Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 22, No. 01, pp. 19 - 29, January - March, 2005

DETERMINATION OF INHIBITION IN THE ENZYMATIC HYDROLYSIS OF CELLOBIOSE USING HYBRID NEURAL MODELING

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(Received: June 15, 2003 ; Accepted: July 26, 2004)

Abstract - Neural networks and hybrid models were used to study substrate and product inhibition observed in the enzymatic hydrolysis of cellobiose at 40ºC, 50ºC and 55ºC, pH 4.8, using cellobiose solutions with or without the addition of exogenous glucose. Firstly, the initial velocity method and nonlinear fitting with Statistica® were used to determine the kinetic parameters for either the uncompetitive or the competitive substrate inhibition model at a negligible product concentration and cellobiose from 0.4 to 2.0 g/L. Secondly, for six different models of substrate and product inhibitions and data for low to high cellobiose conversions in a batch reactor, neural networks were used for fitting the product inhibition parameter to the mass balance equations derived for each model. The two models found to be best were: 1) noncompetitive inhibition by substrate and competitive by product and 2) uncompetitive inhibition by substrate and competitive by product; however, these models' correlation coefficients were quite close. To distinguish between them, hybrid models consisting of neural networks and first principle equations were used to select the best inhibition model based on the smallest norm observed, and the model with noncompetitive inhibition by substrate and competitive inhibition by product was shown to be the best predictor of cellobiose hydrolysis reactor behavior.
Key words: Neural networks; Enzymes; Modeling; Product inhibition; Substrate inhibition; Cellobiose.

INTRODUCTION

Because of its abundance and easy renovation, cellulosic waste has recently been receiving lately increasing attention as an alternative energy source and raw material for industries. Cellulose corresponds to about 38% of all agricultural waste and 45% of all municipal waste (Bezerra, 1995).

Cellulose is a linear polymer of D-anhydro-glucose units linked by 1,4-β-D-glucosidic bonds that can be hydrolyzed by cellulosic enzymes or by acids and bases. Cellulosic enzymes catalyze the cellulose hydrolysis at 40 to 50ºC without forming undesirable products. The enzyme system for the conversion of cellulose to glucose comprises endo-1,4-β-glucanase, cellobiohydrolase and β-glucosidase (also called cellobiase), which act sequentially and cooperatively to degrade crystalline cellulose to glucose. Cellobiase hydrolyzes the hydrolysis end dimmer, cellobiose, which as a rate-limiting factor is generally responsible for regulation of the entire cellulose hydrolysis process, because both endoglucanase and cellobiohydrolase activities are often inhibited by cellobiose. Thus, cellobiase produces glucose from cellobiose and also reduces cellobiose inhibition during cellulose hydrolysis, allowing the cellulolytic enzymes to function more efficiently (Saha et al., 1994). Therefore, there is a big incentive to develop suitable modeling for cellobiose hydrolysis that depends on reaction

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conditions (Aguado et al., 1995).

An important feature in the kinetics of the hydrolysis of cellobiose is the occurrence of substrate and product inhibition. Several alternative models have been used for this kinetics (e.g. Grous et al., 1985; Dekker, 1986; Woodward and Wohlpart, 1982).

The objective of our work was to use neural network modeling to determine which of a set of alternative inhibition models provides the best data fit. The proposal is founded on the capability of artificial neural networks to be used as universal function approximators, and the technique is appropriate because its model structure can be considered generic in the sense that a little knowledge of the system is required for its determination (Willis et al., 1990).

NEURAL NETWORKS AND HYBRID MODELING

Neural networks are parallel-distributed systems formed of several elements, known as neurons or nodes, placed in layers. Generally, neural network processing corresponds to a weighed sum of the neuron input values with their weights, which will be the argument of a function, usually the sigmoid or logistic function, whose value is the neuron's output.

When neural networks are used to model a system, this model is said to be a black-box model. It was given this name due to the absence of any type of information about the system. On the other hand, conventional modeling is known as white-box because it is based on equations that describe the system and demonstrate some of its characteristics. The gray-box or hybrid models are a combination of the black-box and white-box models. In this type of model, system equations and neural networks are used simultaneously to describe the behavior of the system. Generally, in these models, neural networks are employed to approximate the parameters of the first principles balance equations.

In process engineering, neural networks have a wide range of applications, such as process design and simulation, process supervision and control (Norgaard et al., 2000). Specifically, in relation to biotechnological processes, several studies can be found in the literature, such as the description of the α-amilase inactivation (Geeraerd et al., 1998), the prediction of the final concentration of ethanol in a batch fermentation process (Saucedo et at., 1994) and as a soft-sensor (McAvoy et al., 1992).

