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VALIDATION OF INHIBITION EFECTS IN THE CELLULOSE HYDROLYSIS: A DYNAMIC MODELLING APPROACH

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Abstract
Enzymatic hydrolysis is one of the main steps in the processing of bioethanol from lignocellulosic raw materials. However, complete understanding of the underlying phenomena is still under development. Hence, this study has focused on validation of the inhibition effects in the cellulosic biomass hydrolysis employing a dynamic mathematical model. A systematic framework for parameter estimation is used for model validation, which helps overcome the problem of parameter correlation. Data sets obtained from carefully designed enzymatic cellulose and cellobiose hydrolysis experiments, were used for parameter estimation (calibration) and validation purposes. The model predictions using calibrated parameters have shown good agreement with the validation data sets, which provides credibility to the model structure and the parameter values.

INTRODUCTION

Production of bioethanol from agricultural waste such as lignocellulosic biomass (i.e. grass, wood, trees among others) forms an alternative renewable energy resource. The process of converting biomass to ethanol is complex with many unit operations including the pretreatment, enzymatic hydrolysis, fermentation of sugars and downstream processes (see Figure 1). Here, the physical pre-treatment and the enzymatic hydrolysis of lignocellulosic material are key steps to release simple sugar molecules, which are subsequently converted into ethanol. However, enzymatic hydrolysis is still facing some problems in terms of production cost, due to specific enzyme and processing issues. Therefore, one of the main challenges is obtaining a significant reduction of the cellulase enzyme cost, which can be achieved by improving enzyme characterization (Rodriguez-Gomez et al., 2011), purification and mutation (via protein engineering). From an enzymatic hydrolysis process point of view, it is nevertheless also necessary to make progress on reactor operation (Hodge et al., 2009), for example by a combination of optimization of reactor conditions, implementation of improved control strategies for enzyme and substrate dosage (Morales-Rodriguez et al., 2010) and enzyme recovery. To our opinion, such efforts should be supported by the use of a mathematical model, where simulated experiments with this model have the advantage of being cheaper in terms of cost, time and resource consumption, when compared with the purely experimental approach.

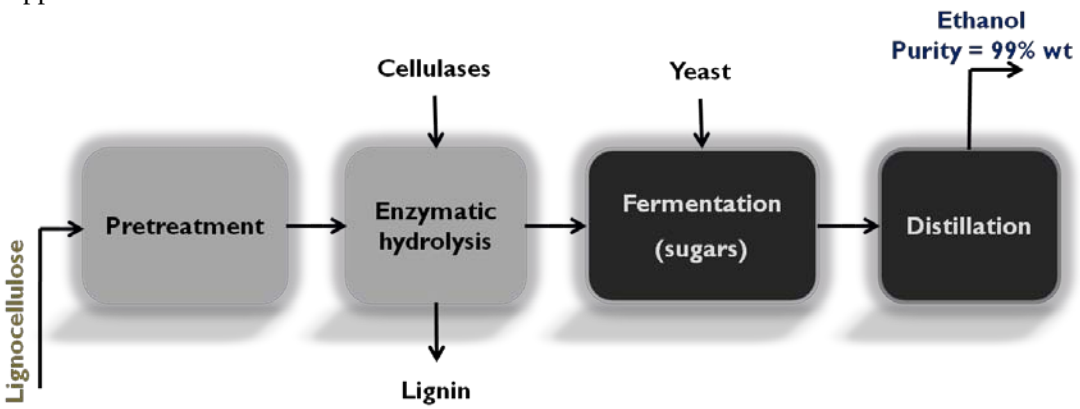

Figure 1 Bioethanol production process from lignocellulosic raw materials.

The mechanism of converting insoluble polymeric substrate into soluble sugars by the action of cellulase enzymes has not yet been completely understood due to the complexity of the involved phenomena (such as, adsorption, desorption, enzyme deactivation, etc.). Nevertheless, a number of mathematical models for enzymatic hydrolysis have been proposed in the literature. However, only few of those models have been independently subjected to experimental validation.

