

MODELING BIOCHEMICAL CONVERSION OF LIGNOCELLULOSIC MATERIALS FOR SUGAR PRODUCTION: A REVIEW

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To deeply understand the factors that affect the conversion of lignocellulosic biomass to fermentable sugars, experimental results should be bridged with process simulations. The objective of this paper is to review published research on modeling of the pretreatment process using leading technologies such as dilute acid, alkaline, and steam explosion pretreatment, as well as the enzymatic hydrolysis process for converting lignocellulose to sugars. The most commonly developed models for the pretreatment are kinetic models with assumptions of a first-order dependence of reaction rate on biomass components and an Arrhenius-type correlation between rate constant and temperature. In view of the heterogeneous nature of the reactions involved in the pretreatment, the uses of severity factor, artificial neural network, and fuzzy inference systems present alternative approaches for predicting the behavior of the systems. Kinetics of the enzymatic hydrolysis of cellulosic biomass has been simulated using various modeling approaches, among which the models developed based on Langmuir-type adsorption mechanism and the modified Michaelis-Menten models that incorporate appropriate rate-limiting factors have the most potential. Factors including substrate reactivity, enzyme activity and accessibility, irreversible binding of enzymes to lignin, and enzyme deactivation at high conversion levels, need to be considered in modeling the hydrolysis process. Future prospects for research should focus on thorough understanding of the interactions between biomass reactants and chemicals/enzymes — the key to developing sophisticated models for the entire conversion process.

Keywords: Lignocellulose; Modeling; Pretreatment; Enzymatic Hydrolysis; Kinetics

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INTRODUCTION

Ethanol, a renewable energy source obtained through fermentation of simple sugars, is one of the sustainable alternatives to gasoline that can be used to enhance energy security and mitigate greenhouse gas emissions (Lynd et al. 2008; Sun and Cheng 2002; Wyman 2007). Current fuel ethanol production in the world is mainly based on the fermentation of glucose derived from food based crops. This approach, however, is limited by the arable land and the food and feed applications for the crops such as corn and sugarcane (Elobied et al. 2007; Somerville et al. 2010; Sun and Cheng 2002).

Lignocellulosic materials such as agricultural residues, perennial grasses, and forestry biomass are major promising energy sources that have the potential to substitute a large portion of fossil fuels use across the globe, particularly being able to replace 30% of current petroleum consumption in the US market by 2030 (Perlack et al. 2005; Wyman 2007).

Ethanol production from lignocellulosic biomass features a biological conversion that involves the hydrolysis of cellulose and hemicellulose to fermentable reducing sugars and the fermentation of the sugars to ethanol (Lynd et al. 2008; Sun and Cheng 2002). Unlike the relatively simple structure of sugar or starch-based feedstocks, biomass recalcitrance is one of the major challenges in realizing the full cellulosic ethanol production potential (Himmel et al. 2007; Zhang et al. 2007). This recalcitrance is due to the complex structure of lignocellulosic biomass and the way specific components (cellulose, hemicellulose, and lignin) interact with each other (Kumar et al. 2009; Balan et al. 2009).

In light of the natural resistance of cellulosic biomass to attack by hydrolytic enzymes, a pretreatment step is required to alter the structure of biomass and increase the digestibility of cellulose and hemicellulose by the enzymes (Mosier et al. 2005; Sun and Cheng 2002; Yang and Wyman 2008). The current costly cellulosic ethanol production is due to the high costs associated with releasing fermentable sugars from biomass through pretreatment and enzymatic hydrolysis (Lynd et al. 2008; O'Dwyer et al. 2007; Yang and Wyman 2008).

Improving conversion efficiency from lignocellulosic biomass to sugars will require new biotechnological approaches developed through a thorough understanding of what factors impede the sugar production (Lynd et al. 2008; O'Dwyer et al. 2007). This deep perception can be acquired via bridging computer simulations and experimental results together to identify common problematic features that hinder the progress of cellulosic ethanol industry. Given the areas being advanced for lignocellulose-to-ethanol conversion, improving the existing models or developing new models should focus on the two technologically and economically critical steps, including pretreatment and enzymatic hydrolysis.

Kinetic modeling is the most widely investigated method for simulating the two processes, as it is developed based on inherent reaction mechanisms (Conner et al. 1985; Dang and Nguyen 2006, 2007; Fuentes et al. 2011; Jacobsen and Wyman 2000; Kadam et al. 2004; Kim and Holtzaple 2006; Keshwani and Cheng 2010; Ladisch et al. 1983; South et al. 1995), while some non-kinetic or fuzzy inference models are of particular interest when considering complex systems such as conversion of cellulosic biomass to ethanol (O'Dwyer et al. 2008; Redding 2009; Keshwani and Cheng 2010).

Although much work has been carried out to model a single or hybrid process for the biochemical conversion of lignocellulosic materials to sugars, there has been a need for a comprehensive review on the studies of modeling the entire process. Therefore, it is of great interest to summarize these valuable works to enlighten potential improvements for process design and optimization as well as economic assessment of the processes. The objective of this paper is to review published research on modeling different pretreatment processes and enzymatic hydrolysis of lignocellulosic biomass for sugar production.

PRETREATMENT PROCESS MODELING

Process Overview

Pretreatment offers a key to unlock lignocellulosic biomass to achieve high sugar yields after enzymatic hydrolysis, despite the fact that it accounts for only 20% of the total cost for cellulosic ethanol production (Yang and Wyman 2008). Pretreatment processes typically reduce cellulose crystallinity, increase biomass porosity, and remove lignin and/or hemicellulose (McMillan 1994; Xu et al. 2011). A variety of pretreatments can be achieved through physical, chemical, physico-chemical, thermo-chemical, or biological processes, with different approaches having distinct modes of action (Kumar et al. 2008; Sun and Cheng 2002). Careful selection of appropriate pretreatment technologies for process design is essential, as pretreatment has pervasive effects on all other operations in the overall conversion process, and these affected units incorporate the choice of feedstock, size reduction, enzymatic hydrolysis, sugar fermentation, product recovery, residue usage, and waste treatment (Wyman 2007; Yang and Wyman 2008).