Recently, neural networks have frequently been used within hybrid models, which provide a better performance for interpolation and extrapolation properties (van Can et al., 1998). Hybrid models have already been applied to bioreactors (Tholudur and Ramirez, 1996; Chen et al., 2000; James et al., 2002; Patnaik, 2003), ethanol production by Sacharomyces cerevisae (Harada et al., 2000), and enzymatic production (Tholudur et al., 1996; Gonçalves, et al., 2002) and other processes.

In this work, neural networks were utilized to describe the behavior of the inhibition parameter ki. The networks had the following inputs: the kinetic parameters, km, V_{max} and ks, and initial substrate and product concentrations, S_0 and G_i. The response of the neural network, i.e., the ki parameter, was fed into the mass balance equations of different inhibition hydrolysis models for the batch reactor to estimate the substrate conversion.

Considering the available literature on cellobiose hydrolysis inhibition (Dekker, 1986; Grous et al., 1985; Woodward & Wohlpart, 1982; Gong et al., 1977), two types of substrate inhibition, namely uncompetitive and noncompetitive, and three types of product inhibition, namely competitive, uncompetitive and noncompetitive, were considered, yielding a combination of six inhibition models. Our goal was to determine which inhibition model best fits our data.

Low Hydrolysis Conversion Models

At the beginning of the cellobiose hydrolysis reaction, product concentration (glucose, G) is very low and only substrate inhibition needs to be taken into account. Therefore, under this condition, the number of inhibition model alternatives is reduced to two and the reaction rate equations can be written as (Calsavara, 1998)

- Model U: uncompetitive substrate inhibition with $G \rightarrow 0$:

$$V = \frac{S\, V_{max}}{S + km + \frac{S^2}{ks}} \tag{1}$$

- Model N: noncompetitive substrate inhibition with $G \rightarrow 0$:

$$V = \frac{S\, V_{max}}{S + km\left(1 + \frac{S}{ks}\right) + \frac{S^2}{ks}} \tag{2}$$

where S and ks are the substrate concentration and substrate inhibition constant, V is the initial reaction rate, V_{max} the maximum reaction rate and km the Michaelis-Menten constant.

Low to High Hydrolysis Conversion Models

For the whole range of hydrolysis conversions, low to high, both the substrate and the product inhibitions must be taken into account. A mass balance on a hydrolysis batch reactor considering each of the six inhibition model alternatives gives (Calsavara, 1998).

- Model UC: uncompetitive inhibition by substrate and competitive by product:

$$t=\left(\frac{S_0}{V_{max}}\right)\left(1+\frac{S_0}{ks}-2\frac{km}{ki}\right)X_A-\left(\frac{km}{V_{max}}\right)\left(1+\frac{G_i}{ki}+2\frac{S_0}{ki}\right)\ln(1-X_A)-\left(\frac{S_0^2}{2\,V_{max}\,ks}\right)X_A{}^2 \quad (3)$$

- Model UN: uncompetitive inhibition by substrate and noncompetitive by product:

$$t=\left(\frac{S_0}{V_{max}}\right)\left(1+\frac{G_i}{ki}+\frac{S_0}{ks}-2\frac{km}{ki}\right)X_A-\left(\frac{km}{V_{max}}\right)\left(1+\frac{G_i}{ki}+2\frac{S_0}{ki}\right)\ln(1-X_A)-\left(\frac{S_0^2}{V_{max}}\right)\left(\frac{1}{2}ks-\frac{1}{ki}\right)X_A{}^2 \quad (4)$$

- Model UU: uncompetitive inhibition by substrate and product:

$$t=\left(\frac{S_0}{V_{max}}\right)\left(1+\frac{G_i}{ki}+\frac{S_0}{ks}\right)X_A-\left(\frac{km}{V_{max}}\right)\ln(1-X_A)-\left(\frac{S_0^2}{V_{max}}\right)\left(\frac{1}{2}ks-\frac{1}{ki}\right)X_A{}^2 \quad (5)$$

- Model NC: noncompetitive inhibition by substrate and competitive by product:

$$t=\left(\frac{S_0}{V_{max}}\right)\left(1+\frac{km}{ks}+\frac{S_0}{ks}-2\frac{km}{ki}\right)X_A-\left(\frac{km}{V_{max}}\right)\left(1+\frac{G_i}{ki}+2\frac{S_0}{ki}\right)\ln(1-X_A)-\left(\frac{S_0^2}{2V_{max}\,ks}\right)X_A{}^2 \quad (6)$$