Bansal et al. (2009) made a review where a collection of diverse mathematical models for enzymatic hydrolysis was presented. The models were classified as follows: i) empirical models; ii) Michaelis-Menten based models; iii) Models accounting for soluble substrate; and iv) models developed for soluble substrates.

Among the published mathematical models, this study has selected the mathematical model proposed by Kadam et al. (2004) that is classified as a Michaelis-Menten based model that also takes enzyme adsorption and product inhibition into account. This mathematical model describes the conversion of cellulose to cellobiose (r_1), the conversion of cellulose to glucose (r_2) and the conversion of cellobiose to glucose (r_3) (see Figure 2). The mathematical model is based on a number of assumptions such as: a) amorphous and crystalline cellulose are lumped and uniform, b) enzyme activity remains constant throughout the reaction, c) enzyme adsorption is described via Langmuir kinetics, d) considers separate endoglucanases (EG), cellobiohydrolases (CBH) and β-glucosidases (BG) activities on cellulose breakdown and competitive inhibition by simple sugars.

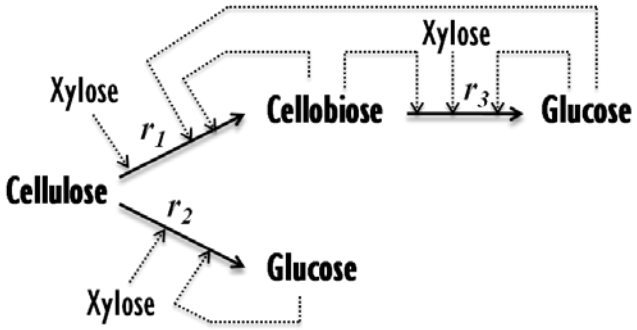

Figure 2 Reaction scheme for modelling cellulose hydrolysis. Enzyme involved in r_1: EG and CBH. Enzyme involved in r_2: CBH and BG. Enzyme involved in r_3: BG (Adapted from Kadam et al., 2004).

This study aims to validate the dynamic mathematical model for cellulosic biomass hydrolysis with particular focus on the validation of the hydrolysis and product inhibition mechanisms. For the experimental setup (Tsai et al., 2011), Avicel® and cellobiose were used as substrates. The end product glucose and/or intermediate cellobiose were added to the initial samples in different concentrations, to identify their inhibition effect. The enzymes used in the experiments were Novozymes 188 (BG) and Celluclast 1.5L (CBH and EG). Three sets of experiments were performed focusing exclusively on three different conversion reactions.

A total of nine parameters (k_{1r}, k_{2r}, k_{3r}, K_{1IG2}, K_{2IG2}, K_{3M}, K_{1IG}, K_{2IG} and K_{3IG}) (Kadam et al., 2004) involved in the conversion reactions were estimated, relying on advanced statistical analysis. The parameter estimation and model solution were done in MatLab (The MathWorks, Natick, MA).

PARAMETER ESTIMATION FOR CELLULOSIC BIOMASS HYDROLYSIS

The estimation of the parameters was performed using eight different experimental setups with two or three repetitions (19 experiments in total) as illustrated in Table 1. The parameters employed in the kinetic reaction for

cellulose conversion to cellobiose and glucose (r_1 and r_2, respectively), were estimated using experiments a.1-a.5. The dissimilarity among the five experiments was found in the initial concentration of inhibitory agents, where glucose and cellobiose were used in this case to quantify their inhibition effects on the cellulosic hydrolysis. The kinetic parameters of the cellobiose to glucose conversion were calculated using experiments b.1-b.3, using only glucose as initial inhibitor since cellobiose is the substrate, and is present in a relatively high concentration (37.5 g/kg).

Table 1. Data sets employed for parameter estimation in the conversion of cellulosic material into glucose.