Successfully modeling compositional changes of biomass during pretreatment is conducive to process design and optimization for better conversion (Jacobsen and Wyman 2000). Previous reviews state that efficient pretreatments need to employ chemicals for high product yields and low costs (Xu et al. 2011; Yang and Wyman 2008). In this respect, kinetic models would shed light on understanding the physical and chemical changes of biomass components and their correlations with chemicals implemented in pretreatments. Apart from the application of kinetic models on pretreatment, a fuzzy-logic-based inference system has been applied to simulate the process (Keshwani and Cheng 2010). Process simulation for acid pretreatment in the context of cellulosic ethanol production has drawn much attention for the last two decades. As is the case with acid pretreatment, modeling compositional changes of lignocellulosic biomass during alkaline pretreatment is also widely investigated, with the focus on delignification kinetics inspired from modeling the alkaline pulping process. A unified model, from which the entire conversion efficiency will benefit, is highly desired for predicting the performance of different pretreatment technologies.

Acid Pretreatment Simulation

Dilute acid pretreatment of lignocellulosic biomass for ethanol production has attracted extensive attention as to technology improvement and process simulation. A critical review on acid-based pretreatment/hydrolysis processes for the conversion of lignocellulosic materials to ethanol was carried out by Taherzadeh and Karimi (2007a), highlighting the dependence of acid-based hydrolysis efficiency on properties of the substrate, acidity, and rate of decomposition of the biomass components during the process. In general, the acid reagent used in this pretreatment technology is sulfuric acid, of which the effectiveness on sugar production after enzymatic hydrolysis has been the focus of research efforts (Canilha et al. 2011; George et al. 2010; Guo et al. 2008; Redding et al. 2011; Saha et al. 2005; Schell et al. 2003; Sun and Cheng 2005; Yang et al. 2009; Yat et al. 2008). The major impact of acid pretreatment is to solubilize hemicellulose to form xylose, with little or high lignin removal for batch or flow-through reactors, respectively (Moiser et al. 2005; Yang and Wyman 2004). With this commonly-

agreed mechanism, predicting the change of hemicellulose content in lignocellulosic biomass becomes the priority for model development. Although dilute acid pretreatment has insignificant influence on the dissolution of cellulose (Redding et al. 2011; Taherzadeh and Karimi 2007a), inclusion of equations relating cellulose saccharification with pretreatment conditions and biomass physical or chemical properties in model development favors the process design of subsequent enzymatic hydrolysis.

Kinetic model approach

Substantial knowledge of the kinetics of cellulose and hemicellulose fractionation is extremely beneficial to the design, development, and modeling of acid pretreatment processes. Figure 1 illustrates the generic kinetic processes representing xylan removal and cellulose saccharification during acid pretreatment (Conner et al. 1985; Jacobsen and Wyman 2000; Mehlberg and Tsao 1979; Saeman 1945). The initial kinetic model developed for predicting the changes of cellulose content and its associated products was reported by Saeman (1945). This model consists of two consecutive reactions, of which the first one has a rate constant more sensitive to the changes of temperature and acid concentration than the second one (Saeman 1945). Instead of studying the kinetic conversion of cellulose to glucose, an approach that simulates cellobiose hydrolysis was used to simplify the process (Mosier et al. 2002). Based on Saeman's study, Jacobsen and Wyman (2000) proposed a simple kinetic model for xylan removal during dilute acid pretreatment. In their model, xylan is considered as a chemically unified component that is hydrolyzed to xylose, from which degradation products are formed. Several studies have applied this simple kinetic model to predicting xylan solubilization for acid pretreatment of corn stover (Lu et al 2008), a variety of timbers and switchgrass (Yat et al. 2008), and sugarcane bagasse (Bustos et al. 2003).

A biphasic reaction kinetic model is also applicable to predict cellulose and xylan hydrolysis during dilute acid pretreatment (Bustos et al. 2003; Carrasco and Roy 1992; Conner et al. 1985; Esteghlalian et al. 1997; Mehlberg and Tsao 1979). The first attempt to model the kinetics of hemicellulose hydrolysis during acid pretreatment was made by Mehlberg and Tsao (1979), who suggested two parallel paths for xylan reacting with hydrochloric acid. According to Conner (1984), the slow-reacting rate of xylan solubilization may be due to the entanglement of a portion of xylan with lignin via intermolecular bonds. The inclusion of oligosaccharides in the parallel reaction model takes into account scenarios where breaking down oligomers to monomers is not necessarily much faster than their formation. A relatively complex model for cellulose saccharification was developed by Conner et al. (1985) who took into consideration the presence of amorphous cellulose, and the reversion reactions of glucose. At high acid concentrations and low temperatures, a remarkable reversible formation of disaccharides from glucose is commonly observed (Pilath et al. 2010).

All the aforementioned kinetic models, as shown in Fig. 1, involve the use of a first-order dependence of reaction rate on the biomass component. The reaction rate equation for the biomass constituent, R_x , is expressed as follows,

$$R_x = -dX/dt = kX \quad (1)$$

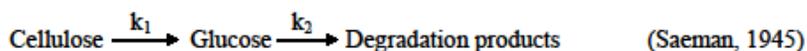
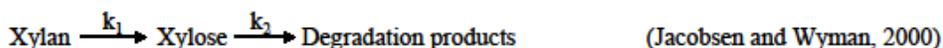
where X is the fraction of original biomass component remaining in the solid residue and k is the rate constant. The commonly used reaction rate constants are assumed to be dependent on temperature and acid concentration and can be calculated from a modified Arrhenius equation (Carrasco and Roy 1992; Jacobsen and Wyman 2000),

$$k = A \cdot C^{m_i} \cdot \exp(-E_a/RT) \quad (2)$$

where A is the Arrhenius constant or the pre-exponential factor (1/time depending on the unit of k); C is the concentration of acid (wt%); m_i is a constant; E_a is the activation energy (J/mol); R is the ideal gas constant, 8.314 J/(mol K); and T is the absolute temperature (K).

The effect of the neutralizing capacity of the biomass was also evaluated and incorporated in the kinetic models due to the presence of ash-forming constituents (Conner et al. 1985; Esteghlalian et al. 1997). In light of this attention, the acid concentration C can be substituted with the molar hydrogen-ion concentration $[H^+]$ (Conner et al. 1985). These kinetic models are applicable not only to single-stage pretreatment but also to multi-stage pretreatment. Baugh et al. (1988) predicted the saccharification of both cellulose and hemicellulose during a multistage acid pretreatment using a typical first-order model indicated in Fig. 1.

