Appl Biochem Biotechnol (2014) 173:1083–1096
DOI 10.1007/s12010-014-0912-4

Modeling the Kinetics of Complex Systems: Enzymatic Hydrolysis of Lignocellulosic Substrates

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Received: 7 February 2014 / Accepted: 7 April 2014 /
Published online: 23 April 2014

Abstract Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin. Fuzzy logic, in turn, is a branch of many-valued logic based on the paradigm of inference under vagueness. This paper presents a methodology, based on computational intelligence, for modeling the kinetics of a complex reactional system. The design of a fuzzy interpolator to model cellulose hydrolysis is reported, within the perspective of applying kinetic models in bioreactor engineering. Experimental data for various types of lignocellulosic materials were used to develop the interpolator. New experimental data from the enzymatic hydrolysis of a synthetic substrate, on the other hand, were used to validate the methodology. The accuracy of the results indicates that this is a promising approach to extend the application of models fitted for specific situations to different cases, thus enhancing their generality.

Keywords Cellulose · Clustering · Enzymatic hydrolysis · Enzyme kinetics · Fuzzy interpolator · Lignin

Introduction

Recently, lignocellulosic materials such as sugarcane bagasse and other agricultural wastes have been used as raw material for various products. The worldwide search for renewable energy sources makes the use of lignocellulosic biomass waste a promising alternative to produce bioethanol fuel on a large scale and at a competitive cost.

One of the most popular alternatives for production of bioethanol in industrial scale is the biochemical route, based on the fermentation of the sugar monomers of cellulose and

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hemicellulose. Two techniques may be used for obtaining fermentable sugars from lignocellulosic materials—acid and enzymatic hydrolyses.

Acid chemical hydrolysis has well-known drawbacks—generation of toxic products and corrosiveness of the medium, requiring the use of more costly reactors. Moreover, it is necessary to recover the acid for making the process economically viable. In general, when concentrated acids are used, the monosaccharides released by hemicellulose are degraded due to the fact that the hemicellulose fraction is hydrolyzed more rapidly than the fraction of cellulose. The sugars present in hemicellulose are exposed for a longer time in the reaction medium, which results in their degradation (for instance, to furfural). On the other hand, hydrolysis with dilute acid requires the use of high temperatures. For this reason, a relatively high amount of soluble sugars and lignin is degraded, leading to inhibition during the fermentation process [1].

In the enzymatic hydrolysis, cellulose is broken down by a pool of cellulolytic enzymes, and there is no formation of by-products due to the specificity of enzymes (resulting in a high yield of fermentable sugars). However, it is necessary to pretreat the biomass, to improve its digestibility, and to use high concentrations of enzymes for achieving high conversions of the cellulose, which makes the process more costly [2].

Enzymatic hydrolysis of cellulose yields glucose, which can be further fermented to provide ethanol. It can be operated under mild conditions, avoiding the formation of byproducts which can inhibit the fermentation, such as hydroxymethylfurfural [3], consequently leading to a reduction of the cost of separation of the products [4].

The presence of lignin and hemicellulose hinders the access of cellulases to their substrate; this can reduce significantly the efficiency of the hydrolysis. Therefore, there is a need for pretreatments that weaken the interaction between the main components of the lignocellulosic material. Pretreatments aim at partially separating lignin and hemicellulose from the cellulose matrix and disrupting the crystalline structure of cellulose. The result is increased accessibility to cellulose particles, as a consequence of the solubilization and/or partial degradation of hemicellulose and lignin [5]. To be considered effective, a pretreatment must meet the following requirements: avoiding the need of reducing the particle size of the biomass, limiting the formation of degradation products that inhibit the microbial fermentation, and minimizing the energy demand. Various types of processes have been used for the pretreatment of lignocellulosic materials [1]. The processes described in the literature include physical processes, such as steam explosion and thermal hydrolysis; chemical processes, such as weak acid and alkaline hydrolysis; biological processes using fungi to solubilize the lignin; finally, a combination of processes, as steam explosion catalyzed by adding H_2SO_4 or CO_2 [6, 7]. However, the most common methods are based on the use of alkali, acid hydrolysis, steam explosion, and hot water, all with the purpose of breaking the associative structure of lignocellulose [8–10].

For the enzymatic hydrolysis to be efficient, it is necessary to optimize the reaction conditions since the yield of hydrolysis is governed by several factors such as the type of pretreatment of the substrate, inhibition of enzymatic activity by end products of the biodegradation, ineffective adsorption of enzymes (on lignin, for instance) thermal stability of enzymes, duration of the hydrolysis, pH, concentration of substrate in the medium, and agitation speed [11].

