Kinetic modeling for enzymatic hydrolysis of pretreated sugarcane straw

J.D. Angarita [a], R.B.A. Souza [b], A.J.G. Cruz [b], E.C. Biscaia Jr. [a], A.R. Secchi [a],*

[a] Chemical Engineering Program, COPPE, Universidade Federal do Rio de Janeiro, UFRJ, Centro de Tecnologia, Bloco G, Cidade Universitária, CP 68502, CEP 21941-972 Rio de Janeiro, RJ, Brazil
[b] Chemical Engineering Graduate Program, Universidade Federal de São Carlos, UFSCar, Via Washington Luiz (SP 310), km 235, CP 676, CEP 13565-905 São Carlos, SP, Brazil

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ABSTRACT

A semimechanistic multi-reaction kinetic model was developed for modeling enzymatic hydrolysis of pretreated sugarcane straw (SCS) at high-solids concentration. The model, including: enzyme adsorption, xylose formation, end-product inhibition and substrate reactivity, was adjusted from a reference condition (enzyme concentration of 10 FPU/g-cellulose and solid loading of 15% w/v, dry basis). A simplified model was also proposed to overcome high parameter correlation and improve model fidelity. Predictive capacity of both simplified and original model was verified for different enzyme concentration (5–60 FPU/g-cellulose), solids loading (10 and 20 %) and end-product sugar inhibition (30 and 60 g/L glucose, 10 g/L cellobiose and 10 g/L xylose). The glucose concentration was predicted with reasonable accuracy by the simplified model, even under critical conditions of sugar-inhibition. The model is able to describe batch saccharification of pretreated SCS at high-solids concentrations.

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1. Introduction

Bioethanol is an alternative fuel produced from biomass with potential to reduce greenhouse gas emissions; in fact the conversion of lignocellulosic crops residues is the most attractive option to get for even larger reductions of greenhouse gas emission in transport and industrial sector [1]. However the availability of both agricultural land and cost-effective conversion technology are the main barriers to produce bioethanol in industrial scale [2].

Brazil is the second largest producer of ethanol with 27.54 billion liters (27% of world production) from sugarcane juice [3]. Actually, ethanol yield per hectare of sugarcane is 6000 L/ha and it could be increased by more than 50% without increasing the cultivated area, if sugarcane residues such as bagasse and straw were used [4].

The biochemical route for the conversion of lignocellulosic biomass to ethanol includes four main stages: pretreatment, saccharification or hydrolysis of polysaccharides present in the substrate, fermentation of sugars, and recovery of ethanol by distillation. Sugar production is the critical step for bioethanol production. Enzymatic hydrolysis is preferred over acid hydrolysis for

its high sugar yield (due to the specificity of enzymes), minimal byproducts formation, low energy requirements, mild operating conditions (pH 4–5 and $T = 45–50\,°C$) and low corrosiveness [5–8]. However the high cost of enzymes [9], the low reaction rate [10], and the difficulty of operating at high solids concentration (>12%) [11] are the main barriers for commercialization of lignocellulosic ethanol through biochemical route.

Several factors related to both enzyme characteristics (adsorption, inhibition, synergism, activity) and substrate characteristics (degree of polymerization, crystallinity, accessible surface area, lignin content) affect the hydrolysis of cellulose, therefore including all of them into a kinetic model to describe the synergistic action of enzymes on lignocellulosic substrates is one of the most challenging subjects in engineering for this process. Many kinetic models have been proposed in the literature to describe the enzymatic hydrolysis of cellulose [12,13]. Such models are mainly motivated by both, demonstrating an understanding of physical process in a mathematical structure that adequately fit the experimental data, and development of model-based applications for process design, simulation and control.

Many efforts have been made to reduce the ethanol cost production, including: improving pretreatment technologies; genetic modifying of plant cell walls, genetically improving the enzyme activity and productivity; applying different production schemes such as separate hydrolysis and fermentation (SHF) and simulta-

Nomenclature

C	cellulose concentration (g/L)
E_B	concentration of bound enzyme onto solids (g/L)
E_{BC}	concentration of bound enzyme onto cellulose (g/L)
E_{BH}	concentration of bound enzyme onto hemicellulose (g/L)
E_F	free enzyme concentration (g/L)
E_{max}	maximum mass of enzyme that can be absorbed onto a unit mass of substrate (g protein/kg substrate)
E_T	total enzyme concentration (g/L)
G	glucose concentration (g/L)
G_2	cellobiose concentration (g/L)
H	hemicellulose concentration (g/L)
K_{3M}	substrate (cellobiose) saturation constant (g/L)
K_{ad}	dissociation constant for the enzyme adsorption/desorption reaction (L/g protein)
K_{iIG}	inhibition constants for glucose (g/L); i = 1, 2, 3, and 4
K_{iIG2}	inhibition constants for cellobiose (g/L); i = 1, 2 and 4
K_{iIX}	inhibition constants for xylose (g/L); i = 1, 2, 3 and 4
k_{ir}	reaction rate constants (L/g·h); i = 1, 2 and 4. k_{3r} (h-1)
L	lignin concentration (g/L)
r_i	reaction rate (g/L·h); i = 1, 2, 3 and 4
R_s	substrate reactivity parameter (dimensionless)
S	solids concentration (g/L)
S_0	initial solid concentration (g/L)
X	xylose concentration (g/L)
α	constant relating substrate reactivity with hydrolysis degree (dimensionless)

neous saccharification and fermentation (SSF); and optimization of the hydrolysis operating conditions. High solids saccharification (>12%) presents potential economic advantage over the conventional hydrolysis at low solids loading such as, lower capital cost due to reduced volume reactor; lower operating cost that result from lower energetic requirements for heating and cooling; lower downstream processing cost because of the higher product concentration and reduced disposal and treatment cost due to lower water consumption [14].