Simple process



Complex process



Fig. 1. Generic kinetic processes of xylan removal and cellulose saccharification during dilute acid pretreatment

The validity of a first-order kinetic model for pretreatment of a mixture of lignocellulosic feedstocks is essential, because in reality a cellulosic ethanol plant may use a mixture of feedstocks for the purpose of cost savings. Jensen et al. (2008) proved the applicability of the kinetics approach in dilute sulfuric acid pretreatment of a mixture of switchgrass and woody biomass, and stated that the impact of the interactions among these biomass varieties on the kinetics of hemicellulose saccharification is negligible. To accurately determine the kinetic parameters, preheating time needs to be as short as possible to fulfill an isothermal condition. In a case of non-isothermal condition, heat transfer limitations resulting from reactor configurations need to be considered when developing a kinetic model for biomass saccharification during pretreatment (Jacobsen and Wyman 2001). The existing kinetic models provide a useful tool for predicting the changes of cellulose and hemicellulose during dilute acid pretreatment under various conditions. However, more progress is needed to make these models more reliable and effective by taking into account the effects of the interactions among lignin and the two carbohydrates.

Severity factor approach

The concept of combining the effects of reaction temperature and residence time into one single factor in empirical models to predict the solubilization of biomass components during acid pretreatments has roots in the pulp and paper industry. Overend and Chornet (1987) first examined the use of a severity parameter that incorporated temperature and time for steam explosion pretreatment. The inclusion of acid concentration into the severity factor was later realized in acid pretreatment of aspen (Chum et al. 1990). This modified severity factor (M) is determined as,

$$M = t \cdot C^n \cdot \exp[(T_r - 100)/14.75] \quad (3)$$

where t is the residence time (min); C is the acid concentration (wt%); T_r is the reaction temperature (°C); and n is an arbitrary constant. For non-isothermal conditions, T_r is not an equilibrium temperature but a temperature profile varying with time. A similar combined severity factor replacing the acid concentration with pH value was derived to effectively model the dilute acid pretreatment of softwoods (Nguyen et al. 2000). The performance of this severity factor approach has been evaluated in different studies (Bower et al. 2008; Jacobsen and Wyman 2000; Silverstein et al. 2007). In particular, Silverstein et al. (2007) found that this approach enables good predictive ability of severity factor-incorporated models in xylan solubilization.

Artificial neural network approach

Artificial neural network (ANN) modeling has apparent advantages over traditional kinetic modeling approaches in predicting the behavior of complex systems, mainly because it avoids the derivation of mathematical equations but behaves analogously to a biological neural structure (O'Dwyer et al. 2008). An ANN is normally composed of three layers including input, hidden, and output, from which a typical feed-forward back-propagation ANN can be built. Figure 2 shows an example of a typical scheme of three-layer neural network. In this network, there are an input layer with three

inputs, a hidden layer consisting of three neurons, scalar weights, biases, and transfer functions (normally sigmoid), and an output layer including one neuron, scalar weights, a bias, and a transfer function (normally linear). The mechanism of feed-forward back-propagation ANN is to feed the weighted inputs to the neuron in the hidden layer and make a summation that will be passed to the neuron in the output layer through a sigmoid transfer function, and then further to the output using a linear transfer function.

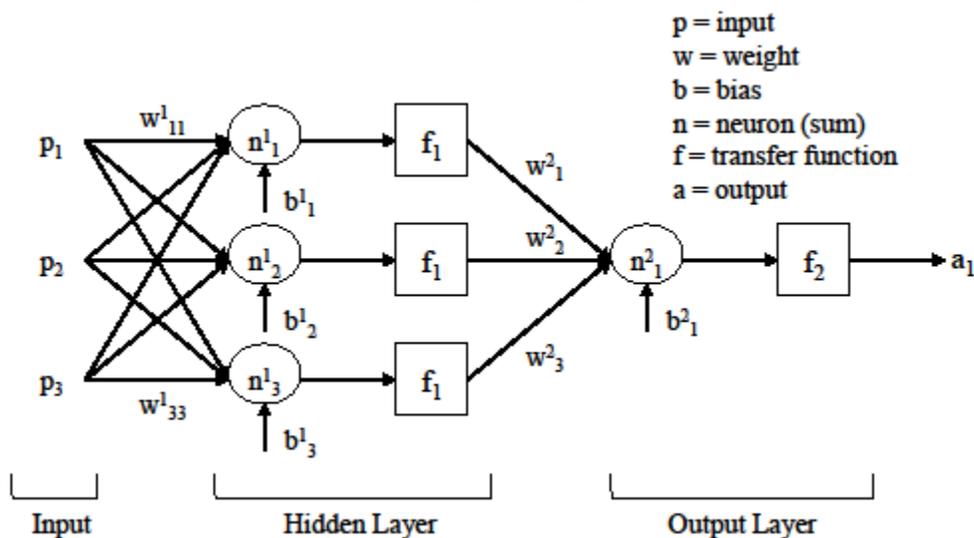


Fig. 2. Typical scheme of three-layer feed-forward back-propagation artificial neural network

The critical step in developing an effective ANN is to train the network with a series of inputs and associated outputs to minimize the error between the predicted and the actual output by adjusting the weights and biases (O'Dwyer et al. 2008; Redding 2009). Over-fitting, one of the most commonly encountered problems during the training process of neural networks, has to be mitigated by regularization to produce an ANN that performs well with any data within the range of training (O'Dwyer et al., 2008). The number of neurons in a hidden layer also needs to be controlled well so that the probability of over-fitting and under-fitting can be maintained at low levels. The successful development of an ANN would bring remarkable value to the design of cost-effective pretreatment and enzymatic hydrolysis processes.

There have been few studies so far applying this approach for predicting and optimizing cellulosic-to-ethanol process. The first if not the only application of ANN for simulating dilute acid pretreatment of lignocellulosic biomass was examined by Redding (2009), who reported that the ANN predicted more accurately the xylose content of the prehydrolyzate than a multiple linear regression model. Additionally, he found that the optimum number of neurons in the hidden layer was six, which was double the number of inputs including acid concentration, temperature, and residence time. O'Dwyer et al. (2008) also successfully applied a feed-forward back-propagation ANN to correlate biomass structure properties such as lignin content, acetyl content, and cellulose crystallinity with glucan and xylan digestibility during enzymatic hydrolysis. Moreover, they showed the good flexibility of ANN in explaining the impact of glucan and xylan hydrolyses have on each other by increasing the dimensionality of the ANN input matrix.