In enzymatic hydrolysis processes, there are some important phenomena that finally control the overall rates of reaction. For hydrolyses in batch-stirred reactors, the overall reaction rate is determined by the rates of three events occurring in sequence: (a) the rate

of (external) mass transfer of the enzyme, (b) the rate of diffusion/adsorption of the enzyme on the substrate surface, and (c) the rate of the cellulase catalytic action. After a first initial stage, the overall reaction rate is subject to (internal) diffusion and adsorption of the enzyme on the solid substrate. Most authors do not consider the external mass transfer resistances. The adsorption of enzymes and the formation of enzyme-substrate complexes are considered crucial steps in this process. Adsorption of cellulase on the insoluble cellulose has been described as reversible, irreversible, and semireversible (thus, literature is inconclusive on this subject [12]).

Modeling the enzymatic hydrolysis of lignocellulosic materials is probably one of the most challenging subjects in bioreactor engineering science. The challenges of this problem can be grouped into three classes—the complexity of the substrate, the action of the enzymes, and the enzyme-substrate interactions. There are different approaches for this modeling. Zhang and Lynd [13] grouped kinetic models of the enzymatic hydrolysis of biomass according to the level of detail of their description of the substrate and/or of the activities of the different enzymes that are acting. According to these authors, models can be classified as nonmechanistic, semimechanistic, functionally based, and structurally based.

Engineering practice for design and optimization of the hydrolysis bioreactor has been based mainly on different empirical and semi-empirical approaches (nonmechanistic and semimechanistic models), including simple kinetic equations [14]. The most common semimechanistic approach is based on pseudohomogeneous Michaelis-Menten models, i.e., despite being actually a solid, the substrate is treated as a soluble reactant, characterized by its concentration. Bezerra and Dias [15] investigated the kinetics of one exoglucanase in the presence of cellobiose using different enzyme/substrate (Avicel) ratios. It was found that the cellulose hydrolysis rate followed a pseudohomogeneous Michaelis-Menten model that takes into account competitive inhibition by cellobiose. A second class of semimechanistic rate equations pictures a system closer to the real one, considering that the substrate is in solid form and that the soluble enzyme has to adsorb to (and desorb from) it. Carrillo et al. [16] studied the kinetics of the hydrolysis of pretreated (with sodium hydroxide) wheat straw using different concentrations of a commercial cellulase (Novozymes A/S). Initial rates of an equation derived from a Michaelis-Menten mechanism were measured, assuming solid substrate and soluble enzyme. The initial rate of hydrolysis can be expressed as a function of the initial enzyme concentration in a modified Michaelis-Menten model.

Finally, in Chrastil's semimechanistic model [17, 18], all time constants of the rate of product formation are ranked, taking into account that in a heterogeneous system the time curves depend strongly on rate-limiting phenomena such as enzyme (internal) diffusion and adsorption.

Many kinetic rate equations were developed for model substrates such as Avicel, CMC, or Solca Floc. However, real substrates do not show the same characteristics as pure cellulose. These substrates may not represent well the hydrolysis of biomass, where cellulose is combined with other components (hemicellulose and lignin). Carvalho et al. [19, 20] investigated the kinetics of the enzymatic hydrolysis of three cellulosic substrates (added to the bioreactor in low and high loads)—filter paper (FP), used as a delignified substrate model, steam-exploded sugarcane bagasse (SB), and acid treated SB. The latter two were also treated with 4 % NaOH. Kinetic models were fit for these different lignocellulosic substrates, and different functional forms of the rate equations (Michaelis-Menten-like and Chrastil equations) were tested for different types of substrates.

However, due to the complexity of the reactional system, it becomes difficult to use a single semimechanistic kinetic model for the different hydrolysis conditions that appear while the reaction proceeds. An interesting idea would be to combine the attributes of the different "local" models adjusted by Carvalho et al. [19, 20] in a "global" system, calculating the rate of hydrolysis (incorporating models fitted for different hydrolysis conditions). A first step towards this development is the classification of the experimental data from Carvalho [19, 20] into an adequate number of clusters. This classification is important for determining kinetically similar regions.

Clustering of numerical data forms the basis of many classification algorithms for preprocessing experimental data. The purpose here is to identify natural groupings in a set of data in order to improve the representation of the system behavior. A popular technique for grouping data is associated with fuzzy logic, and is known as fuzzy C-means (FCM), as described in the "Theory" section of this paper.

The aim of this paper is to develop a fuzzy interpolator for kinetic modeling based on simple models to obtain a "global" model (combining these simple models), covering a broad range of hydrolysis conditions. The present paper intends to assess how the interpolation of simple semimechanistic models adheres to experimental data of enzymatic hydrolysis of cellulosic substrates. Three kinetic models were considered—pseudohomogeneous Michaelis-Menten, modified Michaelis-Menten, and Chrastil models, all previously fitted by Carvalho et al. [19, 20].