	Substrate	Reaction	Enzyme	Enzyme Concentration (mg/g)	Inhibitor	No	Inhibitor concentration (g/kg)	Sampling time (hr)
Estimation	Avicel (100 g/kg)	$r_1 + r_2$ (k_{1r}, k_{2r}, K_{1IG2}, K_{2IG2}, K_{1IG}, K_{2IG})	Celluclast 1.5L	10.5	No	a.1	-	0, 3, 6, 12, 24, 48, 72, 120, 168
					Glucose	a.2	25	
						a.3	50	
					Cellobiose	a.4	15	
						a.5	30	
	Cellobiose (37.5 g/kg)	r_3 (k_{3r}, K_{3M}, K_{3IG})	Novozymes 188	3.9	No	b.1	-	0, 1, 3, 6, 12, 24, 48, 72
					Glucose	b.2	25	
						b.3	50	

Previously, Sin et al. (2010) performed an analysis of the same enzymatic hydrolysis mathematical model (Kadam et al., 2004), and found a generally poor identifiability of the mathematical model parameters as well as a high correlation between the estimated parameters. This means in practice that any change in one parameter could be compensated by a change in another one, hence making it extremely difficult (if not impossible) to find a unique estimate for these correlated parameters. To overcome this issue, a framework for parameter estimation was then introduced consisting of three steps for the estimation of the parameters and one for validation (see Figure 3). First of all, the estimation of the parameters for r_3 is performed. The second step of the procedure consists of estimating the unknown parameters for r_1 and r_2. Estimated parameters are then combined, followed by the validation of the mathematical model and the estimated parameter, on the basis of an independent data set.

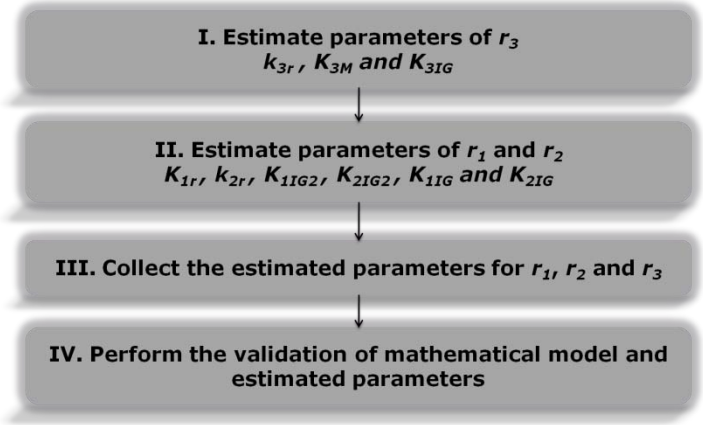

Figure 3 Framework for parameter estimation in the cellulosic biomass hydrolysis mathematical model.

The estimation of the parameters was done using MatLab (The Mathworks, Natick, MA). Initial values for the estimation were taken from Kadam et al. (2004). The Levenberg-Marquardt method for nonlinear least squares

curve-fitting was used in this study. The results of the parameter estimation according to the procedure outlined in Figure 3 are illustrated in Table 2.

Table 2. Parameter values obtained for the reaction network for cellulosic hydrolysis.

Parameter	Value	Parameter	Value	Parameter	Value
k_{1r}, $kg/g \cdot h$	22.3	k_{2r}, $kg/g \cdot h$	7.17	k_{3r}, h^{-1}	263.9
K_{1IG2}, g/kg	0.0035	K_{2IG2}, g/kg	131.9	K_{3M}, g/kg	0.0277
K_{1IG}, g/kg	0.6494	K_{2IG}, g/kg	0.019	K_{3IG}, g/kg	0.0061

The comparison of the model predictions (using the estimated parameters) versus the experimental data is illustrated in Figure 4. The predictions for reaction r_1 and r_2 (Figure 4.a) shows high-quality results where the highest deviation from the experimental results is found for data set a.5, which in fact started with a slightly high concentration of cellobiose (37.5 g/kg), if compared with the other experiments. As far as reaction r_3 is concerned (Figure 4.b), all the results are in agreement with exception of data set b.3. It is observed that a small deviation between experimental data and the model predictions is present for r_3 when the initially added glucose concentration is increasing.