Alkaline Pretreatment Simulation

Alkaline pretreatment includes the use of sodium hydroxide, lime, and ammonia water at dilute concentrations. Tarkow and Feist (1969) examined the mechanism of alkaline pretreatment and found that intermolecular ester bonds crosslinking hemicellulose and lignin are broken down, and the resulting structural disruptions increase biomass porosity. Delignification is considered to be the major impact of alkaline pretreatment to enhance the reactivity of the remaining carbohydrates (Iyer et al. 1996; Kim and Holtzaple 2005; Kim and Lee 2005; Wang et al. 2010; Wang and Cheng 2011; Xu et al. 2010a,b). In addition to separating lignin from biomass, alkaline pretreatment has the capability of effectively removing acetyl groups and uronic acid substitutions on hemicellulose, which also contributes to improvement in enzymatic hydrolysis (Chang and Holtzaple 2000; Selig et al. 2009). A novel study by Xu and Cheng (2011) found that combining the use of sodium hydroxide and lime can remarkably improve the cost-effectiveness of alkaline pretreatment of switchgrass at room temperature.

Delignification kinetics approach

Efforts aimed at modeling changes of biomass composition during alkaline pretreatment began with studies of delignification kinetics in wood pulping processes. Gustafson et al. (1983) briefly summarized the kinetic models developed during the late 1950s to the late 1970s, and pointed out that these kinetic models mostly lack considerations of mass transfer limitations. In response to this issue, Gustafson et al. (1983) developed a theoretical delignification model incorporating kinetics and diffusion and provided a good fit of the model to the pulping behavior of softwood. In their study, Fick's law for diffusion, which is applicable to homogeneous systems, was used to estimate the mass transfer rate of alkalis during the pulping process. However, the assumption of a homogeneous process for the alkaline pulping may not be necessarily appropriate due to the complexity of pulping reaction systems.

In light of the heterogeneous nature of the alkaline pulping, the delignification process can also be viewed as a superposition of three consecutive stages including initial, bulk, and residual phases. The three phases represent distinct fractions of lignin, and are described as first-order reaction sub-processes (Chiang et al. 1990). A mathematical formula that depicts the general delignification kinetics in kraft pulping is shown as,

$$W_L = a_i \cdot \exp(-k_i \cdot t) + a_b \cdot \exp(-k_b \cdot t) + a_r \cdot \exp(-k_r \cdot t) \quad (4)$$

where W_L is the fraction of the residual lignin (g lignin remaining/g lignin in raw biomass); a_i is the maximum fraction of lignin fragments released in the initial stage; a_b is the maximum fraction of lignin fragments released in the bulk stage; a_r is the maximum fraction of lignin fragments released in the residual stage; and k_i , k_b , and k_r are the reaction rate constants for the initial, bulk, and residual delignification stage, respectively (Chiang et al. 1990; Kim and Holtzaple 2006). Note that $a_i + a_b + a_r = 1$ since $W_L = 1$ at $t = 0$. The well-known Arrhenius-type temperature dependence can be applied to relate the rate constants with reaction temperature.

Kim and Holtzaple (2006) and Fuentes et al. (2011) have examined the performance of the three-stage delignification kinetics on simulating lignin content change during lime pretreatment of corn stover, and sugarcane bagasse, respectively. The main difference between the two studies is that Kim and Holtzaple (2006) developed a modified kinetic model with the initial phase excluded for pretreatment time of weeks, while Fuentes et al. (2011) established a first-order reaction model without inclusion of the initial and residual phases for pretreatment time up to 108 h. Parameter estimation for constants (a_i , a_b , and a_r) and activation energy (E_a), and pretreatment conditions are summarized in Table 1. The results demonstrated the applicability of the first-order reaction corresponding to the initial, bulk, and residual phases of delignification to lime pretreatment under a wide range of pretreatment conditions. Depending on the variety of biomass feedstock and the pretreatment conditions, this delignification model may need to be modified in order to accurately predict compositional changes of biomass during alkaline pretreatment. Instead of the model based on three parallel first-order reactions, a kinetic model based on time-dependent rate constant was developed by Montane et al. (1994) to reasonably simulate kraft pulping delignification. This approach described a distribution of activation energies using the Kohlraush distribution function by assuming a continuous distribution of parallel first-order processes in the pulping system.

Table 1. Pretreatment Conditions and Parameter Estimation for Delignification Kinetic Models of Lime Pretreatment

Pretreatment conditions & model parameters	Corn stover (Kim and Holtzaple 2006)		Sugarcane bagasse (Fuentes et al. 2011)
	Non-oxidative	Oxidative	
Lime loading (g Ca(OH) ₂ /g dry biomass)	0.5		0.15, 0.25, 0.4, 0.55, 0.65
Temperature (°C)	25, 35, 45, 55		60, 70, 80, 90
Time	up to 16 weeks		up to 108 h
a_b	0.28	0.27	1
a_r	0.63	0.57	N/A
$E_{a(b)}$ (kJ/mol)	70.24	50.15	31.47
$E_{a(r)}$ (kJ/mol)	10.74	54.21	N/A

In a review of delignification kinetics, Bogren et al. (2007) described five successive events that occur during the delignification process by including the rates of mass transport of the involved species. In the delignification of a wood chip, as explained by Bogren et al. (2007), the chemicals initially transfer from the bulk liquor to the surface of the chip, and then transport to the reactive site for facilitating reactions and dissolution of wood components, after which the dissolved components move from the reactive site to the surface and finally to the bulk liquor. The concentrations of hydrosulphide, hydroxide ion, sodium ion, dissolved wood components, and lignin-carbohydrate complex (LCC) that influence the performance of pulping system have to be considered in a model representing kraft delignification (Bogren et al. 2007).