Although the effects of high solids concentration over both enzyme inhibition [15] and slurry viscosity [16] have been studied, few models have been used to describe the enzymatic hydrolysis performance under these special operating conditions. The goal of this work is the development of a semimechanistic kinetic model to describe the complex enzymatic hydrolysis of sugarcane straw at high solids concentration with potential to be used in model-based applications such as simulation and optimization of the production process of lignocellulosic ethanol.

2. Enzymatic hydrolysis models

Cellulose saccharification is a complex heterogeneous reaction catalyzed by the synergistic action of enzyme pool, including three major types of enzymatic activities: endoglucanases, exoglucanases, and β-glucosidases [17]. Many models have been proposed to describe the cellulose enzymatic hydrolysis [12,13] based on the reactional scheme of Fig. 1. Zhang and Lynd [13] classified the enzymatic hydrolysis models based on the number of solubilizing activities and substrate state variables, as: (1) nonmechanistic model; (2) semimechanistic model; (3) functionally based model; and (4) structurally based model. Models based on a defensi-

ble adsorption model and using only the concentration variable to describe the substrate state and/or using a single cellulose activity are called semimechanistic. Many hydrolysis models used for the design of industrial systems are included into this category [21]. Although semimechanistic models can be limited to provide a detailed description about the effects of substrate features (crystallinity, degree of polymerization, surface area) and multiple hydrolyzing activities on enzymatic hydrolysis [13], these models are useful once minimal structural information is required for descriptive purpose such as reactor design and model-based applications such as simulation and process optimization.

An early kinetic model for enzymatic hydrolysis of lignocellulosic substrate (rice straw) was proposed by Wald et al. [5]; enzyme adsorption, product inhibition, and a multi-enzyme system were considered into the model. The substrate was modeled assuming two regions (crystalline and amorphous) with different properties related to the enzyme adsorption and hydrolysis. The enzyme adsorption was expressed as function of available sorption sites and the accessible surface area following the Langmuir isotherm. This model was able to describe enzymatic hydrolysis of rice straw at high substrate (up to 333 g/L) and enzyme concentration (up to 9.2 FPU/mL). The modeling of enzyme adsorption on substrate was neglected by researcher in the beginning and generally a Michaelis–Menten (M–M) model was used to describe the enzymatic hydrolysis, though the substrate is not in excess in this heterogeneous system. Gusakov et al. [18,19] model followed this approach, considering also cellulase composition, structural complexity of cellulose, products inhibition, and enzyme inactivation due to unproductive adsorption to substrate. The model satisfactorily predicted batch glucose and cellobiose concentration in the substrate concentration range of 5–100 g/L and enzyme concentration range of 5–60 g/L.

Philippidis et al. [20] proposed a SSF-based kinetic model to simulate enzymatic hydrolysis of pure cellulose. The kinetic parameters were estimated by nonlinear regression from experimental data. Since neither an adsorption model, enzyme inactivation, nor substrate complexity were considered in this model, the cellulose and cellobiose concentration predictions were overestimated and underestimated, respectively, by the model after 24 h of hydrolysis.

One of the most complete semimechanistic kinetic models for enzymatic hydrolysis of cellulose was proposed by Kadam et al. [9], who developed a multireaction kinetic model for batch enzymatic hydrolysis of corn stover pretreated with dilute acid. This model was the first to include xylose inhibition beside of glucose and cellobiose inhibition and included the direct conversion of cellulose to glucose. The temperature effect and the reactivity substrate (R_s) were also incorporated. Model predictions about the effect of inhibition sugar were successfully described for initial concentrations of glucose (30 and 50 g/kg), cellobiose (30 g/kg) and xylose (30 and 40 g/kg). This model has proved to be useful in the simulation and optimization of enzymatic hydrolysis of lignocellulosic substrate [9,21], and could describe successfully the enzymatic hydrolysis of bagasse and straw of sugarcane.

2.1. Model description

The Kadam et al. model [9] is considered sophisticated enough to describe the enzymatic hydrolysis of lignocellulosics and has been chosen to model the enzymatic hydrolysis of sugarcane straw. This model has been experimentally verified [11,21], evaluated in applications of simulation and optimization process [11,22], and recently used to model the enzymatic hydrolysis of sugarcane bagasse [23]. The proposed model is based on the biochemical of enzymatic hydrolysis and include enzyme adsorption, product inhibition, substrate reactivity, and conversion of hemicellulose to