- Model NN: noncompetitive inhibition by substrate and product:

$$t=\left(\frac{S_0}{V_{max}}\right)\left(1+\frac{G_i}{ki}+\frac{S_0}{ks}+\frac{km}{ks}-2\frac{km}{ki}\right)X_A-\left(\frac{km}{V_{max}}\right)\left(1+\frac{G_i}{ki}+2\frac{S_0}{ki}\right)\ln(1-X_A)-\left(\frac{S_0^2}{V_{max}}\right)\left(\frac{1}{2}ks-\frac{1}{ki}\right)X_A{}^2 \quad (7)$$

- Model NU: noncompetitive inhibition by substrate and uncompetitive by product:

$$t=\left(\frac{S_0}{V_{max}}\right)\left(1+\frac{G_i}{ki}+\frac{S_0}{ks}+\frac{km}{ks}\right)X_A-\left(\frac{km}{V_{max}}\right)\ln(1-X_A)-\left(\frac{S_0^2}{V_{max}}\right)\left(\frac{1}{2}ks-\frac{1}{ki}\right)X_A{}^2 \quad (8)$$

where ki is the product inhibition parameter, t is the batch reaction time, X_A is the substrate conversion, S_0 is the substrate initial concentration and G_i is the product initial concentration.

MATERIALS AND METHODS

Experimental System

The neural network modeling technique was employed in the enzymatic hydrolysis of cellobiose, in which the enzyme β-glucosidase (EC 3.2.1.21), also known as cellobiase, hydrolyzes the β-glucosidic bond of cellobiose producing two molecules of glucose (Calsavara et al., 1999b).

The substrate was cellobiose from Sigma and it contained a very low level of glucose contamination (0.133% [w/w]). The enzyme was Novozym 188, a

β-glucosidase produced by the microorganism Aspergillus niger, containing 170 mg/mL of protein and a specific activity of 9.5 U/ mg protein. More details about the experimental data utilized in this work, the reactor system and the enzyme can be found in Calsavara (1998) and Calsavara et al (1999a, 1999b).

Two data clusters were used in this work. In the first group, which is associated with low conversions, the effect of substrate concentration on the initial rate of cellobiose hydrolysis at 40ºC, 50ºC and 55ºC, pH 4.8, was studied using cellobiose solutions from 0.4 to 2.0 g/L (1.2 to 58.5 M). The second group is associated with low to high cellobiose conversions and contains data on hydrolysis test runs in a batch reactor for up to very long reaction times under the experimental conditions shown in Table 1.

Table 1: Cellobiose hydrolysis conversion test conditions.

Test Condition	Temperature [ºC]	Initial Substrate Concentration S_0 [mM]	Initial Product Concentration G_i [mM]
01	40	5.85	0.0
02		5.85	10.0
03		58.5	0.0
04	50	5.85	0.0
05		5.85	10.0
06		58.5	0.0
07	55	5.85	0.0
08		5.85	10.0
09		58.5	0.0

Training and Validation Data for the Neural Network

The data group used for training and validation had 1198 cases, separated randomly into 1098 training cases and 100 validation cases, as recommended by Statistica Neural Networks (SNN)® (StatSoft Inc.). This data group was generated using Equations (3) to (8), having as input the concentrations S_0 and G_i under each test condition, and the values of the parameters km, ks and V_{max}, determined as described below. The output variable of the training data was the product inhibition parameter, ki.

Neural Network Creation and Training

The architecture and weights of the neural networks built for each hybrid model were determined with the software Statistica Neural Networks (SNN)® (StatSoft Inc.). This software determines the best architecture for a neural network by conducting preliminary training in different architectures and comparing their performances. All of the neural networks were multilayer perceptrons (MLP) with one hidden layer. The activation function used in the first layer was linear, while for the hidden and outer layers the logistic (or sigmoid) function was used. The number of hidden neurons was determined by SNN.

Four training algorithms were utilized: back propagation, quasi-Newton, Levenberg-Marquardt and conjugate gradients. All six networks were trained with the four algorithms. Thus, the difference between the six neural networks developed consisted in the number of neurons in the hidden layer and in the selected training algorithm.