Severity factor approach

In the history of development of kinetic models for the kraft pulping process, the H factor, which combines the reaction temperature and reaction time into one variable, as

well as the effective alkali concentration, has been regarded as the core of process control schemes (Gustafson et al. 1983). The work conducted by Montane et al. (1994) indicated that the kinetic model based on a time-dependent rate constant is useless for non-isothermal processes. The reason is that the model does not incorporate the dependence of the reaction order regarding the SH^- concentration and the distribution of activation energies with the reaction temperature. This limitation prompted a formulation of a severity factor, combining reaction temperature, reaction time, and chemical (one or two active species) concentration (Montane et al. 1994). Satisfactory prediction in compositional changes and pulp yield using this severity factor has been demonstrated for sodium hydroxide, sodium bisulfate, and kraft pulping processes (Montane et al. 1994). The severity factor for acid pretreatment developed by Chum et al. (1990) has also given good performance in predicting the change of lignin content during alkaline pretreatment of cotton stalk (Silverstein et al. 2007). Based on the concept behind the severity factor, a simplified model, which is able to simulate and control the residual lignin content by simply determining the residual alkali concentration during the entire pulping cycle, was derived by introducing a correlation between the alkali concentration of a cooking liquor and the time or the time-temperature variable of pulping characterized as H factor (Masura 1999). The model parameters have different values for the initial, bulk, and residual phases according to Masura's (1999) results.

Nuclei growth model approach

The aforementioned studies of modeling delignification during alkaline pretreatment lack an intensive investigation on simulating carbohydrate losses of lignocellulosic materials. With this concern, Dang and Nguyen (2006, 2007) developed a universal kinetic model, based on the power law of growth and nuclei growth concepts proposed by Avrami (1940), to predict the changes of both lignin and carbohydrate for a variety of alkaline pulping conditions. This model describes the heterogeneous characteristics of alkaline pulping kinetics, taking into account the heterogeneous mass transport phenomenon and the effects of active alkali concentration and reaction temperature, and can be expressed as the following rate equation,

$$-dX/dt = knt^{n-1} C^b X \quad (5)$$

where X is the amount of the component (lignin, cellulose, or xylan) in the biomass during the course of the pretreatment; k and n are constants that are determined by the heterogeneous nature of the entire reaction system; and C is the chemical concentration (Dang and Nguyen 2006, 2007). Model (5) can be transformed to yield,

$$-dX/dt = a \cdot \exp[-E/(RT)]^n t^{n-1} C^b X \quad (6)$$

by assuming that the temperature effect follows the Arrhenius rule of thermodynamics (Dang and Nguyen 2006, 2007). The parameter C was then replaced with the dimensionless dielectric loss tangent of the alkali reagent for modeling compositional changes during microwave-based alkali pretreatment of switchgrass (Keshwani and Cheng 2010). Both kinetic models developed in these two studies show accurate predictions in

delignification and xylan loss but not in cellulose loss. This is because cellulose has the most uniform interlinked structure among the three components, thus resulting in the least variability of the rate coefficient of cellulose loss with respect to time (Keshwani and Cheng 2010).

Fuzzy-logic-based model approach

Due to the inherent variability in lignocellulosic biomass characteristics and heterogeneity of pretreatment reactions, an alternative approach other than kinetic modeling that is able to deal with non-random uncertainty is highly desirable. Fuzzy-logic-based modeling is such a method that has been successfully utilized for predicting the behavior of complex systems in the real world. Factors that may contribute to the success of this approach include (1) it is based on natural language for qualitative description in a fast and efficient way; (2) it is able to model nonlinear functions of arbitrary complexity; (3) it is tolerant of imprecise data; and (4) it can build a model based on the experienced understanding on the system or process being modeled. The development of fuzzy-logic-based models can be fulfilled using the fuzzy logic toolbox embedded in MATLAB.

Of various types of fuzzy relational models, the Mamdani-type fuzzy inference system was applied for the first time by Keshwani and Cheng (2010) to model microwave-based alkali pretreatment of switchgrass. A typical Mamdani-type fuzzy inference system comprises three components including input and output membership functions and the rule-base that maps the input space to the output space. Each rule in the Mamdani-type fuzzy inference system is developed based on an IF-THEN relationship between inputs and output. These components need to be determined based on expert opinion of the system being modeled and actual relationships between inputs and outputs observed from experimental data. In general, both input and output membership functions are represented by linguistic terms such as *low*, *medium*, and *high* which depict certain ranges in each dimension of data space. The fuzzy output set predicted by the rule-base has to be converted to crisp values by defuzzification routines for comparison with experimental values.

In Keshwani and Cheng's (2010) work, the levels of input factors were used to define the number of input membership functions and their location in the data space, while the number of output membership functions and their location were determined by clustering experimental output data with subtractive clustering followed by a fuzzy C-means algorithm. The rule-base was then established based on observations from the training data set. Their fuzzy-logic-based model has several improvements over the kinetic model using a time-dependent rate coefficient. They were able to predict multiple outputs using the same inference system, and reflect uncertainty and changes in the process by modifying membership functions and rules. The results obtained by Keshwani and Cheng (2010) have demonstrated the ability of the fuzzy inference system in predicting cellulose loss during the pretreatment with a high degree of accuracy. This good performance of the fuzzy-logic-based modeling approach in predicting compositional changes during alkali pretreatment suggests its potential application in other types of pretreatment of lignocellulosic biomass.

Physico-chemical Pretreatment Simulation

Steam explosion (autohydrolysis), ammonia fiber explosion (AFEX), and CO₂ explosion are the three common physico-chemical pretreatment technologies used in the lignocellulose-to-ethanol process (Alizadeh et al. 2005; Balan et al. 2009; Kim and Hong 2001; Lee et al. 2010; Negro et al. 2003; Teymouri et al. 2005; Zheng et al. 1998). The most promising pretreatment options, among the above three, are steam explosion and AFEX (Mosier et al. 2005; Yang and Wyman 2008); therefore, investigations on developing models with good performance for these two pretreatments are valuable for process control and optimization.

Kinetic model approach

Developing useful models for steam explosion of lignocellulosic biomass has seen abundant efforts during the past two decades (Conner 1984; Garrote et al. 2001, 2002; Mittal et al. 2009; Overend and Chornet 1987; Rogalinski et al. 2008; Zimbardi et al. 1999). Steam explosion can be viewed as a hydrolysis process in acidic media, since acetate present in biomass generates acetic acid during the course of hydrothermal reaction (Conner 1984). Accordingly, the pseudo-homogeneous kinetics with an Arrhenius-type temperature dependence of reaction rate constants, which has been successfully employed for acid pretreatment, is applicable to hydrothermal process (Garrote et al. 1999). The kinetic model that combines two parallel pseudo-first order reactions delivered the best fit to the removal of xylan (Conner 1984), while both the two-parallel-reaction model and the one considering only the degradation of the reactive part of xylan accurately predicted the yields of xylooligomer, xylose, furfural, and decomposition products (Garrote et al. 2001, 2002; Mittal et al. 2009). Apart from xylan solubilization simulation, modeling of glucan hydrolysis can be achieved using the pseudo-first-order reaction kinetics (Rogalinski et al. 2008).