Materials and Methods

Fuzzy Interpolator Design

Experimental data from Carvalho [19, 20], of hydrolysis with different lignocellulosic substrates (filter paper; exploded bagasse treated with 4 % NaOH, low and high load; bagasse treated with 1 % H_2SO_4 and 4 % NaOH, low and high load), were grouped using the FCM clustering technique. The data were clustered into different classes, so that each different class matches a different kinetic model. For clustering, the variables used were percentage of cellulose load in the reactor and lignin content (percentage) with regard to the substrate composition.

Initially, Carvalho [19, 20] carried out filter paper studies in 250-mL Erlenmeyer flasks, in a total reaction volume of 20 mL, with 0.5 FPU $mL^{-1}_{solution}$, at pH 4.8, in 50 mM sodium citrate buffer. The experiments were run using a refrigerated incubator (Marconi MA-832) with agitation set at 250 rpm at 50 °C. The initial substrate concentration of 3.85 % ($w_{cellulose}/w_{total}$) was considered. For the exploded sugarcane bagasse treated with 4 % NaOH, hydrolysis experiments were also performed in 250-mL Erlenmeyer flasks, with a liquid volume of 30 mL, by adding 0.85 FPU $mL^{-1}_{solution}$, at pH 4.8, in 50 mM sodium citrate buffer. The initial percentage of 2.9 % ($w_{cellulose}/w_{total}$), corresponding to a potential glucose concentration of 33.33 $g_{Glucose}\ L^{-1}_{solution}$, was taken into account. Also, an assay was performed by setting the initial substrate at 6.54 % ($w_{cellulose}/w_{total}$), corresponding to a potential glucose concentration of 77.77 $g_{Glucose}\ L^{-1}_{solution}$. For the bagasse treated with 1 % H_2SO_4 and delignified with NaOH 4 %, similar procedures were carried out. For the determination of glucose concentration from samples taken during the hydrolysis assays, an enzymatic kit was used by Carvalho [19, 20]. To calculate the cellulose load in the reactor and the lignin content with regard to the substrate composition along time, taking into account the initial chemical characterization of the substrates (for cellulose, lignin, hemicellulose, and ashes) and the

consumption of cellulose along time in each experiment of Carvalho [19, 20], the following calculations were performed:

$$\%\ \text{of cellulose in the reactor} = \frac{100 \times \text{remaining cellulose (g)}}{w_{\text{total}}\ \text{(g)}} \quad (1)$$

$$\text{lignin content} = \frac{100 \times \text{lignin (g)}}{\text{lignin (g)} + \text{remaining cellulose (g)} + \text{hemicellulose (g)} + \text{ashes (g)}} \quad (2)$$

Based on the clustering, a fuzzy interpolator was generated for the interpolation of different simple semimechanistic models (those presented in Table 1). The study was conducted using the software MATLAB.

The command line function *fcm* of MATLAB starts with an initial estimate for the cluster centers, which will be the average position of each cluster. The initial estimate for these cluster centers is probably incorrect. Furthermore, the function *fcm* calculates, for all data points, 1 degree of membership with regard to each cluster. By iterative updating the position of these cluster centers and the degree of membership of each data point, the function *fcm* iteratively moves the cluster centers within a dataset. These iterations are based on minimizing an objective function that represents the distance from any given data point to a cluster center, weighted by the degree of membership of that point.

The information returned by FCM was used to build a fuzzy inference system by creating membership functions to represent the fuzzy qualities of each cluster. The classification was used to generate an inference algorithm to represent the system behavior (rate of hydrolysis according to the conditions of hydrolysis) using a minimum number of *fuzzy* rules. The predictions of the "global" model proposed here were compared with experimental data of enzymatic hydrolysis of a synthetic (cellulosic) substrate.

Table 1 Semimechanistic kinetic models and kinetic parameters used to develop the fuzzy interpolator for the enzymatic hydrolysis of lignocellulosic materials [19, 20]

Model	Equation	Parameters
Model 1 Pseudohomogeneous Michaelis-Menten (MM) model, with inhibition—studies originated with filter paper	$v = \frac{V_{\max} S}{K_m[1+(P/K_I)]+S}$	$V_{\max}$: 0.09 g L^{-1} min^{-1} K_m: 45.11 g L^{-1} K_I: 4.13 g L^{-1}
Model 2 Pseudo-homogeneous MM with competitive inhibition	$v = \frac{V_{\max} S}{K_m[1+(P/K_I)]+S}$	$V_{\max}$: 0.105 g L^{-1} min^{-1} K_m: 8.78 g L^{-1} K_I: 5.91 g L^{-1}
Model 3 Modified MM Model with inhibition	$v = \frac{K\ E\ S}{K_m[1+(P/K_I)]+E}$	K: 0.0033 min^{-1} K_m: 22.06 g L^{-1} K_I: 20.18 g L^{-1}
Model 4 Chrastil	$P=P_\infty[1-\exp(-k'E_0t)]^n$	$P\infty$: 33.33 g L^{-1} k': 2.49E-7 L g^{-1} min^{-1} Eo: 7.83 g L^{-1} n: 0.33
Model 5 Chrastil b		$P\infty$: 77.77 g L^{-1} k': 4.96E-8 L g^{-1} min^{-1} Eo: 13.05 g L^{-1} n: 0.30