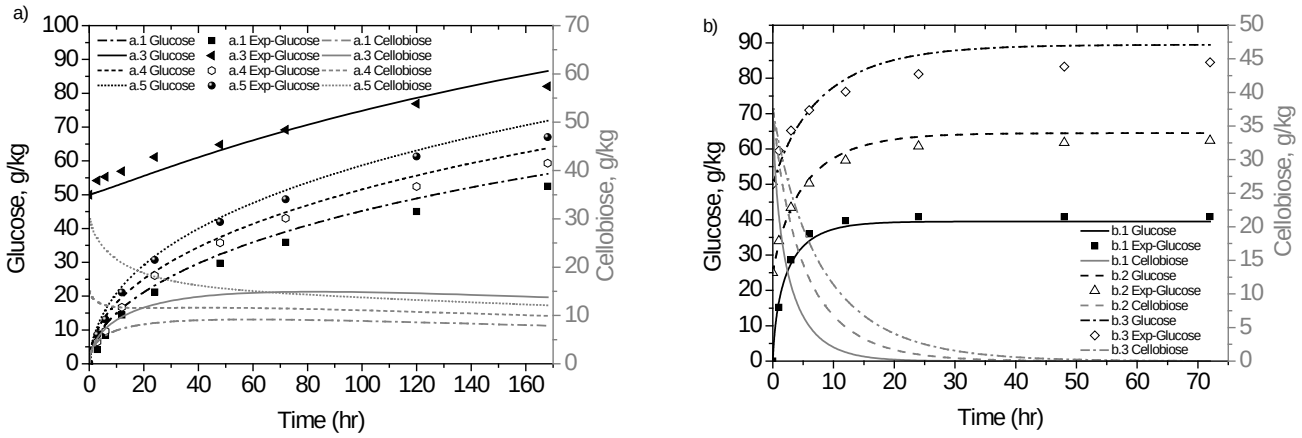

Figure 4 Results for parameter estimation for a) r_1 and r_2, and b) r_3.

VALIDATION OF INHIBITION EFFECTS IN THE CELLULOSIC BIOMASS HYDROLYSIS KINETIC

The validation of the obtained results to describe the inhibition effects present in the enzymatic hydrolysis of cellulosic material was performed using different data sets with two repetitions for each of them (see Table 3). In experiments c.1 and c.2, simultaneous enzyme addition (Celluclast 1.5L and Novozyme188) was performed, in a first experiment with no inhibitor present and in a second one with cellobiose (as inhibitor) present at the starting point of the experiment. Simultaneous enzyme addition results in synergistic cellulose degradation in the presence of three kinds of cellulases. For experiment d.1 only Celluclast 1.5L was added to the reacting mixture, and no inhibitor was introduced at the initial phase of the experiment.

All estimated parameters were combined in order to perform the mathematical model validation (steps III and IV, Figure 3). The resulting simulations with those parameters show a good agreement with experimental data (Figure 5). The mathematical model predictions for reactions r_1 and r_2 demonstrate an excellent model fit for the

data set d.1. The simulation results with cellobiose present as an inhibitor (c.2), are also properly represented by the model, thereby, illustrating the predictive ability of the model when an inhibitor has a high concentration in the reaction mixture. As far as experiment c.1 is concerned, this experiment was not entirely well predicted by the mathematical model, even though no inhibitor was present at the start of the experiment.

Table 3. Data sets employed for parameter validation in the conversion of cellulosic material to glucose.

	Substrate	Reaction	Enzyme	Enzyme Concentration (mg/g)	Inhibitor	No	Inhibitor concentration (g/kg)	Sampling time (hr)
Validation	Avicel (100 g/kg)	$r_1 + r_2 + r_3$	Celluclast 1.5L + Novozyme 188	15.8 + 5.9	No	c.1	-	0, 3, 6, 12, 24, 48, 72, 120, 168
					Cellobiose	c.2	30	
		$r_1 + r_2$	Celluclast 1.5L	21.1	No	d.1	-	

