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Kinetic Modelling for Enzymatic Hydrolysis of Pre-treated Sugarcane Straw

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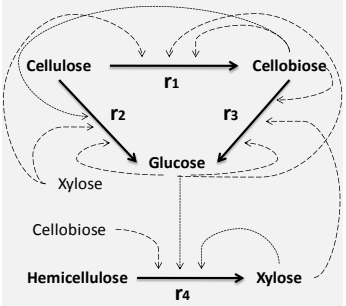

A semimechanistic kinetic model has been used to describe the enzymatic hydrolysis of pre-treated sugarcane straw. This model considers one homogeneous reaction of cellobiose to glucose and two heterogeneous reactions of cellulose to cellobiose and cellulose to glucose. The Langmuir isotherm was used to model the enzyme adsorption on substrate. The competitive enzyme inhibition by the products and the conversion of hemicellulose to xylose were also incorporated in the model. Experimental data were used for both parameters estimation and model verification. The model was found to have the ability to predict with good accuracy the concentration of hydrolysis products. A model parameters simplification proved to be a good option to overcome the parametric correlation reported in the literature for this type of model, without affecting the prediction accuracy.

Introduction

Mathematical modelling of enzymatic hydrolysis of lignocellulosic biomass is a challenging engineering topic and this is reflected in the large number of proposed models [1]. Multiple reactions in a heterogeneous system are carried out during enzymatic hydrolysis. Insoluble cellulose is initially degraded at the solid-liquid interface by the synergistic enzymatic action of cellulases, followed by the hydrolysis of soluble intermediates (cellobiose and short-chain oligosaccharides) in the liquid phase by β-glucosidase [2].

The hydrolysis depends on enzymatic characteristics such as: (1) adsorption on the substrate; (2) inhibition by the products; (3) synergism, and (4) mass transfer limitations affecting transport of the enzyme to the substrate. Substrate characteristics including composition and distribution of components (i.e. lignin, hemicellulose, fats and proteins), particle size, and crystallinity also affect the hydrolysis. Incorporating all these factors into a single model is highly complicated [3].

Different semimechanistic models have been proposed in the literature to describe the enzymatic hydrolysis of pre-treated biomass [1-2]. Recently, Kadam *et al.* (2004) [4], developed and validated a kinetic model for batch enzymatic hydrolysis of diluted acid pre-treated corn stover. This model considers one homogeneous reaction of cellobiose to glucose and two heterogeneous reactions of cellulose to cellobiose and to glucose. The enzyme adsorption was incorporated by a Langmuir-type adsorption isotherm, competitive end-product inhibition, and substrate reactivity, without including the enzyme inactivation. An extension of this model was proposed by Câmara (2012) [5] to take into account both the formation and inhibition by xylose, according to Figure 1.

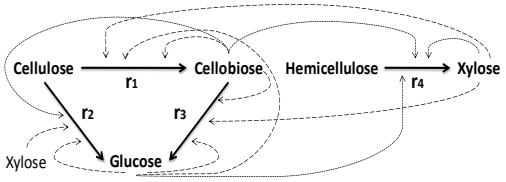

Figure 1. Reaction Scheme for modelling the hydrolysis of lignocellulose. Cellulases (EG and CBH) are involved in r_1 and r_2, β-glucosidase in r_3, and hemicellulases in r_4. Dashed and dotted lines show the sugars inhibition on enzymes. Both r_4 and dotted line were adopted by [5], modified from [4].

The model proposed by Câmara (2012) (see Table 1) was chosen to describe the enzymatic hydrolysis of pre-treated sugarcane straw (PSS), since the original model has been verified experimentally [6,7], analyzed statistically [8] and has also been used in the enzymatic hydrolysis modelling of sugarcane bagasse [5].

Materials and Methods

The experimental data for parameters estimation and model verification were generated using hydrothermal PSS at 195ºC for 10 min [9]. The commercial enzymatic complex Cellic CTec2 (203 FPU/ml, 36 mg-protein/ml; Novozymes, Araucária, PR, Brazil) was used in the experiments without β-glucosidase complementation. Other conditions are listed in Table 2.