Validation Test

Enzyme

The commercial complex of cellulases Accellerase® 1500, from *Trichoderma reesei*, donated by Genencor ® (Palo Alto, CA, USA), was used. According to the manufacturer, this complex contains multiple enzymatic activities, different exoglucanases and endoglucanases, beta-glucosidase, and hemicellulase. This complex operates at 50 °C and pH 4.8. In long-term experiments, 50 °C is a good choice to minimize temperature inactivation. To determine the complex overall activity, the method of total reducing sugars (TRS) was applied, making use of 3,5-dinitrosalicylic acid (DNS) and Whatman filter paper no. 1 [21–23].

Enzymatic Hydrolysis of a Synthetic Substrate

Two cellulosic materials were used to compose a synthetic substrate—qualitative filter paper (Satelit, Brazil) and *in natura* sugarcane bagasse (*Saccharum officinarum*), donated by the Center for Sugarcane Technology (CTC), Piracicaba, SP, Brazil.

The synthetic substrate was prepared from the *in natura* sugarcane bagasse and the filter paper with a ratio of 1:1 (*w*/*w*). This was done in order to obtain a substrate with a lignin percentage between 0 and 20 % (12 %) in order to validate the model interpolator (since this is a new condition, different from Carvalho's [19, 20]).

Experimental Conditions

The hydrolysis experiment was carried out in Erlenmeyer flasks with 50 mL of 50 mM sodium citrate buffer, at pH 4.8 and 20 FPU $g^{-1}_{cellulose}$ of Accellerase® 1500, corresponding to 1.2 FPU $mL^{-1}_{solution}$. The experiment began with the addition of 4.32 g of synthetic substrate (50 % of paper filter–50 % of sugarcane bagasse) in order to obtain an initial concentration of potential glucose of 66.66 g L^{-1}. The synthetic substrate was hydrolyzed at 50 °C and 250 rpm for a total time of 72 h. Experiments were run in triplicate.

Sugar Quantification

Glucose and cellobiose were determined by high performance liquid chromatography (HPLC) using a Shimadzu SCL-10A chromatograph with a refractive index detector Shimadzu RID-10A, column Aminex HPX-87H (300×7.8 mm, Bio-Rad) with 0.005 mol L^{-1} H_2SO_4 as mobile phase at a flow rate of 0.6 mL min^{-1} and oven temperature of 45 °C.

Theory

FCM is a technique for grouping data where each data point belongs to all clusters, but in a different intensity—which is specified by the degree of membership of this point to each cluster. Such a technique was originally introduced by Bezdek [24] as an improvement in prior methods [25]. Here, the number of cluster is predefined. There are other techniques that can be used when the number of clusters is not previously known [26].

The FCM algorithm performs the steps listed in the following [25, 27, 28]:

1. Centers of the clusters are chosen randomly, and for each point in the dataset the distance to each center is calculated by Eq. (3).

$$D_{ij}^2 = \left(u(i) - c_j\right)^T \sum \left(u(i) - c_j\right) \tag{3}$$

where $u(i)$ is a data point, j is the number of clusters, and c is the chosen center.

2. After that, the degree of membership of each data point (for each cluster) is calculated, using Eq. (4).

$$\mu_{ij} = \frac{1}{\sum_{l=1}^{C} \left(D_{ij}^2 / D_{il}^2\right)^{\frac{1}{\nu-1}}} \tag{4}$$

where μ is the degree of membership, ν is a weighting exponent, between 1 and ∞ (when there is no prior knowledge, 2 is a good estimate [25, 27, 28]), and C is the number of centers.

3. After this step, the centroid is computed for each cluster, which can be obtained from Eq. (5).

$$c_j = \frac{\sum_{i=1}^{N} \mu_{ij}^{\nu} u_i}{\sum_{i=1}^{N} \mu_{ij}^{\nu}} \tag{5}$$

N represents the number of existing data. The centers are modified until an objective function, represented by I in Eq. (6) is minimized.

$$I = \sum_{j=1}^{C} \sum_{i=1}^{N} \mu_{ij}^{\nu} \left(u(i) - c_j\right)^T \sum \left(u(i) - c_j\right) \tag{6}$$

Results and Discussion

Clustering of numerical data forms the basis of many classification and system modeling algorithms. Initially, natural groupings (clusters) from experimental data of Carvalho [19, 20] were identified. Figure 1 shows the experimental dataset, percentage of cellulose in the reactor *versus* percentage of lignin with regard to the substrate composition, during the hydrolysis processes, i.e., along time, to determine the centers of the clusters. It is worth noticing that although we refer to the variable time, the time itself is not an input variable for grouping the dataset (only percentage of cellulose in the reactor and percentage of lignin). The data are for the five different lignocellulosic substrates previously mentioned (filter paper (1); exploded bagasse treated with 4 % NaOH, low (2), and high load (3); bagasse treated with 1 % H_2SO_4 and 4 % NaOH, low (4) and high load (5)) (Fig. 1).