RESULTS AND DISCUSSION

Determination of the Kinetic Parameters

From the first data group, on the initial hydrolysis reaction rate (V) vs. cellobiose concentration (S_0), the Michaelis-Menten (km) and substrate inhibition (ks) constants and also the maximum rate of reaction (V_{max}) can be determined. These data were obtained using the initial velocity method (Dixon and Web, 1979) when the product concentration in the reaction medium is negligible ($G \rightarrow 0$). The reaction rate can be described by Equations (1) and (2) converted into their parabolic form,

- Model U: uncompetitive substrate inhibition with $G \rightarrow 0$:

$$\frac{S}{V} = \frac{km}{V_{max}} + \left(\frac{1}{V_{max}}\right)S + \left(\frac{1}{ks\ V_{max}}\right)S^2 \qquad (9)$$

▪ Model N: noncompetitive substrate inhibition with $G \rightarrow 0$:

$$\frac{S}{V} = \frac{km}{V_{max}} + \left(\frac{1}{V_{max}}\right)\left(1 + \frac{km}{ks}\right)S + \left(\frac{1}{ks\ V_{max}}\right)S^2 \quad (10)$$

The parabola (Equation 11) was fit to the first group data using the software Statistica®(StatSoft Inc.). From the value of the coefficients a, b and c, the kinetic constants were determined for the two kinds of inhibition (Equations 9 and 10). Figure 1 shows the good fitting obtained for the experimental data at all the temperatures tested.

$$\frac{S}{V} = a + b\,S + c\,S^2 \quad (11)$$

Table 2 shows the values of the kinetic constants determined by this work and compares them with some values found in the literature. Our results shown in Table 2 are of the same magnitude as those obtained by Grous et al. (1985) and Dekker (1986).

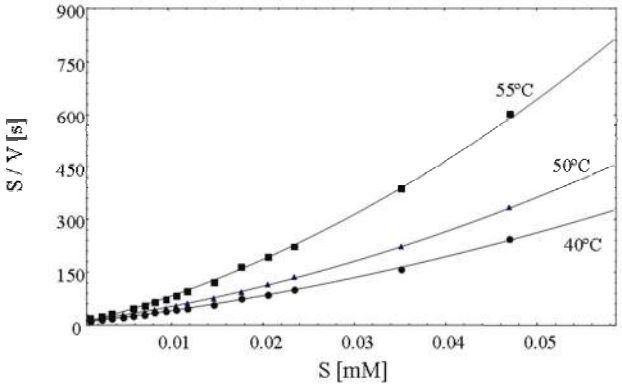

Figure 1: Fitting of Equation (11) to the data on the initial rate of cellobiose hydrolysis under the conditions shown in Table 1 to determine the kinetic parameters: Michaelis-Menten constant (km), maximum rate of reaction (V_{max}) and substrate inhibition constant (ks).

Table 2: Kinetic parameters calculated by fitting the substrate inhibition models, Equations (9) and (10), to data on the initial rate of the enzymatic hydrolysis of cellobiose.

Author		Temperature [ºC]	Km [mM]	V_{max} [μmol glucose s^{-1} mg protein^{-1}]	ks [mM]
This work	Model U: Uncompetitive Substrate Inhibition	40	0.683	0.1582	52.84
		50	2.43	0.2823	54.48
		55	2.31	0.3498	70.05
	Model N: Noncompetitive Substrate Inhibition	40	0.692	0.1603	52.14
		50	2.55	0.2962	51.93
		55	2.39	0.3622	67.66
Dekker (1986)		50	5.63	0.5623	-
Grous et al. (1985)		50	1.66	-	43.4

Determination of the Product Inhibition Parameter

After determining the value of the parameters km, ks and V_{max}, ki can be obtained from the second experimental data group, which is on low to high cellobiose hydrolysis conversion (X_A) vs. batch reaction time (t), obtained at 40, 50 and 55ºC. In this work, the parameter ki was fit as a function of the reaction temperature and initial substrate concentration, working accordance with (Calsavara et al., 1999b; Grous et al., 1985). The software Statistica®(StatSoft Inc.) was used to fit the hydrolysis inhibition model Equations (3) to (8) to the data at each temperature, and the estimated parameter values are shown in Table 3.

The models that gave the best fit were: 1) uncompetitive inhibition by substrate and

competitive by product (UC), and 2) noncompetitive inhibition by substrate and competitive by product (NC), whereas for some models, the experimental data could not be fit and this was highlighted by a horizontal bar (-) placed at the R^2 data entry. The calculated values for the product inhibition parameter, ki, are shown in Table 3. It can also be observed that the correlation coefficients obtained for these two models (UC and NC) are quite close. This result makes the choice between these two models more difficult, and the neural hybrid models will be applied in the next section to discriminate between the two best-fit models.

