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Kinetic Modeling of Enzymatic Hydrolysis of Pretreated Sorghum Bicolor and Rice Husk

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ABSTRACT

In this study, hydrolysis of pretreated sorghum stem and rice husk was investigated at various initial enzyme concentrations and substrate loadings. The slowdown in enzymatic hydrolysis of lignocellulosic materials with conversion has often been attributed to decreasing the activity of enzyme. A kinetic model was developed and expressed mathematically based on enzyme deactivation for enzymatic hydrolysis of lignocellulosic materials. The decline in activation of the adsorbed enzyme is represented by a second order reaction. The models were used to fit experimental data of sorghum stem and rice husk hydrolysis. The kinetic parameters of model were determined by experimental results and evaluated. The model performed well in predicting hydrolysis trends at experimental condition.

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1. INTRODUCTION

Enzymatic hydrolysis of lignocellulosic material is still considered as one of the main limiting steps in bioethanol production [1]. The main challenges associated with enzymatic hydrolysis are slow reaction rates, high enzyme costs, and poor understanding of enzyme kinetics on lignocellulosic substrates [2]. Lignocellulosic materials are insoluble and consist of components resistant to enzymatic degradation [3]. Successful hydrolysis of these materials needs synergistic actions of different cellulase components through heterogeneous reactions [4]. These facts indicate that the mechanisms of the hydrolysis reactions are sequential and indeed very complicated. Kinetic modeling of enzymatic hydrolysis is necessary to better understand the interactions between enzyme and substrate, to integrate present and future enzyme improvements, and to design optimized reactors. Toward this aim, extensive studies of enzymatic hydrolysis kinetics including empirical and mechanistic models have been carried out over the last years [5-8]. The empirical models have been generally used to correlate hydrolysis with either the structural properties of the substrate or with time [2]. These models however, could not provide any understanding of the mechanistic details of the process and therefore, they cannot be applicable outside the conditions under which they are developed [9]. The mechanistic models can be based on Michaelis-Menten, soluble substrate and adsorption. They could overcome the empirical models drawbacks. The classical Michaelis-Menten model has been usually used for the kinetic studies of enzymatic hydrolysis by the initial velocity approach [10, 11], although, it has been shown that this model is not suitable for the enzymatic hydrolysis of substrate with the heterogeneous structures, especially when the enzymatic reaction is diffusion limited [12]. However, they can be applied to describe the hydrolysis of soluble substrates, but due to heterogeneous nature of enzyme action on insoluble cellulosic biomass, extension to insoluble substrates is not straightforward. Some of the mechanistic models of enzymatic hydrolysis have been achieved with the Langmuir adsorption isotherm, or

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with the help of kinetic equations [4, 13]. Although these models are useful for recognizing surface phenomenon, however, most of them provide no information on the adjustments of enzyme concentration as well as the hydrolysis product as a function of hydrolysis time. Another important phenomenon which has not been considered in many mechanistic models is cellulase deactivation. Above-mentioned factors are critical for economy of enzyme related industries [14]. While it is important that a mathematical demonstration of reaction kinetics should incorporate these factors, but on the other hand, the model should not be overcomplicated by attempting to cover all system complexities. Many of relevant previous modeling efforts contain several differential equations where determinations of corresponding parameters are often very difficult [2, 13, 15]. By far, only a few simple models have been proposed considering these factors [9, 16], while still lacking to describe the biomass decomposition to xylose as a main pentose sugar which can be metabolized by some microorganism for ethanol production. The cellulase enzyme derived from *Trichoderma reesei* contains minor amounts of endo-1,4-β-xylanase and β-xylosidase, which can catalyze the degradation of hemicellulose. Therefore, the xylose formation need to be considered in the developed model.