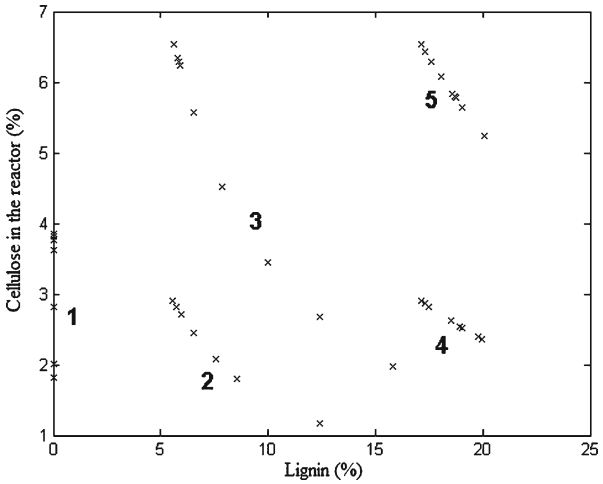

Fig. 1 Percentage of cellulose in the reactor versus percentage of lignin with regard to the substrate composition, during the hydrolysis processes, for the experimental data of Carvalho [19, 20]. The data are for five different lignocellulosic substrates (filter paper (*1*); exploded bagasse treated with 4 % NaOH, low (*2*) and high load (*3*); bagasse treated with 1 % H2SO4 and 4 % NaOH, low (*4*) and high load (*5*))

We use MATLAB to show the experimental data graphically, where the *Y*-axis represents the percentage of cellulose load in the reactor (%) and the *X*-axis the percentage of lignin with regard to the substrate composition (%). In MATLAB, invoking the command-line function *fcm*, it was possible to find five clusters in this dataset (until the objective function, objFcn, was no longer decreased). Function *fcm* returned a vector containing the coordinates of the five cluster centers, other vector

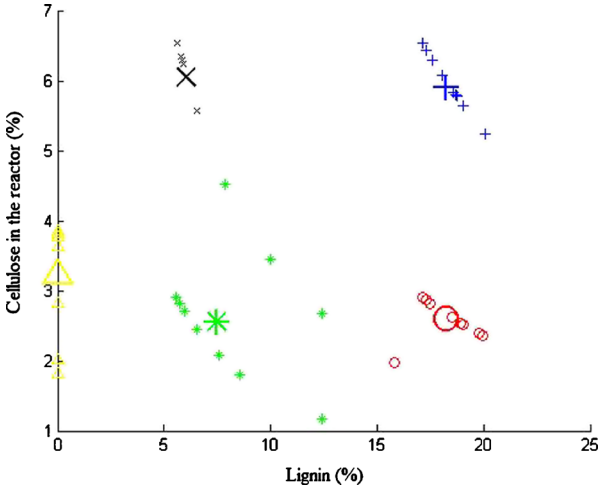

Fig. 2 First output for the clustering process. *Empty triangle* Center of the cluster for filter paper; *asterisk* center of the cluster for exploded bagasse treated with 4 % NaOH (low percentage of cellulose); *multiplication symbol* center of the cluster for exploded bagasse treated with 4 % NaOH (high percentage of cellulose); *empty circle* center of the cluster for bagasse treated with 1 % H2SO4 and 4 % NaOH (low percentage of cellulose), *positive symbol* center of the cluster for bagasse treated with 1 % H2SO4 and 4 % NaOH (high percentage of cellulose)

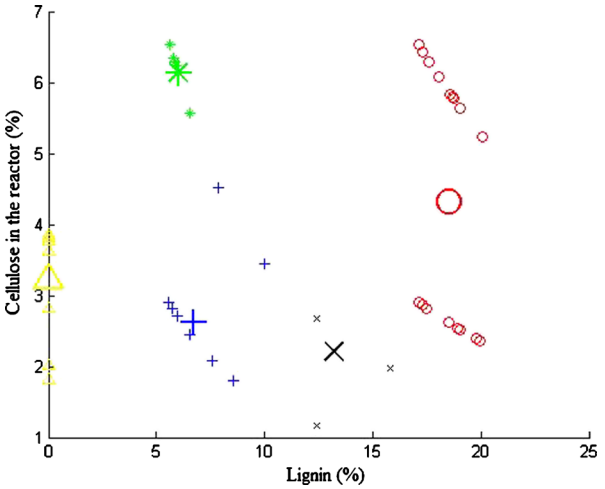

Fig. 3 Second output for the clustering process

containing the membership degrees for each one of the data points, and a history of the objective function along the iterations.