Still, according to the quality of fitting observed, some considerations, such as the following, can be made:

- Product inhibition has more influence on the kinetics of cellobiose hydrolysis; this is confirmed by the similar results shown in Table 3 for the three product inhibition hypothesis independently of the substrate inhibition hypothesis considered.
- Of the three product inhibition types considered, the competitive one seems to be the most adequate, followed by uncompetitive inhibition.

Figures 2 to 4 show the experimental data and fitting results for the NC model (noncompetitive inhibition by substrate and competitive by product). The UC model (uncompetitive inhibition by substrate and competitive by product) gave similar results.

Table 3: Correlation coefficients (R^2) and ki values calculated by fitting the inhibition models, Equations (3) to (8), to the cellobiose hydrolysis batch conversion data under the experimental conditions given in Table 1.

Test Conditions	Inhibition Model											
	UC*		UU*		UN*		NC*		NU*		NN*	
	R^2	ki	R^2	ki	R^2	ki	R^2	ki	R^2	ki	R^2	ki
01	0.9837	2.0650	0.9645	0.5322	0.6667	0.8100	0.9827	2.0417	0.9644	0.5378	0.6778	0.8222
02	0.9858	1.2444	0.6647	0.9283	0.9122	1.2606	0.9855	1.2400	0.6733	0.9339	0.9155	1.2728
03	0.9879	0.8750	0.9759	0.6022	0.9666	0.6228	0.9841	0.8022	0.9759	0.6094	0.9079	0.6311
04	0.9937	11.4100	0.9753	0.5500	-	-	0.9936	11.3367	0.9753	0.5728	-	-
05	0.9614	6.8361	0.6849	0.9228	0.4755	2.6372	0.9612	6.8267	0.6762	0.9450	0.5444	2.7872
06	0.9953	2.8200	0.9536	0.5850	0.2995	0.6628	0.9947	2.7328	0.9536	0.6128	0.3821	0.6856
07	0.9443	13.1594	0.9658	0.4361	-	-	0.9463	13.3994	0.9658	4.4964	-	-
08	0.7571	10.1706	0.4263	0.7800	-	-	0.7595	10.0683	0.4731	2.6283	-	-
09	0.8273	4.8450	0.9434	0.4544	-	-	0.8471	5.0900	0.9434	0.4694	-	-

*UC: uncompetitive substrate and competitive product inhibition; UN: uncompetitive substrate and noncompetitive product inhibition; UU: uncompetitive substrate and product inhibition; NU: noncompetitive substrate and uncompetitive product inhibition; NC: noncompetitive substrate and competitive product inhibition; NN: noncompetitive substrate and product inhibition

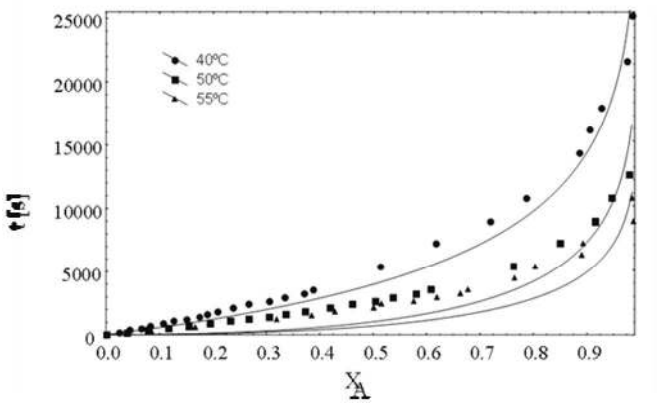

Figure 2: Fitting of the NC model (noncompetitive inhibition by substrate, and competitive by product), Equation (6), to determine ki with S_0 = 5.85 mM and G_i = 0 mM for the three test temperatures.

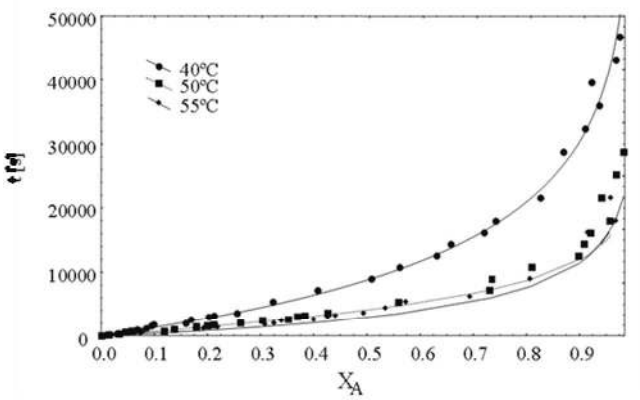

Figure 3: Fitting of the NC model (noncompetitive inhibition by substrate and competitive by product), Equation (6), to determine ki with S_0 = 5.85 mM and G_i = 10.0 mM for the three test temperatures.