Iran is an agricultural country and produces a number of cellulosic materials that can be used to produce good quality ethanol. Rice husk, one of the agricultural residues, can be used as an excellent prospective source of raw material for the production of bioethanol, because of the cheapness, relatively high content of holocellulose (57-61% by weight) and wide availability of this lignocellosic biomass [10, 17]. Sorghum bicolor (broomcorn) ranks fifth in global cereal production [18]. It is drought resistant and needs only limited water and normal conditions to grow [19]. This plant is valued for its grain, stems and leaves [20]. Sorghum stem has been used to make various types of brooms, brushes, building material, fencing and decorative items for several hundred years. Due to the high content of carbohydrates in sorghum stem, it has become a potential feedstock for ethanol production. In recent years, several efforts have been made to use rice husk and sorghum stem as the feedstock for production of bioethanol [19, 21-23]. Also some progress has been made on the utilization of sorghum stem and rice husk, including pretreatment and saccharification [17, 24, 25], however, less attention has been paid to the kinetics study of their enzymatic hydrolysis. The objective of this work was to study the kinetics of enzymatic hydrolysis of rice husk and sorghum stem, after being pretreated by NaOH. A kinetic model based on enzyme deactivation was deduced to describe the relationship between products (glucose and xylose) concentration and enzymatic hydrolysis conditions.

2. MATERIALS AND METHODS

2. 1. Substrates and Enzymes Sorghum bicolor and rice husks (Hashemi Tarom) were obtained from local market in (Amircola) Babol and (Katehposht) Amol, Iran. The husks were separated using a mechanical paddy de-husker. The sorghum stems after removal of leaves, were cut to nominally 3-4 cm in length. Then samples were ground with a food homogenizer (Black & Decker, Model No. FX350, England) and then passed through a 40 mesh screen (particle size≤0.42 mm). Finally, the screened materials were stored in tightly sealed plastic bags at room temperature until further use. The fresh raw materials were oven dried at 105 °C for 24 h to a dry matter content of 93.09 and 89.66% for sorghum stem and rice husk, respectively. The compositional analysis of native sorghum stem and rice husk was carried out by two stage acid hydrolysis protocol developed by National Renewable Energy Laboratory [26]. The constituent of untreated materials in this study is presented in Table 1.

Two commercial enzymes, cellulase (Celluclast 1.5L) and *β*-glucosidase (Novozyme 188) were purchased from Novozymes A/S (Bagsvaerd (Denmark) and Sigma-Aldrich Co. (St. Louis, USA), respectively. Activity of Cellulase was measured according to the standard procedure provided by the NREL [27]. The activity of celluclast 1.5L (cellulase) was measured as 45 (FPU)/mL. The activity of β-glucosidase (Novozym 188) was 250 IU/mL as reported by the supplier.

2. 2. Pretreatment and Enzymatic Hydrolysis Sorghum stem and rice husk were pretreated with dilute NaOH solution in an autoclave at 121°C. Optimization of parameters for alkaline pretreatment of biomasses was performed in our previous studies [28, 29]. The optimum operating conditions (sodium hydroxide concentration, pretreatment time and solid loading) were found to be 1.7%, 60.4 min and 4.2% for sorghum stem and 2.28%, 60.53 min and 6.8% for rice husk, respectively.

TABLE 1. Constituent of sorghum stem and rice husk in this study (dry-weight basis)

Component	Dry weight (%)	
	Sorghum stem	Rice husk
Cellulose	47.58	37.55
Hemicellulose	24.66	15.24
Total lignin	24.73	19.22
Ash	1.93	16.00
Extractive	1.10	11.90

The solid fraction resulted from the pretreatment of raw materials at the optimum conditions was washed with distilled water to neutralize pH and kept at 5 °C for use in saccharification stage.

The defined amounts of pretreated biomasses were soaked in 50 mM sodium citrate buffer at pH 4.8 and then incubated at 50 °C for 10 min before adding enzyme. After reaching to 50 °C, cellulase and *β*-glucosidase were added. The cellulase was supplemented with Novozyme 188 to reduce inhibition effect of cellobiose on cellulase. Enzymatic hydrolysis was carried out at 50 °C and 120 rpm in a shaker incubator (IKA, Germany) for 24 h. The enzymatic hydrolysis experiments were carried out by different initial enzyme concentrations (2.87, 5.75, 8.63 and 11.5 g/L) and different solid loadings of rice husk (3, 6, 9, 12 and 15% w/v) and sorghum stem (4, 6, 8 and 10% w/v), respectively. Samples were taken at certain time interval and were stored at -20° C until used for analysis of sugar content. Each experiment was replicated twice and the reported results indicate the average values.