Table 1. Kinetic model for lignocellulose hydrolysis.

Langmuir-type isotherm	$E_{BS} = \frac{E_B}{S} = \frac{E_{\max} K_{ad} E_F}{1 + K_{ad} E_F}$	(1)
Enzyme adsorbed on cellulose	$E_{BC} = E_B \frac{C}{S}$	(2)
Enzyme adsorbed on hemicellulose	$E_{BH} = E_B \frac{H}{S}$	(3)
Total enzyme	$E_T = E_F + E_B$	(4)
Total solids	$S = C + H + L$	(5)
Substrate reactivity	$R_s = \alpha \frac{S}{S_0}$	(6)
Cellulose (C) to cellobiose (G2)	$r_1 = \frac{k_{1r} E_{BC} R_S S}{1 + \frac{G2}{K_{1IG2}} + \frac{G}{K_{1IG}} + \frac{X}{K_{1IX}}}$	(7)
Cellulose (C) to glucose (G)	$r_2 = \frac{k_{2r} E_{BC} R_S S}{1 + \frac{G2}{K_{2IG2}} + \frac{G}{K_{2IG}} + \frac{X}{K_{2IX}}}$	(8)
Cellobiose (G2) to glucose (G)	$r_3 = \frac{k_{3r} E_F G2}{K_{3M}\left(1 + \frac{G}{K_{3IG}} + \frac{X}{K_{3IX}}\right) + G2}$	(9)
Hemicellulose (H) to xylose (X)	$r_4 = \frac{k_{4r} E_{BH} R_S S}{1 + \frac{G2}{K_{4IG2}} + \frac{G}{K_{4IG}} + \frac{X}{K_{4IX}}}$	(10)
Cellulose balance	$\frac{dC}{dt} = -r_1 - r_2$	(11)
Cellobiose balance	$\frac{dG2}{dt} = 1.056 r_1 - r_3$	(12)
Glucose balance	$\frac{dG}{dt} = 1.111 r_2 + 1.053 r_3$	(13)
Hemicellulose balance	$\frac{dH}{dt} = -r_4$	(14)
Xylose balance	$\frac{dX}{dt} = 1.136 r_4$	(15)

Liquid supernatant samples were collected at sampling time = 1, 2, 6, 12, 24, 48 and 72h for measuring glucose, cellobiose and xylose concentration by HPLC. The protein concentration in the solution was measured by the Bradford protein assay. Experiments for model verification were conducted with different enzyme loading (5-60 FPU/g-cellulose), dry solid loadings (10-20% w/v) and background sugars concentrations, including glucose (30 and 60 g/l), cellobiose (10 g/l) and xylose (10 g/l); experimental conditions outside the range of the reference condition were carried out to validate the model predictions and to assess the model fidelity in describing the sugar inhibition problem.

All experiments were performed by the Laboratory of Bioprocess Development and Automation (LaDABio) at the Universidade Federal de São Carlos (UFSCar).

Computational Methodology. The EMSO software [10] was used to estimate both adsorption and kinetic parameters. The type-Langmuir isotherm parameters were estimated independently from experimental data of protein in the supernatant for the hydrolysis with different enzymatic loading according to Table 2. The substrate reactivity constant (α) was assumed as unit according to the value reported in the literature for similar substrate [5].

Results and Discussion

Parameters were estimated minimizing the Weighted Least Squares (WLS) function using the flexible polyhedron method. The two types of parameters estimated: adsorption and kinetic parameters are showed in Table 3.

This set of parameters provided a good fit to the data according to Figure 2. However it was not possible to estimate the significance, the confidence interval (CI), and the correlation matrix of the parameters due to the strong correlation between them, which generated ill-conditioning of the Fisher information matrix. Other studies [8] have founded that all parameters have too large CI, this means that the parameters are statistically unidentifiable and a likely model overfitting was achieved.

Table 2. Experimental conditions for the development and verification of the kinetic model[a].