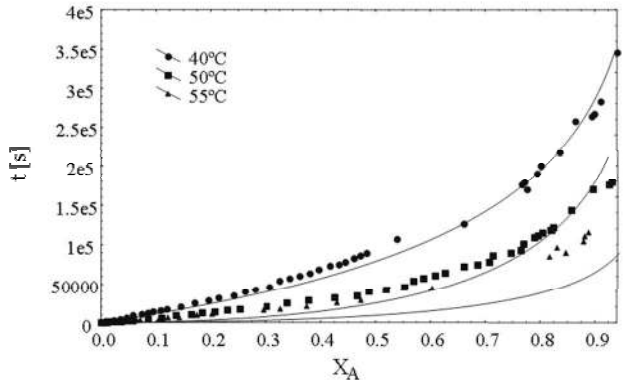

Figure 4: Fitting of the NC model (noncompetitive inhibition by substrate and competitive by product), Equation (6), to determine ki with S_0 = 58.5 mM and G_i = 0 mM for the three test temperatures.

Hybrid Neural Modeling

Six hybrid models were built, one for each combination of substrate and product inhibition hypothesis, for the purpose of comparison. The aim was to use the ability of the neural networks to verify which hybrid model would reproduce the batch reactor data most closely. As mentioned above, hybrid models were composed of a neural network and one of the mass balance equations (Equations (3) to (8)). The inputs and outputs used for these networks were the same as those above and the ki output was also fed to the model equations.

To compare the six hybrid models, the maximum norm was used. According to Willianson et al. (1972), the maximum norm can be defined, for any $\mathbf{x} = (x_1,...,x_n)$, by

$$\|\mathbf{x}\| = \max\{|x_1|,...,|x_2|\} \tag{12}$$

As a valuable tool for error analysis, maximum norms are recommended for measuring error in vectors and matrices (Higham, 1986; Anderson et al., 1995). The maximum norm defined by the relation $\|\mathbf{x} - \hat{\mathbf{x}}\| = \max\{|x_1 - \hat{x}_1|,...,|x_2 - \hat{x}_2|\}$ was calculated for each one of the six models for every experimental curve. It measures the maximum absolute discrepancy between the experimental and predicted values, and therefore we should look for the model that gives the lowest maximum norm. In a probabilistic approach the maximum norm is interpreted as a "worst case performance" (Maiorov and Wasilkowski, 1994). The predicted behaviors for the six models were very different, allowing establishment of the most appropriate inhibition model for the enzymatic hydrolysis of cellobiose. Table 4 shows the maximum norm values for every model under all hydrolysis conditions. It can be observed that for any experimental curve, the lowest average value of the calculated maximum norm occurred for the NC model (noncompetitive substrate inhibition and competitive product inhibition).

The trend in model performance observed when ki was determined was also seen with the hybrid models. The hypotheses of noncompetitive inhibition by substrate and competitive by product (NC) and

uncompetitive inhibition by substrate and competitive by product (UC) had the best results. But, using the maximum norm criterion, the model that considers noncompetitive inhibition by substrate and competitive by product (NC model) clearly stands out.

Figures 5 to 7 compare the predicted behavior of the NC model with the cellobiose hydrolysis experimental data, and Figures 8 to 10 do the same for the UC model.

Table 4: Maximum norm values calculated by fitting the inhibition models, Equations (3) to (8), to the cellobiose hydrolysis batch conversion data under the experimental conditions given in Table 1.

Maximum norm values						
Test Conditions	**Inhibition Model**					
	UU*	**UN***	**UC***	**NU***	**NN***	**NC***
01	0.3186	0.1737	0.1693	0.2267	0.2203	0.0526
02	0.2899	0.3105	0.3605	0.4483	0.7649	0.0595
03	0.7662	0.1100	0.0679	0.0299	0.1358	0.0461
04	0.3427	0.1940	0.0160	0.6971	0.0490	0.0143
05	0.3832	0.2603	0.0762	0.3541	0.5572	0.0222
06	0.4685	0.1695	0.0295	0.3005	0.7761	0.0283
07	0.3874	0.1154	0.058	0.7442	0.2460	0.0351
08	0.3553	0.3103	0.2999	0.3506	0.6287	0.0265
09	0.3139	0.2742	0.0398	0.5716	0.8539	0.0348
Arithmetic Mean	0.4029	0.2131	0.1112	0.4137	0.4702	0.0485

