose identification was performed on the basis of the three different substrate loads. It can be noticed that the kinetics constants for AWS are greater than the ones for KWS, except the inhibition constant. These values agree with the following experimental facts: it can be said that μ_{MAX} for KWS is greater than for AWS because the process is faster; therefore, and recalling that a greater amount of enzyme is used for KWS than for AWS, the only way of KWS's μ_{MAX} to be greater than the one of AWS corresponds to a greater K_c. On the other hand, because K_S for KWS is smaller than for AWS, the substrate-enzyme affinity is greater for KWS than for AWS, which consequently must drive to a greater yield. Finally, K_I is greater for KWS than for AWS, meaning a smaller inhibition effect.

It is worth to say that a model without an inhibition effect was tried, but it did not fit any experimental trajectory. Also, a model was tried taking into account the actual initial holocellulose concentration, but fitting was not achieved. Together with the assumptions about inhibition and an available holocellulose biomass, these

outcomes drove us to propose the mechanistic model (3) as a first step on the mechanistic description of the enzymatic hydrolysis of lignocellulosic biomass. On the other hand, the very different values on the model parameters between substrates make evident that a mechanistic model must be constructed for each specific substrate-enzyme system.

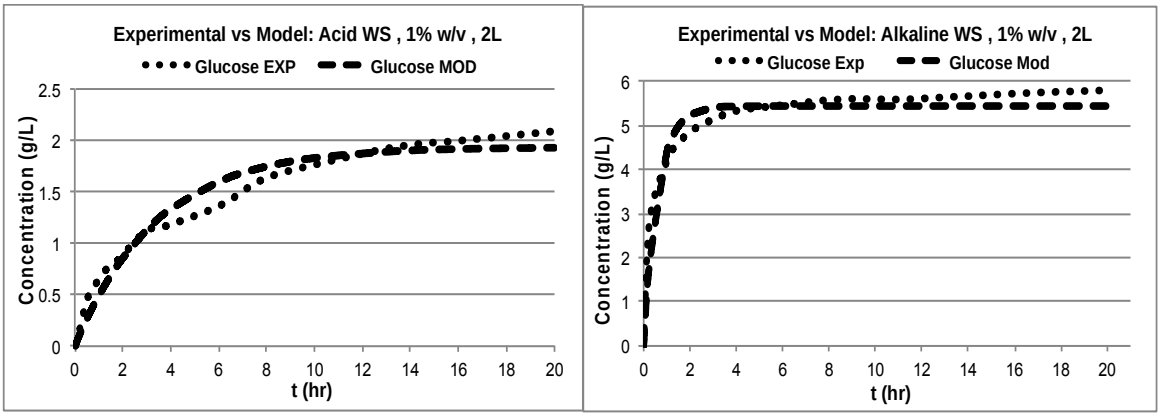

Figure 2. Comparison of trajectories between performance model and experiments.

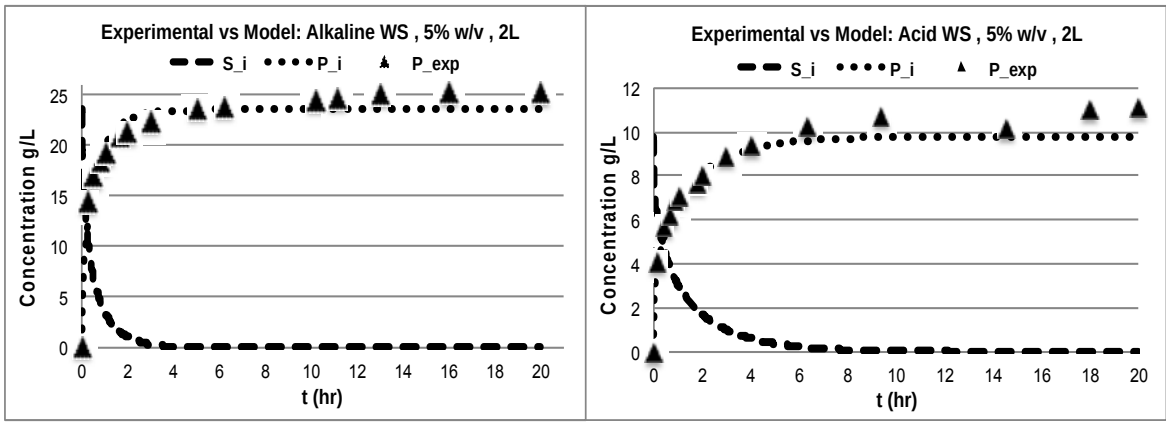

Figure 3. Comparison of trajectories between mechanistic model and experiments.

Table 2. Kinetics model parameters for mechanistic model.

Parameters	Acid WS	Alkaline WS
K_C	27.294	11.301
K_S	6.416	2.875
K_I	0.011	0.094

5. Conclusions

This work proposed two mathematical models; one of them is of simple structure that quantitatively determines through two parameters the performance of the enzymatic hydrolysis of certain substrate-enzyme system. Its usefulness was illustrated by comparing the performance of two systems: one processing wheat straw pretreated by acid, and other one processing wheat straw pretreated by alkaline-oxidative medium, resulting that the hydrolysis of wheat straw pretreated by alkali is faster and yields more reducing sugar than the one with acid wheat straw. The other mathematical model can describe the behaviour of the process at different loads of wheat straw; thus, there exists certain confidence on estimating the trajectories for another wheat straw loads. Finally, although the models are specific for a particular enzymatic hydrolysis system, methodologies for constructing simple but useful models were delineated.

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