After running *fcm* function, two results were obtained due to the initial random choice of the cluster centers. The two possible outputs are shown in Figs. 2 and 3.

The first output was selected (Fig. 2) since each different cluster center is associated with a different hydrolysis substrate (filter paper (Δ); exploded bagasse treated with 4 % NaOH, low (*) and high (X) load; bagasse treated with 1 % H_2SO_4 and 4 % NaOH, low (O) and high (+) load), providing a more intuitive response.

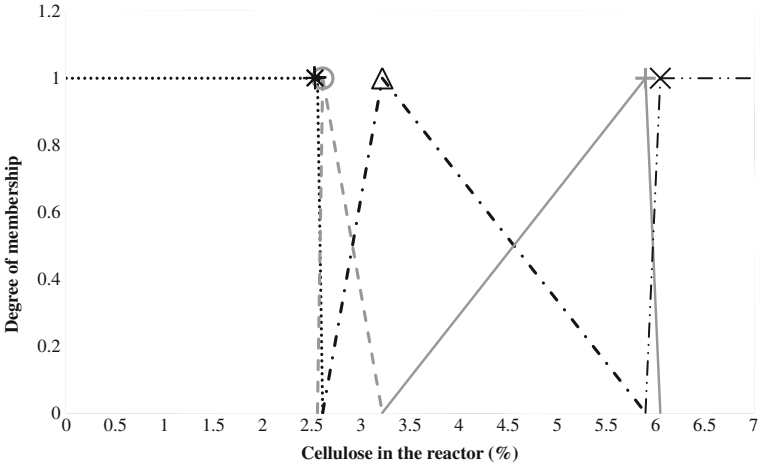

Fig. 4 Membership functions for determining the degree of membership (for cellulose load). Each cluster center corresponds to the maximum degree of membership (=1) of a particular linguistic value (for the linguistic variable cellulose load). The *straight lines* connecting the data points (cluster centers) characterize intermediate degrees of membership within two adjacent linguistic values

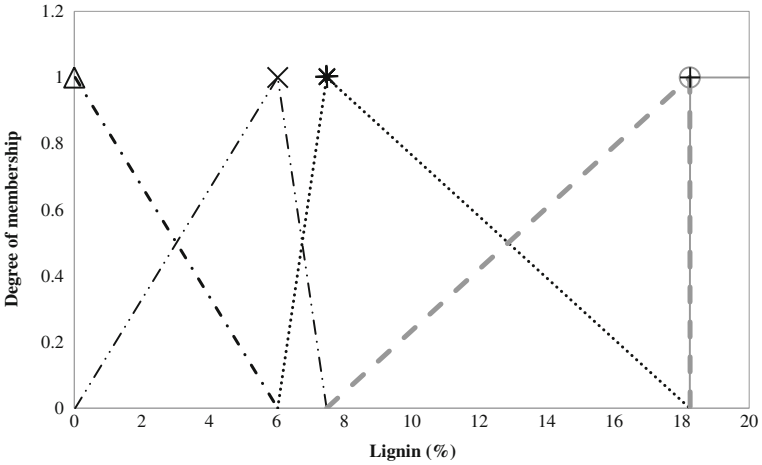

Fig. 5 Membership functions for determining the degree of membership (for lignin content). Each cluster center corresponds to the maximum degree of membership (=1) of a particular linguistic value (for the linguistic variable lignin content). The *straight lines* connecting de data points (cluster centers) characterize intermediate degrees of membership within two adjacent linguistic values

In addition to the figures, the program also provides the coordinates of the cluster centers, which were then used in the creation of functions for determining the degree of membership for the two inputs, cellulose load in the reactor and lignin content, as shown in Figs. 4 and 5.

Each cluster center corresponds to the maximum degree of membership (=1) of a particular linguistic value (for the linguistic variables cellulose load and lignin content).

As one can see in Figs. 4 and 5, some maximum degree of memberships (=1) were very close to each other, making it suitable to fuse some linguistic values. The final membership functions are shown in Figs. 6 and 7.