*UC: uncompetitive substrate and competitive product inhibition; UN: uncompetitive substrate and noncompetitive product inhibition; UU: uncompetitive substrate and product inhibition; NU: noncompetitive substrate and uncompetitive product inhibition; NC: noncompetitive substrate and competitive product inhibition; NN: noncompetitive substrate and product inhibition

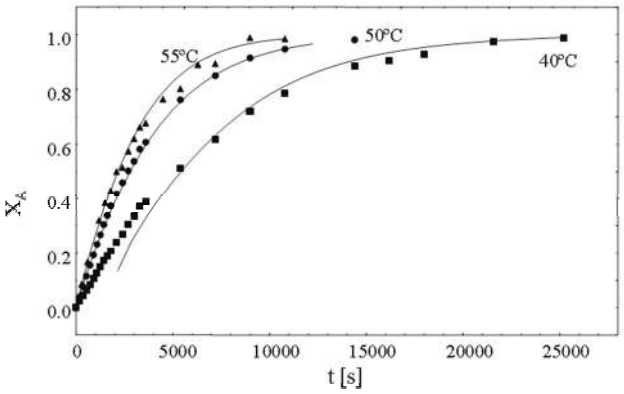

Figure 5: Predicted vs. experimental cellobiose hydrolysis conversions observed with the NC model (noncompetitive inhibition by substrate and competitive by product), Equation (6), with S_0 = 5.85 mM and G_i = 0 mM for the three test temperatures

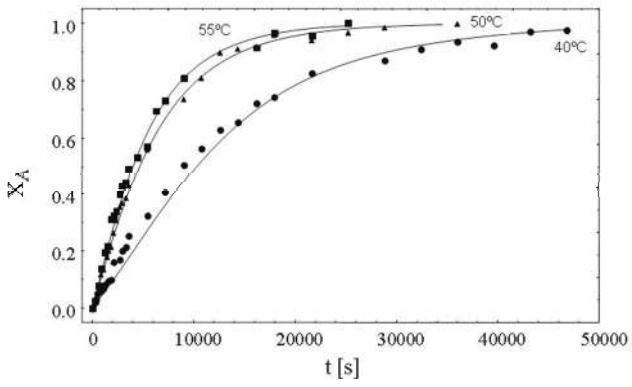

Figure 6:Predicted vs. experimental cellobiose hydrolysis conversions observed with the NC model (noncompetitive inhibition by substrate and competitive by product), Equation (6), with S_0 = 5.85 mM and G_i = 10.0 mM for the three test temperatures.

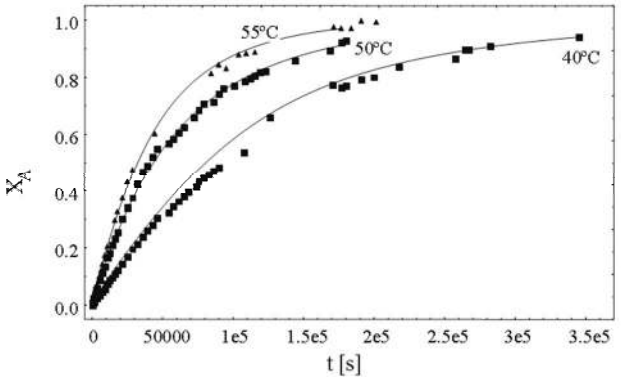

Figure 7: Predicted vs. experimental cellobiose hydrolysis conversions observed with the NC model (noncompetitive inhibition by substrate and competitive by product), Equation (6), with S_0 = 58.5 mM and G_i = 0 mM for the three test temperatures.

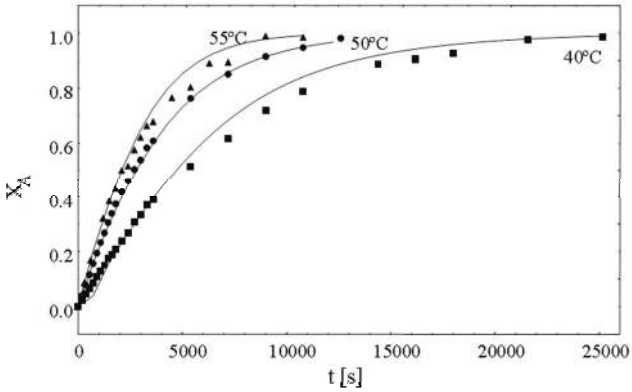

Figure 8: Predicted vs. experimental cellobiose hydrolysis conversions observed with the UC model (uncompetitive inhibition by substrate and competitive by product), Equation (3), with S_0 = 5.85 mM and G_i = 0 mM for the three test temperatures.