Severity factor approach

Based on the theory of H factor applied in the pulping industry, Overend and Chornet (1987) developed a reaction ordinate factor (P),

$$P = t \exp[(T_r - 100)/14.75] \quad (7)$$

where T_r is the temperature of reaction, by combining the effects of steam temperature and residence time. The applicability of the P factor in simulating changes of biomass composition during steam explosion has been demonstrated for a variety of lignocellulosic materials (Overend and Chornet 1987; Ramos 2003; Zimbardi et al. 1999). Different types of reaction systems such as batch and continuous processes can result in varying P factors, which can be correlated with each other using a relationship proposed by Zimbardi et al. (1999). Considering non-isothermal conditions caused by the different temperatures during the heat-up periods, a modified severity factor that includes a temperature profile in the reaction system was developed by Rogalinski et al. (2008). This work is considerably useful for a wide range of hydrothermal pretreatment conditions, regardless of the length of heating or cooling times. Similar to the H factor,

the *P* factor is limited in its use for pretreatments relying on chemicals because it does not incorporate the effect of chemical concentration (Ramos 2003).

ENZYMATIC HYDROLYSIS PROCESS MODELING

Process Overview

Enzymatic hydrolysis of lignocellulosic biomass involves the use of cellulase and hemicellulase enzymes to convert cellulose and hemicellulose into hexoses (glucose, galactose, and mannose) and pentoses (xylose and arabinose). Cellulases are a mixture of three different cellulolytic enzymes, including endoglucanase (1,4- β -D-glucan glucanohydrolase), exoglucanase (1,4- β -D-glucan cellobiohydrolase), and cellobiase (β -glucosidase), that act synergistically to convert cellulose into glucose (Ladisich et al. 1983; Taherzadeh and Karimi 2007b). Endoglucanase randomly attacks and cleaves the β -1-4 glycosidic bonds of cellulose to produce cello-oligosaccharides and glucose. Exoglucanase releases cellobiose from the nonreducing ends of cello-oligosaccharides. This particular enzyme is able to work on both amorphous and crystalline celluloses. Once cellobiose is released into the hydrolysis liquor, cellobiase will degrade cellobiose into glucose. Cellulases generally act on amorphous regions of cellulose, and very few isolated cellulases have shown the ability to hydrolyze crystalline cellulose (Shewale 1982). The inhibition of endoglucanase and exoglucanase by cellobiose (Holtzapple et al. 1984) as well as the inhibition of β -glucosidase by glucose (Gong et al. 1977) can be significant factors affecting the performance of cellulolytic enzymes in the process. In the hydrolysis of xylan, which is the dominant hemicellulose in many lignocellulosic feedstocks, three major enzymes including endo- β -1-4-xylanase, which catalyzes the hydrolysis of the β -1-4 bonds between D-xylose residues of heteroxylans and xylo-oligosaccharides, exoxylanase which releases xylobioses, and β -xylosidase which degrades xylo-oligosaccharides and xylobiose to xylose are involved (Saha and Bothast 1999). The activity of xylanase is likely to increase the accessibility of cellulose to cellulase by enhancing the removal and solubilization of xylan (Berlin et al. 2005; Xu et al. 2011). Depending on the variety of biomass feedstock and the type of pretreatment technology used, different combinations of the aforementioned enzymes need to be carefully selected for efficient enzymatic hydrolysis (Xu et al. 2011).

In enzymatic hydrolysis, the first step is the formation of an enzyme-substrate complex, which involves the mass transfer of enzyme from bulk aqueous phase to cellulose/hemicellulose surface and the formation of an enzyme-substrate complex following enzyme adsorption. The subsequent hydrolysis of cellulose/hemicellulose has two possible modes of action with respect to the location of enzyme and substrate. One mode of action focuses on the movement of substrate and incorporates three major steps (O'Dwyer et al. 2007). First, the reactant molecules are transferred to the active site of the enzyme-substrate complex. Then reaction is catalyzed by the enzyme, followed by release of soluble products to the bulk aqueous phase. The other mode of action illustrated by Ladisich et al. (1983) refers to the location of enzyme, which either moves to the next reaction site along the surface of cellulose or desorbs and then readsorbs onto cellulose. In cellulose hydrolysis, the conversion of cellulose to cellobiose and glucose

involves two heterogeneous reactions, while the degradation of cellobiose to glucose is considered to be a homogeneous reaction (Kadam et al. 2004).

Factors Affecting Enzymatic Hydrolysis of Lignocellulosic Biomass

Different pretreatments can result in various consequences involving changes in physical and chemical characteristics (cellulose crystallinity index, degree of polymerization, lignin content, pore volume and size, and surface area accessibility) of lignocellulosic materials, and the changes in these features will have impact on the kinetics of cellulolytic enzymes (Chang and Holtzaple 2000; Zhang and Lynd 2004). Substrate concentration in the hydrolysis slurry, activity of cellulolytic enzymes, and hydrolysis conditions including temperature, pH, and mixing also have impact on the effectiveness and efficiency of enzymatic hydrolysis of cellulosic biomass (Taherzadeh and Karimi 2007b). Chang and Holtzaple (2000) stated that both lignin content and crystallinity index of cellulosic biomass have remarkable impact on ultimate sugar yield, while the initial hydrolysis rate is predominantly affected by crystallinity index. Later studies by Gollapalli et al. (2002) and Laureano-Perez et al. (2005) confirmed the findings, but the overall conversion efficiency of carbohydrates was reported to be mainly dependent on the amount of residual lignin in pretreated biomass (Laureano-Perez et al. 2005). During pretreatment, hydrogen bonds that contribute to the high degree of cellulose crystallinity can be broken down, which is beneficial for enhancing the initial hydrolysis rate.