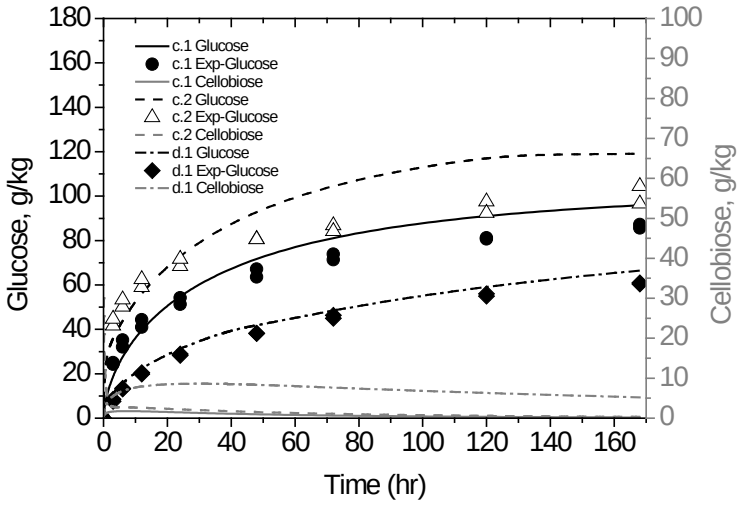

Figure 5 Validation of inhibition effects of the enzymatic hydrolysis of cellulosic biomass

CONCLUDING REMARKS

This study has presented the validation of a mathematical model describing enzymatic hydrolysis of cellulose and including inhibition effects of products (cellobiose and glucose). The validation study has involved the development of a detailed experimental design. The information obtained from the experiments has been used to first perform parameter estimation and then perform a model validation. A systematic approach for parameter estimation was introduced consisting of four main steps, to overcome the correlation issues of the parameters.

The validation of the mathematical model employing the estimated parameters has been performed using three different and independent data sets (not employed in the estimation), which have in general illustrated a good agreement between the model predictions and the experimental data. There are just a few exceptions where the

model fit is less good: the model over-predicted product concentrations for an experimental data set where a high glucose concentration was present.

An introduction of other inhibitory agents (i.e. xylose, furfural, etc.) present in the bioethanol production process, should also be taken into account in future experiments, in order to complete the analysis and understanding of the phenomena involved in the bioethanol production process. An extended version of this manuscript containing additional details is in preparation.

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REFERENCES

1. Andric, P., Meyer, A.S., Jensen, P.A., Dam-Johansen, K. (2010). Effect and Modelling of Glucose Inhibition and In Situ Glucose Removal during Enzyme Hydrolysis of Pretreated Wheat Straw. *Appl. Biochem. Biotechnol*. **(160)** 280-297.
2. Bansal, P., Hall, M., Realff, M.J., Lee, J.H., Bommarius, A.S. (2009). Modelling cellulases kinetics on lignocellulosic substrates. *Biotechnol. Advances*. **(27)** 833-848.
3. Hodge, D.B., Karim, M.N., Schell, D.J., McMillan, J.D., 2009. Model-Based Fed-Batch for High-Solids Enzymatic Cellulose Hydrolysis. *Appl. Biochem. Biotechnol*. **(152)** 88-107.
4. Kadam, K.L. Rydholm, E.C. McMillan, J.D., 2004. Development and Validation of a Kinetic Model for Enzymatic Saccharification of Lignocellulosic Biomass. *Biotechnol. Prog.* **(20)** 698-705.
5. Morales-Rodriguez, R., Capron, M., Huusom, J.K., Sin, G. (2010). Controlled fed-batch operation for improving cellulose hydrolysis in 2G bioethanol production. *Comput.- Aided Chem. Eng*. **(28)** 1497-1502
6. Rodriguez-Gomez D., Lehmann, L., Schultz-Jensen, N., Thomsen, A.B., Hobley, T. (2011). Cellulase and xylanase production by *Trichoderma reesei* Rut C30 growing on plasma pretreated wheat straw and Avicel. *Applied Biochemistry and Biotechnology*. ***Submitted***.
7. Sin, G., Meyer, A.S., Gernaey, K.V. (2010). Assessing reliability of cellulose hydrolysis models to support biofuel process design—Identifiability and uncertainty analysis. *Computers and Chemical Engineering*. **(34)** 1385-1392.
8. Tsai, C.-T., Morales-Rodriguez, R., Balduck, G., Gernaey, K.V., Meyer, A.S., Sin, G. (2011). A Dynamic Model for Cellulosic Biomass Hydrolysis: Validation of Hydrolysis and Product Inhibition Mechanism. ***To be submitted***.