2. 3. Analytical Method Liquid samples taken from enzymatic hydrolysis stage were centrifuged at 13000 rpm for 5 min (Hermle, Germany) and the supernatants were used for HPLC analysis. The glucose and xylose concentration in the hydrolyzate were determined by HPLC (Knauer, Germany) using a Eurokat H (300×8 mm) column coupled with a refractive index (RI) detector (Knauer, Smartline RI Detector 2400, Germany). The mobile phase was 0.01N H_2SO_4 at a flow rate of 0.4 mL/min. The column temperature was 75 °C. For characterizing Langmuir adsorption behavior, free enzyme concentrations in the supernatant was measured by Bradford assay using Coomassie blue dye (Pierce Biotechnology, Rockford, IL).

3. KINETIC MODELING

3. 1. Model Assumption In this study, Langmuir model was used for describing the enzyme adsorption onto the solid substrates. The physical structure of lignocellulosic material consists of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are polysaccharides which can be converted to fermentable sugars. Cellulose fraction in lignocellulosic biomass is composed of crystalline and amorphous components, but hemicellulose is naturally amorphous. Each of them show different digestibility against enzymatic attack. In this work, the structure of the insoluble substrate was considered uniform and homogeneous (the substrate structure was not divided into amorphous and crystalline fraction) [16]. This assumption simplifies enzyme adsorption modeling by using the Langmuir formulation.

Cellulase refers to a group of enzymes that contribute to the degradation of cellulose and hemicellulose to glucose and xylose, respectively. Endo-1,4-β-glucanase, exo-1,4-β-glucanase, exo-1,4-β-glucosidase, β-glucosidase, endo-14-β-xylanase and β-xylosidase synergistically hydrolyze lignocellulosic substrates [4, 16]. The first four enzymes produce glucose; hemicellulose is hydrolyzed to xylose by the action of endo-14-β-xylanase and β-xylosidase. In this study, cellulase was assumed to be a single enzyme which hydrolyzes insoluble substrate to produce glucose and xylose. This assumption obviates the need to independently measure distinct enzyme component concentration.

It has been generally accepted that partial cellulase deactivation occurs during the enzymatic hydrolytic process. Cellulase can be deactivated by many factors including shear force, temperature, substrate crystallinity and product inhibition [9, 14, 30]. The ineffective adsorption of cellulase to substrate and formation of a non-productive enzyme-substrate complex can deactivate enzyme. In this study, product inhibition was elaborated in cellulase deactivation. The enzyme deactivation was considered in developing the model and was assumed a second order reaction. Under similar assumption, Zhang et al. assumed both first and second order reaction for enzyme deactivation. Their comparison indicated that the second order model is much more suitable to describe enzyme deactivation [9]. The above mentioned assumptions were made to simplify the enzymatic hydrolysis model.

3. 2. Model Mechanism Based on the considered assumptions, the enzymatic hydrolysis mechanism involves cellulase (E) (g/L) adsorpsion on the active sites of the cellulose (C) (g/L) and hemicellulose (H) (g/L) to form complexes (CE^*) (g/L) and (HE^*) (g/L), respectively. They are shown as following equations:

$$C + E \overset{K_1}{\Leftrightarrow} CE^*, \quad K_1 = \frac{k_1}{k_{-1}} \tag{1}$$

$$H + E \overset{K_1'}{\Leftrightarrow} HE^*, \quad K_1' = \frac{k_1'}{k_{-1}'} \tag{2}$$

where K_1 and K_1' are the equilibrium constant of enzyme adsorption on cellulose and hemicellulose, respectively. k_1 and k_1' (L/h.g) are the rate constants of forward reaction and k_{-1} and k_{-1}' (L/h.g) are the rate constants of backward reaction, respectively. The (CE^*) and (HE^*) complexes will in turn produce free enzyme and glucose (G) (g/L) and xylose (X) (g/L), respectively, as expressed in the following reactions:

$$CE^* \xrightarrow{k_2} 0.9G + E \quad (3)$$

$$HE^* \xrightarrow{k_2'} 0.88X + E \quad (4)$$

where k_2 and k_2' (h^{-1}) are the rate constants of glucose and xylose formation, respectively. The concentration of the cellulose and hemicellulose is related to the concentration of the corresponding monomeric sugars by a conversion coefficient of 0.90 (or162/180) for glucose and 0.88 (or 132/150) for xylose, respectively. Some part of cellulase is adsorbed on the insoluble cellulose to produce ineffective complex (CE_{in}^*) (g/L) causing reduced enzymes activity and therefore is not effective in the hydrolysis:

$$C + E \xrightarrow{k_3} CE_{in}^* \quad (5)$$

where k_3 (L/h.g) is the rate constant of ineffective complex formation. According to mass action law, the reaction rate can be the n^{th} power of the reactants. In Equations (1) to (4), the value of n was considered 1. So, the complex forming rates from these equations are:

$$\frac{dCE^*}{dt} = k_1 C \times E - k_{-1} CE^* - k_2 CE^* \quad (6)$$

$$\frac{dHE^*}{dt} = k_1' H \times E - k_{-1}' HE^* - k_2' HE^* \quad (7)$$

Mass balance on the cellulose and hemicellulose yields the substrate concentrations, C and H:

$$C = C_0 - CE^* - CE_{in}^* - 0.9G \quad (8)$$

$$H = H_0 - HE^* - 0.88X \quad (9)$$

where C_0 and H_0 are the initial concentrations of cellulose and hemicellulose, respectively. Following equations were obtained by substituting Equations (8) and (9) into Equations (6) and (7), respectively:

$$\frac{dCE^*}{dt} = k_1\left(C_0 - CE^* - CE_{in}^* - 0.9G\right) \times E - k_{-1} CE^* - k_2 CE^* \quad (10)$$

$$\frac{dHe^*}{dt} = k_1'\left(H_0 - HE^* - 0.88X\right) \times E - k_{-1}' HE^* - k_2' HE^* \quad (11)$$

Applying the quasi-steady state condition to above equations and assuming $C_0 >> CE_{in}^*$, the complex concentrations are:

$$CE^* = \frac{(C_0 - 0.9G)E}{K_E + E} \qquad K_E = \frac{k_{-1} + k_2}{k_1} \quad (12)$$

$$HE^* = \frac{(H_0 - 0.88X)E}{K_E' + E} \qquad K_E' = \frac{k_{-1}' + k_2'}{k_1'} \quad (13)$$

where K_E and K_E' (g/L) are the equilibrium constants. Considering the enzyme mass balance, the free enzyme concentration, E, is given by:

$$E = E_0 - CE^* - CE_{in}^* - HE^* \quad (14)$$

where E_0 is the initial concentration of enzyme. Based on Reactions (3) and (4), the glucose and xylose concentrations during the enzymatic hydrolysis process are obtained by following equations:

$$\frac{d(G)}{dt} = k_2 CE^* \quad (15)$$

$$\frac{d(X)}{dt} = k_2' HE^* \quad (16)$$

In order to calculate the accurate value of glucose and xylose concentration from above equations, it is required to define the enzyme activity variation during the hydrolysis time. In this study, the enzyme deactivation by the insoluble substrate was assumed independent and considered as a second order reaction, which is given by:

$$-\frac{dE}{dt} = \frac{dCE_{in}^*}{dt} = \left(k_3 E^2\right) \quad (17)$$

where k_3 (h^{-1}) is the rate constant of enzyme deactivation. Integrating Equation (17) with the boundary conditions $E=E$ at $t=t$ and $E=E_0$ at $t=0$ produces:

$$E = \frac{E_0}{1 + k_3 E_0 t} \quad (18)$$

By substituting Equations (12), (13) and (18) into Equations (15) and (16), the following equations are developed:

$$\frac{d(G)}{dt} = \frac{k_2 (C_0 - 0.9G) E_0}{K_E (1 + k_3 E_0 t) + E_0} \quad (19)$$

$$\frac{d(X)}{dt} = \frac{k_2' (H_0 - 0.88X) E_0}{K_E' (1 + k_3 E_0 t) + E_0} \quad (20)$$

Integration of Equations (19) and (20) by applying boundary conditions $G=X=0$ at $t=0$ and $G=G$ and $X=X$ at $t=t$ gives the following equations for glucose and xylose concentration during enzymatic hydrolysis:

$$G = \frac{C_0}{0.9}\left\{1 - \left[\frac{K_E + E_0}{K_E (1 + k_3 E_0 t) + E_0}\right]^b\right\}, b = \frac{k_2}{K_e k_3} \quad (21)$$

$$X = \frac{H_0}{0.88}\left\{1 - \left[\frac{K_E' + E_0}{K_E' (1 + k_3 E_0 t) + E_0}\right]^{b'}\right\}, b' = \frac{k_2'}{K_e' k_3} \quad (22)$$