In addition to the major contributions of delignification and decrystallization to biomass digestibility, deacetylation facilitates the overall hydrolysis efficiency by improving hemicellulose solubilization (Chang and Holtzaple 2000). As the reaction progresses, several factors including end-product inhibition, low substrate reactivity, enzyme inactivation, and loss of enzyme because of irreversible adsorption on lignin, will slow down hydrolysis rates (O'Dwyer et al. 2007). Specifically, the substrate reactivity is dependent on physical and chemical characteristics of the biomass. After pretreatment, the accessibility of reactants to the active site of enzyme tends to increase, but the degree of polymerization is likely to decrease. These changes would improve the overall substrate reactivity. A common theme for studying the effects of biomass properties on enzymatic hydrolysis efficiency is to build a mathematical correlation through statistical analysis, with some models showing good predictive ability (Chang and Holtzaple 2000; Laureano-Perez et al. 2005) but others not being able to accurately predict the digestibility of biomass (Gollapalli et al. 2002).

Adsorption Based Kinetic Models Approach

The kinetics of enzymatic hydrolysis of lignocellulosic substrates is very complex because of multiple hydrolytic enzyme activities encompassed in the process and the heterogeneous nature of substrate (Kadam et al. 2004). To successfully model the cellulase kinetics, a thorough fundamental understanding of physical and chemical properties of the reacting substrate and its relevant enzyme, and a full investigation of rate-limiting factors are required (Bansal et al. 2009). Both Zhang and Lynd (2004) and Bansal et al. (2009) have conducted comprehensive reviews on understanding the basic assumptions of a number of kinetic models that have been developed for hydrolysis of

lignocellulosic substrates by cellulolytic enzymes; they identified shortcomings and potential improvements for these models. In particular, Bansal et al. (2009) classified all the models into four categories including empirical models, Michaelis-Menten based models, adsorption based models, and soluble substrates based models. Previous work on empirical models has seen limitations on their applicability to conditions outside those used for model development. Moreover, they are useless in terms of disclosing the mechanism of enzymatic hydrolysis. Likewise, soluble substrates based models are not applicable to the enzymatic hydrolysis of insoluble lignocellulosic substrate. Therefore, adsorption based and Michaelis-Menten based kinetic models are the focus of this paper.

Adsorption of cellulases onto insoluble substrates is an essential part of the kinetics of cellulose hydrolysis (Bernardez et al. 1993; Zhang and Lynd 2004), which can be assumed to follow a Langmuir-type isotherm. The first attempt to model the kinetics of cellulose hydrolysis at high substrate and enzyme concentrations was by Wald et al. (1984) who incorporated an enzyme adsorption mechanism into their model. A typical example of the Langmuir isotherm can be given as,

$$E_B = (E_{\max} K_{ad} E_f S) / (1 + K_{ad} E_f) \quad (8)$$

where E_B is the adsorbed enzyme concentration (mg cellulase/L), E_{\max} is the maximum adsorption capacity in the unit of mg cellulase per gram cellulose, K_{ad} is the dissociation constant for adsorption, E_f is the free enzyme concentration, and S is the substrate concentration (Bansal et al. 2009; Kadam et al. 2004). A simplified expression of Equation (8) is: [adsorbed enzyme] = constant \times [free enzyme] \times [substrate], according to South et al. (1995). Although the underlying assumptions (including uniform binding sites and independence of the adsorbing molecules) for the Langmuir isotherm are invalid for cellulolytic enzymes adsorption onto lignocellulosic substrates, the Langmuir equation generally provides a good fit to the cellulase adsorption data for cellulosic substrates (Zhang and Lynd 2004), with standard microcrystalline cellulosic substrate such as Avicel showing an excellent fit (Boussaid and Saddler 1999). The capacity to bind cellulase onto Avicel was reported to be lower than that for pretreated mixed hardwood, as hemicellulose and lignin can also bind cellulase (Bernardez et al. 1994).

High cellulase adsorption capacity is critical for pretreated biomass to bind a sufficient amount of cellulase and then render high hydrolysis rate and sugar yield (Kumar and Wyman 2009a,b). According to Kumar et al. (2009) who studied cellulase adsorption capacity of corn stover and poplar solids pretreated with leading pretreatment technologies, lime pretreated corn stover and flowthrough pretreated poplar had considerably higher cellulases adsorption capacity than dilute acid pretreated corn stover and ammonia fiber explosion (AFEX) pretreated poplar. The study also found that the lignin remaining in lime-pretreated biomass has higher cellulase adsorption capacities than that left in AFEX pretreated biomass. The mechanism for cellulase adsorption onto lignin deserves extensive research efforts because lignin interferes with the performance of cellulases by acting as a competitive cellulases adsorbent that reduces the amount of cellulases available to hydrolyze cellulose (Bernardez et al. 1993; Ooshima et al. 1990) or by blocking enzymatic access to the substrate (Eriksson et al. 2002).

The adsorption model parameters are believed to differ accordingly for different pretreated lignocellulosic substrates and various enzymatic reaction schemes. Table 2 summarizes the values of cellulase adsorption parameters obtained for different substrates pretreated using leading pretreatment technologies.

Table 2. Cellulase Adsorption Parameters for Enzymatic Hydrolysis of Lignocellulosic Substrates Determined by Published Studies

References	Feedstock	Pretreatment	Maximum adsorption capacity (mg/g substrate)	Dissociation constant (L/g substrate)
Kumar and Wyman (2009a)	Corn stover	AFEX	99.7	1.86
		Dilute acid	90.7	2.49
		Lime	133.6	0.88
Kumar and Wyman (2009b)	Poplar wood	AFEX	107.4	0.21
		Dilute acid	170.9	0.94
		Lime	150.8	0.09
Kadam et al. (2004)	Corn stover	Dilute acid	60	2.22

These results show that the type of feedstock, the variety of pretreatment method, and the associated pretreatment conditions can result in the marked variability of adsorption parameters. As compared to pure cellulose substrates, lignocellulosic substrates tend to have more noticeable changes in adsorption attributes such as the maximum adsorption capacity (Bansal et al. 2009). Therefore, when applying adsorption isotherm equation to model kinetics of enzymatic hydrolysis of lignocellulosic biomass, it is important to validate the model against experimental values of the adsorbed enzyme concentration during the course of hydrolysis. Several studies have considered this scenario and indicated that the adsorption characteristics can be caused by not only the properties of enzymes used but also the nature of substrates (Liao et al. 2008; Nidetzky and Steiner 1993; Shao et al. 2009). The effects of temperature and competitive sugar inhibitions also need careful consideration in developing a successful kinetic model for enzymatic hydrolysis of lignocellulose (Kadam et al. 2004).