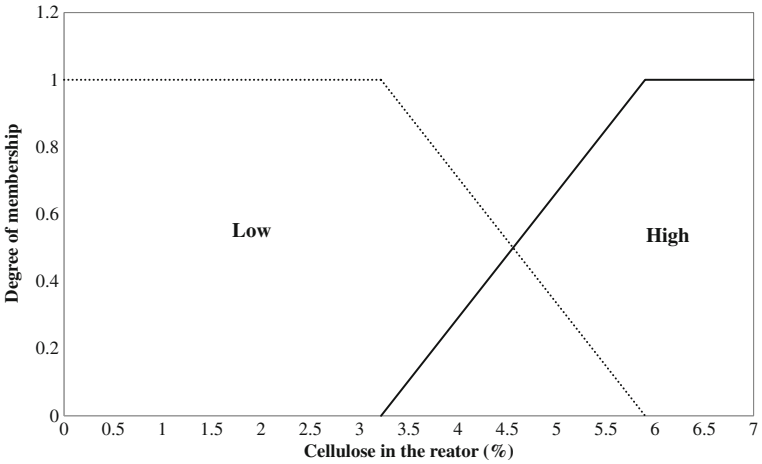

Fig. 6 Final membership functions to determine the degree of membership (for cellulose load)

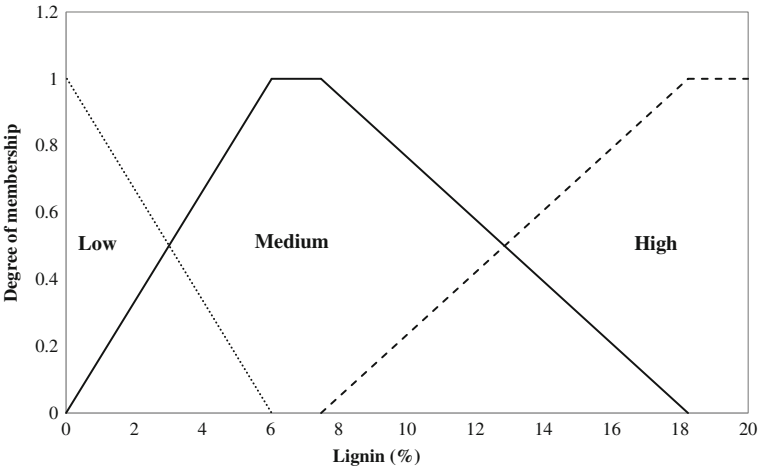

Fig. 7 Final membership functions to determine the degree of membership (for lignin content)

For input variable percentage of cellulose in the reactor, the left trapezoidal membership function in Fig. 6 was assigned the linguistic value "low load", and the function on the right was assigned the value "high load". For the input variable percentage of lignin (Fig. 7), in turn, the triangular function on the left was assigned the value "low lignin," the central trapezoidal region, "medium lignin", and the right, "high lignin."

With the equations of the straight lines (linear equations) associated with the linguistic values of the linguistic variables, a fuzzy inference system was generated representing the system behavior (rate of hydrolysis depending on the conditions of hydrolysis) using a minimum number of rules. These rules are:

Rule (1) If the percentage of cellulose is low or high and the percentage of lignin is low, the hydrolysis rate follows kinetic model 1.

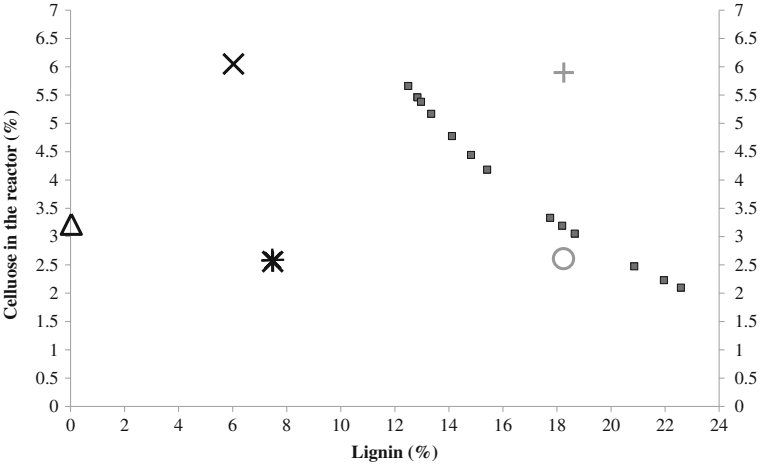

Fig. 8 Experimental data (percentage of cellulose in the reactor and percentage of lignin with regard to the substrate composition) for the validation experiment, in relation to cluster centers