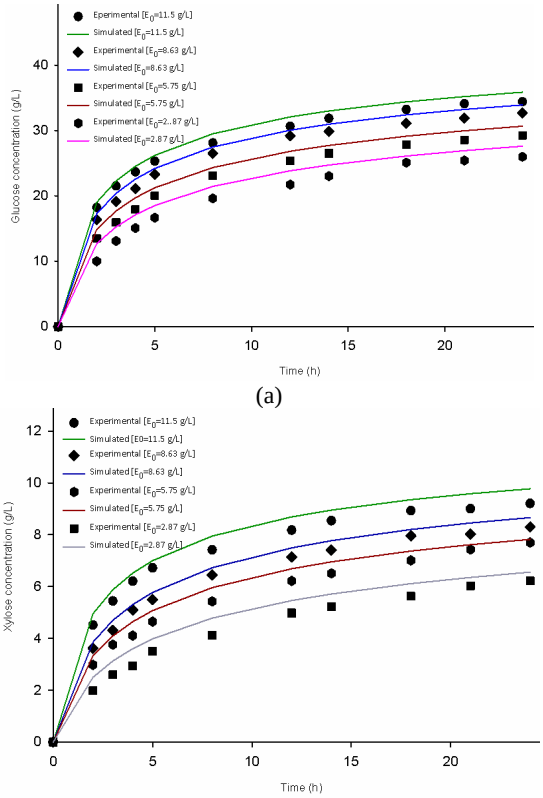
Equations (21) and (22) are two parameter models which describe the relationship between glucose (G)

and xylose (X) concentrations and hydrolysis time t, at different initial concentration of enzyme (E_0), cellulose (C_0) and hemicellulose (H_0). The proposed models take into account the enzyme deactivation during the hydrolysis and the decomposition of substrate. In addition, they represent enzyme adsorption via a Langmuir-type isotherm. Compared to other sophisticated models developed for enzymatic hydrolysis of lignocellulosic biomass that contain many parameters, these models have simple equation and few parameters.

4. RESULTS AND DISCUSSION

4. 1. Effect of Initial Enzyme Concentration

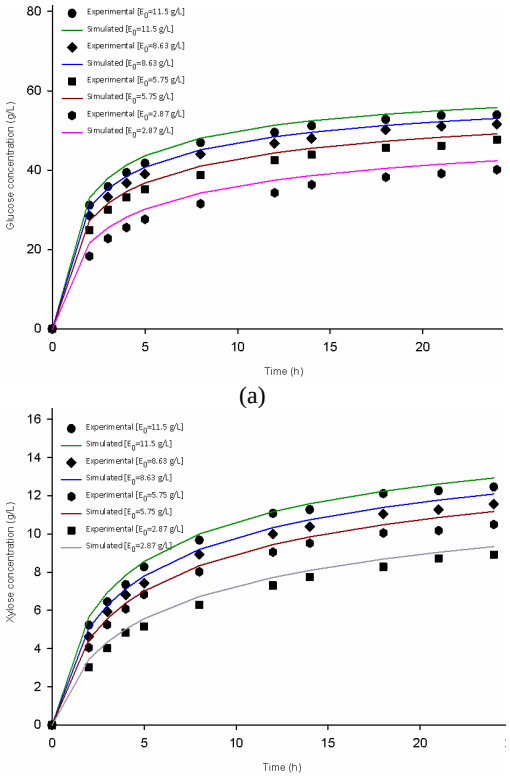
Different initial concentrations of enzyme were used for hydrolysis of pretreated substrates. Figure 1 and 2 illustrate the effect of the initial enzyme concentrations on production of glucose and xylose during the rice husk and sorghum stem hydrolysis, respectively. Increasing initial concentration of enzyme resulted in a faster initial reaction rate, and higher amount of glucose and xylose production.



