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Corn stover semi-mechanistic enzymatic hydrolysis model with tight parameter confidence intervals for model-based process design and optimization

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HIGHLIGHTS

- A semi-mechanistic model is proposed for pretreated corn stover saccharification.
- The model considers high-solid saccharification and washed or unwashed solids.
- A subset of identifiable parameters was found showing tight confidence intervals.
- Uncertainty in parameters estimates was used to predict bands for glucose yield.
- The model reliably describes the saccharification kinetics of corn stover' glucan.

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ABSTRACT

Uncertainty associated to the estimated values of the parameters in a model is a key piece of information for decision makers and model users. However, this information is typically not reported or the confidence intervals are too large to be useful. A semi-mechanistic model for the enzymatic saccharification of dilute acid pretreated corn stover is proposed in this work, the model is a modification of an existing one providing a statistically significant improved fit towards a set of experimental data that includes varying initial solid loadings (10–25% w/w) and the use of the pretreatment liquor and washed solids with or without supplementation of key inhibitors. A subset of 8 out of 17 parameters was identified, showing sufficiently tight confidence intervals to be used in uncertainty propagation and model analysis, without requiring interval truncation via expert judgment.

1. Introduction

The cell walls of plants comprising lignocellulosic biomass are a complex and heterogeneous matrix composed primarily of the biopolymers: cellulose, hemicelluloses, and lignin (Chundawat et al., 2011). These cell wall biopolymers offer the potential as feedstocks for the sustainable production of renewable fuels, chemicals, and biomaterials with a diverse range of biochemical, thermochemical, and catalytic routes. One promising conversion route involves the deconstruction of the cell wall polysaccharides into fermentable monosaccharides by a pretreatment and polysaccharide hydrolysis, followed by biological conversion of sugars to fuels such as ethanol (Galbe and Zacchi, 2012). Cellulose hydrolysis of pretreated lignocellulose can be performed using a cocktail of cooperative cellulase enzymes containing glycosyl hydrolases (Lynd et al., 2002) as well as a recently recognized class of lytic polysaccharide monooxygenases (Harris et al, 2014) that are responsible for

Abbreviation: CBH, cellobiohydrolases; EG, endoglucanases; FPU, Filter Paper Unit; PCS, pretreated corn stover.

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Nomenclature

a_{iad}	adsorption decreasing factor (kg/g)
b_i	activity decreasing factor (kg/g)
$CI^{1-\alpha}$	confidence interval at α significance level
$\boldsymbol{COV}$	$m \times m$ covariance matrix
E_{1max}	maximum mass of enzyme that can be adsorbed onto a unit mass of substrate: 0.06 (g/g)
E_{2max}	maximum mass of enzyme that can be adsorbed onto a unit mass of substrate: 0.01 (g/g)
E_{1B}	bound concentration of CBH and EG (g/kg)
E_{2B}	bound concentration of β-glucosidase (g/kg)
E_{2F}	free concentration of β-glucosidase (g/kg)
E_T	total enzyme concentration (g/kg)
E_{1T}	concentration of CBH and EG (g/kg)
E_{2T}	concentration of β-glucosidase (g/kg)
f_2	fraction of β-glucosidase protein in Spezyme CP
$f_{\beta G}$	fraction of the maximum β-glucosidase activity
G	glucose concentration (g/kg)
G_2	cellobiose concentration (g/kg)
$J(\boldsymbol{\theta})$	cost function for parameter estimation
K_{3M}	cellobiose saturation constant (g/kg)
K_{1ad}	dissociation constant for the enzyme adsorption–desorption reaction: 0.4 (g/g)
K_{2ad}	dissociation constant for the enzyme adsorption–desorption reaction: 0.1 (g/g)
K_{ilA}	inhibition constant for acetic acid (g/kg)
K_{ilG}	inhibition constant for glucose (g/kg)
K_{ilG2}	inhibition constant for cellobiose (g/kg)
K_{ilX}	inhibition constant for five carbon sugars (g/kg)
k_{ir}	reaction rate (g· kg^{-1} · h^{-1})
R_S	substrate reactivity
$rCI^{1-\alpha}$	relative half confidence interval
S	cellulose concentration (g/kg)
$\boldsymbol{V}$	$n \times m$ derivative matrix
$\boldsymbol{W}$	$n \times n$ diagonal matrix of weights