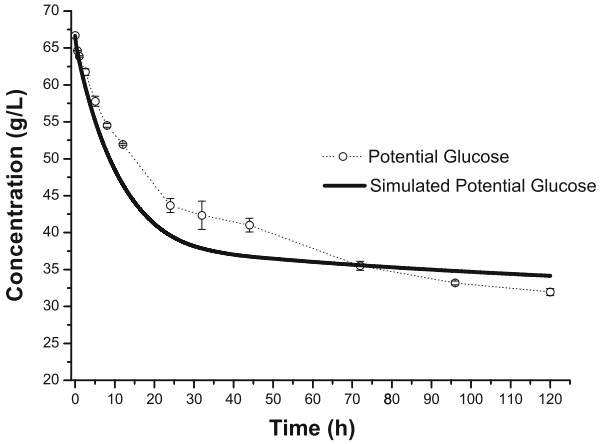

Fig. 9 Potential glucose concentration profiles. Comparison of experimental and simulated data for synthetic substrate. *Error bars* are standard errors of independent triplicate experiments. Operating conditions are 50 °C, pH 4.8, enzyme concentration of 1,200 FPU $L^{-1}_{solution}$, and initial potential glucose concentration of 66.66 $g_{potential\_glucose}$ $L^{-1}_{solution}$

Rule (2) If the percentage of cellulose is low and the percentage of lignin is medium, the hydrolysis rate follows kinetic model 2.

Rule (3) If the percentage of cellulose is high and the percentage of lignin is medium, the hydrolysis rate follows kinetic model 3.

Rule (4) If the percentage of cellulose is low and the percentage of lignin is high, the hydrolysis rate follows kinetic model 4.

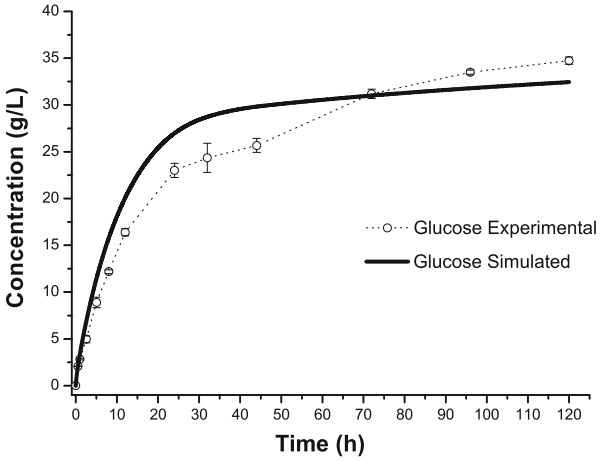

Fig. 10 Product concentration profiles. Comparison of experimental and simulated data for synthetic substrate. *Error bars* are standard errors of independent triplicate experiments. Operating conditions are 50 °C, pH 4.8, enzyme concentration of 1,200 FPU $L^{-1}_{solution}$ and initial potential glucose concentration of 66.66 $g_{potential\_glucose}$ $L^{-1}_{solution}$

Rule (5) If the percentage of cellulose is high and the percentage of lignin is high, the hydrolysis rate follows kinetic model 5.

In these rules, the kinetic models 1–5 are:

Kinetic model 1 Pseudohomogeneous Michaelis-Menten (MM) model, with inhibition studies originated with filter paper.
Kinetic model 2 Pseudo-homogeneous MM with competitive inhibition.
Kinetic model 3 Modified MM Model with inhibition.
Kinetic model 4 Chrastil model.
Kinetic model 5 Chrastil model b.

Validation Test

Figure 8 shows the experimental data for percentage of cellulose in the reactor and percentage of lignin with regard to the substrate composition for the validation experiment (in relation to cluster centers).

Figures 9 and 10 show the comparison of the "global" model predictions compared to experimental data of enzymatic hydrolysis of the synthetic (validation) substrate, which was not used in the processes of clustering and model building. The simulated responses shown in these figures, provided by the integrator, were built initially considering the relevance of models 2, 3, 4, and 5. With the progress of the experiment, the interpolator gently tended to consider mainly Chrastil model 4.

Figure 10 compares the glucose simulated by the fuzzy interpolator ("global" model) and glucose obtained experimentally for a total time of 120 h of hydrolysis. It can be seen that between 0 and 15 h, the interpolator represents the experimental data very well. However, within the interval between 15 and 45 h, the interpolator response does not adhere perfectly to the experimental data due to the higher standard error associated with the experimental measurements of glucose concentration within this interval. Finally, after 45 h, the experimental and simulated data increased gradually up to ~33 g L^{-1}, until hydrolysis is completed. A major advantage in using this interpolator is that retuning of the parameters of the simple "local" models is not necessary.

Conclusion

The presented results support the idea that a fuzzy interpolator for enzymatic hydrolysis of lignocellulosic materials can be an important modeling tool and a feasible methodology for representing the process in a robust and reliable way. Other important factor to be noticed is that this methodology can be applied for enzymatic hydrolysis of lignocellulosic materials different from sugar cane bagasse, such as soy hulls and sugar cane straw. After "local" kinetic models are adjusted and characterized for the lignocellulosic materials, it is possible to develop a fuzzy interpolator for kinetic modeling based on simple models for obtaining a "global" model covering a broad range of hydrolysis conditions.

Acknowledgments The authors would like to thank the support of Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP-BIOEN), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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