(a)

(b)

Figure 1. Simulated values versus experimental values of (a) glucose and (b) xylose concentration for hydrolysis of rice husk at different initial enzyme concentrations [solid loading=12% (w/v)]



(a)

(b)

Figure 2. Simulated values versus experimental values of (a) glucose and (b) xylose concentration for hydrolysis of sorghum stem at different initial enzyme concentrations [solid loading=10% (w/v)]

For pretreated rice husk, the glucose and xylose concentrations increased from 27.11 to 36.84 g/L and 4.23 to 7.94 g/L when the initial enzyme concentration was increased from 2.87 to 11.5 g/L, respectively. Likewise, for pretreated sorghum stem, in the same initial concentrations range of enzyme, the glucose and xylose concentrations increased from 42.65 to 57.29 g/L and 9.01 to 12.46 g/L, respectively. When initial enzyme concentration is above 8.63 g/L, small difference is observed probably due to saturation of the substrate-enzyme complex.

At all enzyme concentrations, over 65% glucose and 75% xylose were produced within the first 4 h of enzymatic hydrolysis. The reduction of hydrolysis rate might be attributed to the enzyme deactivation and substrate recalcitrance [11].

Table 2 presents an analysis of the efficiency of enzyme utilization by calculating the yield of glucose and xylose concentration per unit mass of enzyme used in the hydrolysis. It shows that enzyme efficiency decreased with raise in the enzyme concentration despite the ultimate increased glucose and xylose productions. Thus, the optimal value of the initial enzyme concentration should be determined on the basis of the yield per gram of enzyme.

TABLE 2. Effect of initial enzyme concentration on the enzyme utilization efficiency

Enzyme concentration (g/L)	Glucose yield (g glucose/g enzyme)		Xylose yield (g xylose/g enzyme)	
	Sorghum stem	Rice husk	Sorghum stem	Rice husk
2.87	13.97	9.06	3.10	2.16
5.75	8.30	5.08	1.82	1.34
8.63	5.98	3.79	1.34	0.96
11.5	4.69	2.99	1.08	0.80

Parameters in Equations (21) and (22) were determined by non-linear least-squares regression using Fitting Toolbox of MATLAB version 7.12 (R-2011a). The parameters K_E, k_2, k_3, b, K_E', k_2' and b' determined from the fitting are listed in Tables 3 and 4. According to Equation (17), k_3 (h^{-1}) is the rate constant of enzyme deactivation. As shown in Tables 3 and 4, this parameter value decreased with increasing the initial enzyme concentration for a constant substrate concentration. At the higher concentration of enzyme, the saturation of non-productive complex occurred and therefore the rest of enzyme is available to attack the substrate. The equilibrium constants K_E and K_E' represent a ratio of the rate constant of complex-dissociation and the rate constant of complex forming reactions. The greater equilibrium constant means the faster complex dissociation or the slower complex formation. Therefore, the complexes from hemicellulose were consumed faster than those from cellulose, because hemicellulose has amorphous structures. The rate constants of products formation k_2 and k_2' are related to initial enzyme concentration. As shown in Table 3 and 4 these parameters values increased at higher initial enzyme concentrations. Figures 1 and 2 show the plot of the simulated glucose and xylose concentrations for pretreated rice husk and sorghum stem. The experimental points are apparently close to the line simulated by Equations (21) and (22). The correlation coefficient R^2 between the experimental data and predicted glucose and xylose concentrations were determined (see Tables 3 and 4). The high values of R^2 for all initial enzyme concentrations indicate the good simulation performance of the proposed models.

4. 2. Effect of Initial Substrate Concentration

The composition of lignocellulosic substrate and the initial substrate concentration affect the rate of sugar production. Therefore, experiments were conducted at different substrate loadings of pretreated sorghum stem and rice husk. The results of glucose and xylose concentrations in enzymatic hydrolysis of pretreated sorghum stem at various substrate loadings of 4, 6, 8, 10% (w/v) are shown in Figure 3.