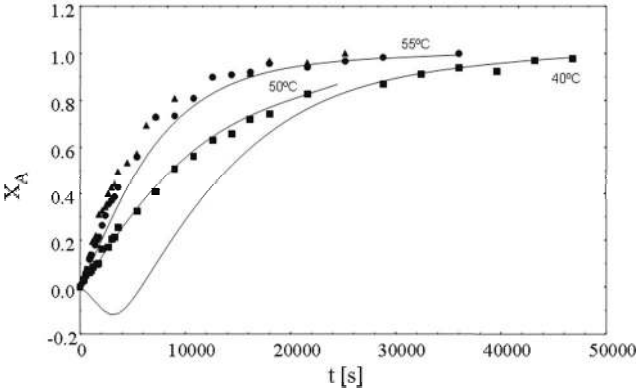

Figure 9: Predicted vs. experimental cellobiose hydrolysis conversions observed with the UC model (uncompetitive inhibition by substrate and competitive by product), Equation (3), with S_0 = 5.85 mM and G_i = 10.0 mM for the three test temperatures.

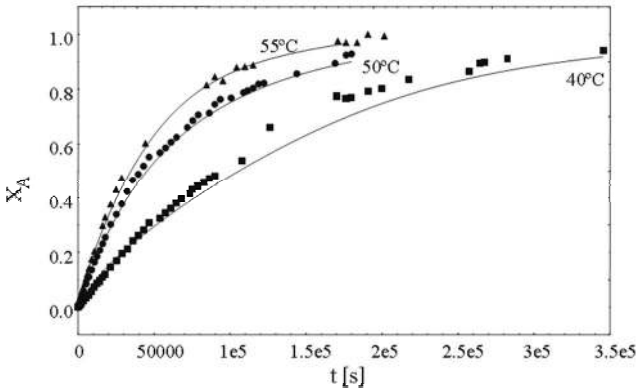

Figure 10: Predicted vs. experimental cellobiose hydrolysis conversions observed with the UC model (uncompetitive inhibition by substrate and competitive by product), Equation (3), with S_0 = 58.5 mM and G_i = 0 mM for the three test temperatures.

CONCLUSIONS

Kinetic parameters were determined for the enzymatic hydrolysis of cellobiose by the initial velocity method, at a negligible product concentration, using either the uncompetitive or the competitive model for substrate inhibition, and the results gave a good fit to the experimental data for both models.

Six models were tested for different combinations of the substrate and product inhibition hypotheses for the enzymatic hydrolysis of cellobiose at low to high conversions in a batch reactor. Through the fitting of the mass balance equations derived for each model, the parameter ki was estimated. The quality of the fitting was different for each model, and the models that considered either noncompetitive inhibition by substrate and competitive by product, or uncompetitive inhibition by substrate and competitive by product showed the highest correlation coefficients (R^2).

The six models were then tested using hybrid models. Six hybrid models were used with the same inhibition hypotheses as above, and the ability of the neural networks in approximating functions was used advantageously with the hybrid models, allowing selection of the best inhibition model based on the smallest norm observed. The model that best

predicted reactor behavior with the hybrid models was the noncompetitive inhibition by substrate and competitive by product model.

ACKNOWLEDGMENTS

We would like to thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior) for the financial support received.

NOMENCLATURE

G	product (glucose) concentration in the reaction medium	[mM]
G_i	product initial concentration	[mM]
ki	product inhibition parameter	[mM]
km	Michaelis-Menten constant	[mM]
ks	substrate inhibition constant	[mM]
NC	noncompetitive substrate and competitive product inhibition	(-)
NN	noncompetitive substrate and product inhibition	(-)
NU	noncompetitive substrate and uncompetitive product inhibition	(-)
S	substrate concentration in the reaction medium	[mM]
SNN®	Statistica Neural Network (StatSoft Inc.)	(-)
S_0	Initial substrate concentration	[mM]
t	batch reaction time	[min]
T	temperature	[ºC]
V	initial reaction rate	[μmol L^{-1} min^{-1}]
V_{max}	maximum rate of reaction	[μmol glucose L^{-1} min^{-1} mg $protein^{-1}$]
X_A	substrate conversion,	$X_A = 1 - S / S_0$
UU	uncompetitive substrate and product inhibition	(-)
UC	uncompetitive substrate and competitive product inhibition	(-)
UN	uncompetitive substrate and noncompetitive product inhibition	(-)

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