Michaelis-Menten Based Kinetic Models Approach

The simplest enzymatic reaction is considered to be irreversible, and no product inhibition exists during the reaction, with the reaction scheme shown as,



where E is the enzyme, S is the substrate, ES is the enzyme-substrate complex, P is the product, k_1 is the forward rate constant for the formation of enzyme-substrate complex, k_{-1} is the dissociation rate constant of the enzyme-substrate complex, and k_2 is the rate constant of product formation. A typical kinetic model for homogeneous enzymatic reaction follows the Michaelis-Menten equation, which is described as,

$$v = (v_{\max} [S]) / (K_M + [S]) \quad (10)$$

where v is the conversion rate of substrate, v_{\max} is the maximum conversion rate of substrate, $[S]$ is the substrate concentration, and K_M is the Michaelis constant.

The quasi-steady state assumption employed in the derivation of Equation (10) cannot be directly applied in enzymatic hydrolysis of insoluble lignocellulosic substrates since it is a heterogeneous reaction system (Bansal et al. 2009). However, the published studies have showcased satisfying applications of Michaelis-Menten based models in simulating the complex enzymatic reaction system for both pure cellulose (Bezerra and Dias 2004; Caminal et al. 1985; Grous et al. 1985; Huang 1975; Ohmine et al. 1983) and lignocellulosic substrates (Brown et al. 2010; Kadam et al. 2004; O'Dwyer et al. 2007).

The identification of inhibition pattern of kinetic models for enzymatic hydrolysis of cellulosic biomass is of particular interest, as product inhibition can limit the sugar production from the biomass. Competitive inhibition has been the primary concern in this context, and the results show that the kinetic models incorporating competitive inhibition are able to fit experimental data very well (Bezerra and Dias 2004; Grous et al. 1985; Huang 1975; Kadam et al. 2004). Another pattern is non-competitive inhibition, which is regarded as the consequence of non-preferential and irreversible binding of enzymes to lignin (Holtzapple et al. 1984; O'Dwyer et al. 2007). This inhibition pattern has been successfully covered by the HCH-1 model, which may be expressed as,

$$V = k[S][E]/(\alpha + \phi[S] + \varepsilon[E]) \quad (11)$$

where $[S]$ is the substrate concentration, $[E]$ is the enzyme concentration, ϕ is the fraction of the substrate surface that is accessible to be hydrolyzed, and k , α , and ε are parameters that represent the degree of substrate reactivity. As reviewed by Bansal et al. (2009), the decreasing rate of enzymatic hydrolysis with increasing conversion can be attributed to enzymatic deactivation, biphasic composition of cellulose, decrease in substrate reactivity and accessibility, fractal or spatially constrained reacting environment, decrease in synergism of cellulase components, and interference by lignin. All these factors need to be considered in the development of any kinetic models for lignocellulose hydrolysis by cellulolytic enzymes in order to satisfactorily simulate the hydrolysis at high conversion levels.

Most of the cellulase kinetic models developed for hydrolysis of cellulosic substrates lack in the involvement of more than one substrate state variable and more than one hydrolyzing activity. In view of this issue, Zhang and Lynd (2006) proposed a functionally based model, taking into account multiple substrate variables and more than one solubilizing activity, for cellulase kinetics using pure substrate. They found that the fraction of accessible β -glucosidic bonds and the degree of polymerization are highly correlated with substrate reactivity, thus influencing the hydrolysis rate. The degree of synergy between endoglucanase and exoglucanase predicted by their model increases as the extents of the two substrate parameters are raised. Regardless of the significant contribution of their model to the fundamental and applied investigation on enzymatic cellulose hydrolysis, more realistic scenarios should be analyzed and included in the development of more rigorous models.

CONCLUSIONS AND OUTLOOK

Sugar produced from lignocellulosic materials has shown a great potential of applications in the production of biofuel and other value-added products such as xylitol, lactic acid, and vanillin. The major challenge associated with the production of these chemicals is how we can effectively release sugars as cheaply as possible from cellulose and hemicellulose present in the recalcitrant biomass. To realize this goal, factors that hinder the conversion of cellulosic biomass to sugar need to be well understood, which will require wisely linking computer simulations with experimental results. Research efforts on modeling the conversion process have been focused on the kinetic behavior of cellulose, xylan, and lignin present in the biomass. Modeling compositional changes of the biomass for the pretreatment process presents variability due to the nature of the heterogeneous materials and the complex reactions involved in the system. The following three points appear to be important for comparison of different models applied in pretreatment of lignocellulosic biomass.

1. In the development of kinetic models for simulating pretreatment processes, the impact of pretreatment conditions such as temperature, chemical concentration or pH value, and residence time, as well as the interactions between lignin and carbohydrates on the reaction rate of each major component needs to be considered. A deeper understanding of pretreatment mechanisms is useful in building robust models that are appropriate for a wide range of pretreatment technologies.
2. A severity factor that combines all pretreatment conditions into one variable can be integrated into kinetic or empirical models to simplify the inputs for saving efforts in process optimization.
3. Non-kinetic models that exclude mathematical formulas, such as artificial neural network and fuzzy-logic-based inference systems, tend to be more efficient than kinetic models in modeling the changes of cellulose, hemicellulose, and lignin in a variety of pretreatment processes.

In light of the inherent complexity of enzymatic reaction of lignocellulose and its associated inhibition patterns, Langmuir-type adsorption and Michaelis-Menten based models have shown good performance in simulating kinetics of cellulolytic enzymes for heterogeneous cellulosic substrates. Factors including substrate reactivity, enzyme activity and accessibility, irreversible binding of enzymes to lignin, and enzyme deactivation at high conversion levels, which considerably influence the kinetics of enzymatic hydrolysis, ought to be investigated and considered in model development. Inclusion of multiple substrate state variables and more than one hydrolyzing activity into a functionally based model development is also believed to be the trend for simulating enzymatic hydrolysis of lignocellulosic biomass. Until the present, however, most of the process models developed in the context of biochemical conversion of lignocellulose to sugar cannot be applied to the entire conversion scheme. In order to accurately predict the behavior of the entire conversion system for efficient process design and optimization, different existing models should be integrated. To make that take place, a thorough

understanding of the conversion system as to physical and chemical properties of biomass, pretreatment reaction mechanism, and enzyme kinetics is also required.

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