TABLE 3. Model parameters determined by fitting of experimental data using Equation (21)

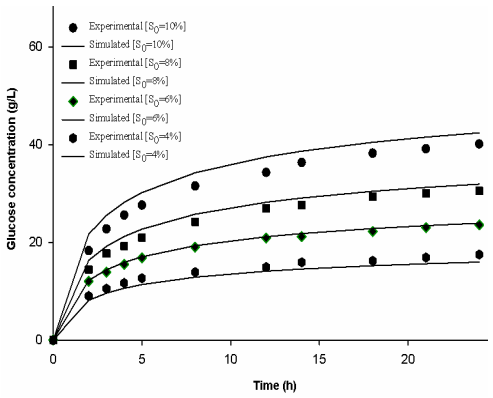
Substrate	E_0 (g/L)	Model parameters				Correlation coefficient (R^2)
		K_E (g/L)	k_2 (h^{-1})	k_3 (L/h.g)	b (-)	
Rice husk (12% (w/v))	2.87	0. 38	0.328	3.78	0.229	0.9893
	5.75	0.46	0.433	3.20	0.265	0.9943
	8.63	0.52	0.463	2.84	0.314	0.9952
	11.5	0.61	0.534	2.54	0.344	0.9937
Sorghum stem (10% (w/v))	2.87	0.521	0.512	3.631	0.271	0.9541
	5.75	0.601	0.658	3.115	0.352	0.9558
	8.63	0.635	0.733	2.736	0.422	0.9556
	11.5	0.688	0.770	2.276	0.492	0.9548

TABLE 4. Model parameters determined by fitting of experimental data using Equation (22)

Substrate	E_0 (g/L)	Model parameters				Correlation coefficient (R^2)
		K_E' (g/L)	k_2' (h^{-1})	k_3 (L/h.g)	b' (-)	
Rice husk (12% (w/v))	2.87	0. 312	0.181	2.62	0.222	0.9991
	5.75	0.392	0.270	2.45	0.271	0.9995
	8.63	0.435	0.303	2.18	0.320	0.9994
	11.5	0.508	0.362	1.94	0.368	0.9993
Sorghum stem (10% (w/v))	2.87	0.312	0.163	2.420	0.216	0.9998
	5.75	0.363	0.217	2.210	0.271	0.9935
	8.63	0.411	0.252	2.040	0.301	0.9947
	11.5	0.472	0.289	1.850	0.332	0.9916

The influence of different substrate loadings [6, 9, 12, 15% (w/v)] on the glucose and xylose concentrations in the hydrolysis of pretreated rice husk is shown in Figure 4. Glucose and xylose concentrations increased with time. For both samples, the hydrolysis rates were maximum at the initial stage followed by a gradual decline by time. The lower reaction rates after initial stage might be due to enzyme deactivation, product inhibition, and depletion of utilizable substrate [9-11, 16, 31]. When substrate loading was increased from 4 to 8% for sorghum stem and from 6 to 12% for rice husk, no significant change in the initial rate of enzymatic hydrolysis was obtained. However, higher substrate loading led to a decline in the overall rate of enzymatic hydrolysis. Nonetheless, more than 70% of total hydrolysates were released during the first 8 h and this value increased slightly over the next 16 h. After 24 h, the maximum glucose concentration for sorghum stem and rice husk were 40.11 and 31.07 g/L, respectively. At similar time, maximum xylose concentration for sorghum stem and rice husk were 8.92 and 7.30 g/L, respectively. The produced glucose and xylose concentrations from pretreated sorghum stem were higher than those obtained from rice husk. This result may be caused by higher cellulose and hemicellulose contents in sorghum stem as well as much lower ash content than those in rice husk. These results indicated that efficiency of enzymatic hydrolysis to some extent is dependent on feedstocks composition.

The performance of the proposed models in the present study was evaluated by obtained data from experiments at different substrate loadings and the predicted values by the model were compared with experimental results. The coefficient of determination (R^2) was also calculated for glucose and xylose concentrations at different substrate loadings (data not shown). The high values for calculated R^2 (> 0.95) indicated that the model performed very well in a wide range of substrate concentrations. The data in Figures 3 and 4 showed that the predictability of the models for



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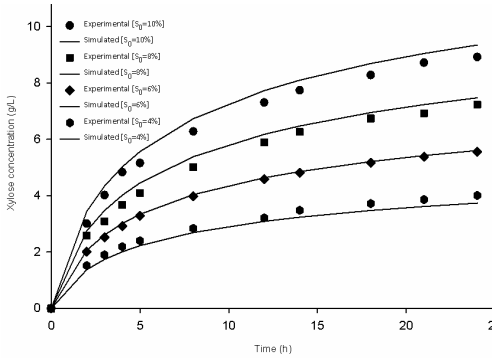