Experimental conditions	Experiments					
	Reference condition	Verification conditions				
		Enzyme loading	Solid loading	Initial glucose	Initial cellobiose	Initial xylose
Enzyme loading (FPU/g-celulose)	10	5,15,20,25, 30,60	10	10	10	10
Solid loading (%w/v)	15	15	10,20	15	15	15
Initial glucose (g/l)	0	0	0	30,60	0	0
Initial cellobiose (g/l)	0	0	0	0	10	0
Initial xylose (g/l)	0	0	0	0	0	10

[a] Temperature, pH, shaking speed, and hydrolysis time were kept constants at 50ºC, 4.8, 250 rpm and 72 h, respectively.

Based on fundamental of enzymatic kinetics, a simplification to the model was proposed to overcoming the parametric correlation. Since the enzyme complex used was modelled as a pseudo-

enzyme with multiple activities, the inhibition constants of the kinetic reactions occurring in the solid-liquid interface (r_1, r_2 and r_4) K_{iIG2}, K_{iIG} and K_{iIX} ($i=1,2$ and 4), could be replaced by a set of inhibition constants (K_{IG2}, K_{IG} and K_{IX}) representing the overall effect of product inhibition on the pseudo-enzyme activities. The estimated parameters for the simplified model and the model fit under the estimation conditions are showed in Table 3 and Figure 3, respectively.

Table 3. Estimated Model Parameters

parameter	value		
Adsorption parameters			
Kad (l/g)	7.16		
Emax (g/kg)	8.32		
Kinetic parameters obtained from saccharification data			
	Original Model	Simplified Model	All data*
k_{1r} (l/g/h)	0.028	0.104	0.015
k_{2r} (l/g/h)	4.78	2.76	0.548
k_{3r} (1/h)	187.8	143.2	170.9
k_{4r} (l/g/h)	15.66	21.42	6.85
K_{1IG2} (l/g)	0.545	1.98	6.02
K_{1IG} (l/g)	2.93	0.546	3.57
K_{1IX} (l/g)	5.07	1.60	5.45
K_{2IG2} (l/g)	117.2		
K_{2IG} (l/g)	0.329		
K_{2IX} (l/g)	0.325		
K_{3M} (l/g)	25.50	45.49	45.6
K_{3IG} (l/g)	0.216	0.409	0.032
K_{3IX} (l/g)	59.07	73.10	79.42
K_{4IG2} (l/g)	20.68		
K_{4IG} (l/g)	0.728		
K_{4IX} (l/g)	196.4		

*Parameters estimated for the simplified model with all available experimental data.

As shown in Figures 2 and 3, the model fit was not affected by the model simplification, even for all verification conditions (data not shown). Significance, CI and correlation matrix were evaluated with the simplified model.

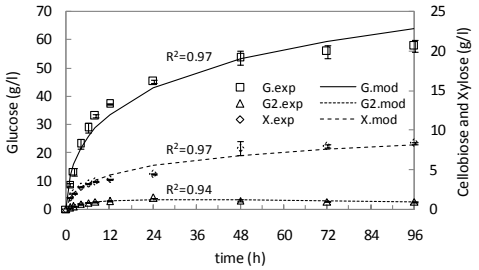

Figure 2. Enzymatic hydrolysis of pre-treated PSS under estimation conditions using all model parameters.

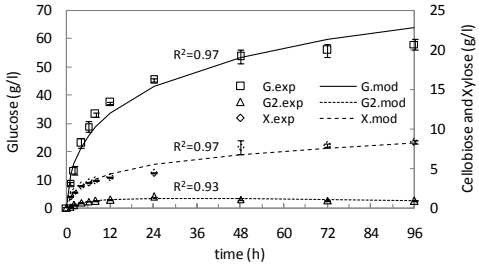

Figure 3. Enzymatic hydrolysis of pre-treated PSS under estimation conditions using simplified model.

Another parameter estimation including all available experimental data (Table 3), improved the significance and CI of the parameters, and reduced the correlation in 50%. Despite some difficulties to predict cellobiose concentration at enzyme loading as high as 60 FPU/g-cellulose, the model predicted with good accuracy (R^2>0.86) the glucose concentration under all different verification conditions of solids, enzyme and initial sugars loading.

Acknowledgements
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