Indices and sets

m	number of parameters
n	number of experimental measures
$\boldsymbol{\theta}$	set of parameters
$\boldsymbol{\theta}_f$	set of parameters with fixed values
$\boldsymbol{K}$	set of combinations of m parameters taken k at a time, each row (K) represents a particular combination of k parameters

Greek symbols

α	significance level for t-test and F-test
δ_{min}	minimum acceptable parameter sensitivity
δ^{msqr}	sensitivity measure
γ_K	collinearity index of parameter subset K
γ_K^C	collinearity index of complement of parameter subset K
γ_K^{max}	maximum allowable collinearity index
$\boldsymbol{\Lambda}'$	matrix of elements of $\boldsymbol{K}$ showing $\gamma_K \leqslant \gamma_K^{max}$

depolymerizing cellulose. Some hemicellulose-retaining pretreatments also require hemicellulose-depolymerizing enzymes to maximize cellulose hydrolysis (Decker et al., 2008). The process is complex due to the number of enzymes that take part and the fact that reactions take place on the surface of a water-insoluble crystalline polymer (i.e. cellulose hydrolysis) as well as reactions in the liquid phase (i.e. cellobiose hydrolysis).

An extensive collection of kinetic models of cellulose enzymatic hydrolysis for model cellulosic substrates and pretreated biomass can be found in literature and have been recently reviewed (Bansal et al., 2009). Models range from simple empirical or black-box models to complex mechanistic models, which attempt to use the current understanding of how the process works to derive causal hypotheses that are incorporated in the mathematical model. While empirical models may have a small number of parameters to adjust (although there are exceptions such as neural network models), mechanistic models can involve a large number of parameters, which need to be found by fitting the model to a large number of purposely generated experimental data (Brun et al., 2001). From a model-based process design point of view; the use of either empirical or mechanistic models depends on whether the user is interested in testing conditions within the experimental data (interpolation), or in testing conditions that lie outside the experimental conditions (extrapolation) where the mechanistic model provide a rational basis for predicting the behavior of the system.

Due to the complexity of the enzymatic hydrolysis process, the changing enzyme formulations made available by the major commercial enzyme producers, as well as the strong influence that pretreatment and feedstock have over the outcome of enzymatic hydrolysis, semi-mechanistic models with the smallest possible number of parameters may be the most adequate choice from a model-based development point of view, thereby reducing the amount of experimental data required to estimate the parameters values. Among the existing semi-mechanistic models, the one developed by NREL researchers in 2004 (Kadam et al., 2004) has been used in a number of biofuel production processes flowsheets evaluation and alternatives comparison (Scott et al., 2013; Morales-Rodriguez et al., 2011; Hodge et al., 2009) and it has been subjected to an identifiability and uncertainty analysis (Sin et al., 2010). Results indicate that only 6 out of 26 parameters are identifiable from the original data, and any attempt to identify a higher number of parameters results in significant errors on their estimates. This is evidenced by the wide confidence intervals for the values of the parameters.

Uncertainty in parameter estimates can arise from a number of sources, including insufficiently informative experimental data, i.e. the model is not sensitive to some of the parameters to be estimated over the experimental data set (Raue et al., 2009) and parameters that are correlated, i.e. parameters are mathematically related to each other through some implicit function (Li and Vu, 2013; Raue et al., 2009). Furthermore, to be used in model-based process development, a cellulose hydrolysis kinetic model should incorporate as many aspects controlling the process behavior as possible. Among them, initial solids loading is a key factor since the higher the concentration of substrate the higher the ethanol titer in the fermentation stage, which decreases the energy needs in the recovery stage. However, it is known that high solid loadings affect the final glucan conversion (Wang et al., 2011; Kristensen et al., 2009; Hodge et al., 2008). Usage of the liquor generated during pretreatment is desirable from a technical and economical point of view, since no capital-intensive separation equipment is required and the sugar oligomers released during pretreatment can be hydrolyzed by the action of enzymes in the saccharification stage, however, enzymes are known to be inhibited by soluble sugar monomers, dimers, and oligomers (Teugjas and Väljamäe, 2013; Qing et al., 2010), organic acids (Hodge et al., 2008), and phenolic compounds (Tejirian and Xu, 2011) contained in the pretreatment liquor. Despite these inherent difficulties and