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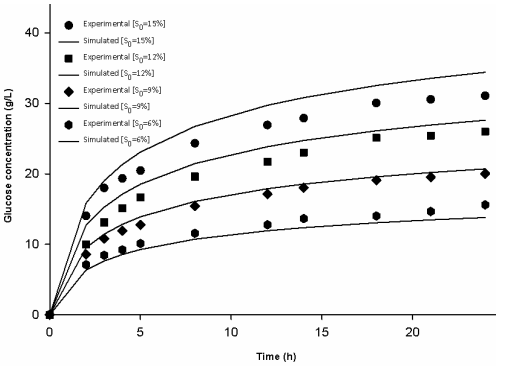
Figure 3. Effect of substrate loading on glucose and xylose production from pretreated sorghum stem during the enzymatic hydrolysis [E_0=2.87 g/L]



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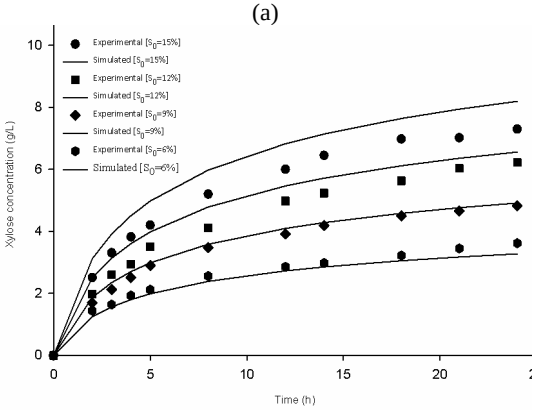

(b)

Figure 4. Effect of substrate loading on glucose and xylose production from pretreated rice husk during the enzymatic hydrolysis [E_0=2.87 g/L]

lower substrate loadings was better than that for higher substrate loadings. The glucose and xylose concentrations were slightly underestimated at lowest substrate loading, whereas these values were overestimated at higher substrate loadings. These results could be caused by the differences in the efficiency of mixing at various substrate loadings and development of

concentration gradients which makes part of substrate less accessible to biocatalyst.

5. CONCLUSION

A modified Langmuir model is proposed to study the kinetic of enzymatic hydrolysis of pretreated sorghum stem and rice husk. This model expresses the relationship between glucose and xylose concentrations and two hydrolytic conditions, i.e., time and initial concentration of enzyme. The proposed model provides a simple method to describe the kinetic behavior of enzymatic hydrolysis of lignocellulosic substrate for industrial applications. It has been successfully used to fit the experimental data of enzymatic hydrolysis of sorghum stem and rice husk. The developed model could help to adjust enzyme loading and hydrolysis time which is helpful for industrial applications.

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Kinetic Modeling of Enzymatic Hydrolysis of Pretreated Sorghum Bicolor and Rice Husk

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چکیده

در کار حاضر، هیدرولیز ساقه سورگوم جارویی و شلتوک برنج پیش‌تیماری شده تحت مقادیر متفاوت از غلظت‌های اولیه آنزیم و میزان بارگذاری سوبسترا مورد بررسی قرار گرفت. کاهش سرعت هیدرولیز آنزیمی مواد لیگنوسلولزی، اغلب به کاهش فعالیت آنزیم نسبت داده شده است. از اینرو یک مدل سینتیکی ریاضی برای هیدرولیز آنزیمی مواد لیگنوسلولزی بر مبنای غیرفعال شدن آنزیم ارائه گردید. در این مدل، کاهش فعالیت آنزیم جذب شده توسط یک واکنش درجه دوم توصیف شد. مدل ارائه شده با داده‌های آزمایشگاهی حاصل از هیدرولیز ساقه سورگوم و شلتوک برنج برازش داده شد. پارامترهای سینتیکی مدل با تطابق نتایج آزمایشگاهی تعیین و مورد ارزیابی قرار گرفتند. عملکرد مدل در تخمین روند هیدولیز آنزیمی تحت شرایط مختلف بسیار خوب ارزیابی گردید.

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