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Fractal kinetic analysis of the enzymatic saccharification of CO_2 laser pretreated corn stover

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ABSTRACT

The enzymatic hydrolyses of laser pretreated corn stover as a novel pretreatment method were examined to establish a simplified kinetic model for the complicated hydrolysis process. The time dependence of the total reducing sugars amount was closely related to the amounts of cellulosic materials and amounts of cellulase. The evaluated model fitted very well with the experimental data of enzymatic hydrolysis of laser pretreated corn stover under different conditions, including cellulase loading, nature of substrate, substrate loading in the reaction medium. The results indicated that the complex kinetics of cellulase enzymatic saccharification could be assessed with the fractal kinetic model. The cellulase enzymatic reaction process was effectively predicted and controlled with the kinetic model. The result showed that the model could effectively reflect dynamic process of enzyme hydrolysis.

1. Introduction

The importance of bioethanol has increased with the exhausted shortage of fossil energy reserves and increased air quality. Lignocellulose is of great interest and latent capacity for the ideal bioethanol production (Gaykawad et al., 2013). Corn stover can be a potential feedstock for bioethanol and comprehensive applications because of its cheap, abundance, renewable and the improving second generation ethanol technologies (Kadam & McMillan, 2003). Corn stover, as one of the most abundant agricultural residue, is estimated that about 300 million tons are obtained annually in China (Liu & Cheng, 2010).

In the conversion of corn stover to bioethanol, how to choose the pretreatment method is an essential procedure (Chen, Zhao, & Xia, 2009). By breaking the physical structural and altering chemical composition of lignocellulosic biomass, the pretreatment can increase the susceptibility of the lignocellulose to enzymolysis and facilitates hydrolysis of lignocellulosic biomass to total reducing sugars effectively (Kaar & Holtzapple, 2000). With a view to converting corn stover materials to bioethanol with high efficiency, suitable pretreatment methods that promote efficient enzymolysis to fermentable reducing sugars drastically should be chosen (Saha, Yoshida, Cotta, & Sonomoto, 2013). In a previous work, using CO_2 laser pretreated corn stover as substrate for enzymatic hydrolysis, relatively cellulase hydrolysis yields (around 30%) were achieved, and found that the saccharification rate of CO_2 laser pretreatment was significantly higher than ultrasonic pretreatment. The fractal kinetic model was supported by the SEM images and Fourier Transform Infrared spectoscopy of laser pretreated corn stover (Tian, Wang, Fan, & Zuo, 2011). However, little work devoted to the enzymatic hydrolysis of corn stover pretreated by CO_2 laser, and many models have been acquired without considering the relationship of cellulase adsorption and enzymolysis (Tian, Wang, Fan, & Zuo, 2012).

It has been shown that the model based on Henri–Michaelis–Menten equation was not suitable for the analysis of cellulase hydrolysis of corn stover structures, especially when the cellulase reaction was diffusion limited (Bajzer & Strehler, 2012). Therefore, we deduced the model based on Langmuir adsorption isotherm equation and Michealis–Menten equation that express the analysis of adsorption process and cellulase hydrolysis of corn stover structures (Ruiz, Vicente, & Teixeira, 2012; Wang & Feng, 2010). Optimization of lignocellulosic materials bioconversion by cellulase requires good knowledge of the reaction kinetics (Monschein, Reisinger, & Nidetzky, 2013). In the present study, we deduced a simple mathematical equation that directly described the relationship between cellulase concentration and CO_2 laser-pretreated corn stover.

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2. Materials and methods

2.1. Materials

Corn stover was obtained from a farm in Harbin, Heilongjiang, China. It was milled and sized using a sieve shaker of 2 mm. The corn stover substrate was pretreated with CO_2 laser. It was pretreated by laser for 68 min at 265 W and liquid to solid ratio, 21:1 (mL/g) (Tian et al., 2011). The pretreated residues was washed three times by deionized water and dried at room temperature. After strained through a 60 meshes sieve, it was used as substrate by crude cellulase powder. Crude cellulase powder was provided by by Gansu Hualing Biological Technology Co., Ltd. of China. The filter paper activity was assayed at 64.5 filter paper unit (FPU)/g, as the measurement described by the description of the NREL Laboratory Analytical Procedure.

2.2. Adsorption experiments

The cellulase was adsorpted by different pretreated corn stover concentrations for 30 min at temperature 50 °C, pH 5.0 (0.2 M acetate buffer) in a shaking bath (160 rpm) and the concentration of cellulase was 0.4 g/L. Initial pretreated corn stover concentrations of 5, 10, 15, 20, 25, and 30 g/L were tested for different times. The protein content of the supernatant was determined using Bradford method (Bradford, Needham, Bulpitt, & Westhead, 2006).

The cellulase was adsorpted for 30 min at temperature 50 °C, pH 5.0 (0.2 M acetate buffer) in a shaking bath (160 rpm), and the concentration of laser pretreated corn stover was 0.4 g/L. Initial cellulase concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12 g/L were tested for different times.

2.3. Enzymatic hydrolysis

The pretreated corn stover was hydrolyzed for 48 h at temperature 50 °C, pH 5.0 (0.2 M acetate buffer) and S/L ratio of 2% (w/v) with the crude cellulase concentration of 0.12 g (8.99 FPU)/g substrate in a shaking bath (160 rpm). 1.5 mL 1 mg/mL Hexadecylpyridinium chloride was added to the dilute buffer solution, for sterilizing the microorganism in the hydrolyzate. After enzymatic hydrolysis, the concentration of total reducing sugars was determinated by the dinitro salicylic acid (DNS) method. The enzymatic hydrolysis reaction of pretreated corn stover was described as that cellulase (E) (g/L) was adsorbed on the active sites of the pretreated substrate (S) (g/L) to form the intermediate complexes (ES) (g/L), which continue to produce total reducing sugar (R) (g/L) and release free enzyme (E). It is shown as the following equation:

$$E + S \underset{}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_2} R + E \tag{1}$$

where k_1 (L/(h g))and k_2 (h^{-1}) are the rate constants of cellulase adsorption and production formation, respectively.

Cellulase enzyme hydrolysis process in the pretreated corn stover cellulose fiber theoretical concentration of the residual solution can be estimated indirectly by utilizing the DNS hydrolysates determination of total amount of reducing sugar, such as shown in Eq. (2) (Ye & Berson, 2011),

$$[S_t] = [S_0] - 0.9[RS] \tag{2}$$

where $[S_t]$ is the theoretical residue values of the fibers in the solution (g/L) during cellulase processing, $[S_0]$ is the initial concentration of the cellulose fibers in the solution (g/L), $[RS]$ is the hydrolysates total reducing sugar concentration (g/L). Therefore, in the process of enzymatic hydrolysis, the enzymatic hydrolysis of cellulose Y (g/L) can be expressed as,

$$[Y] = [S_0] - [S_t] \tag{3}$$

2.4. Model development

Three components of cellulase is assumed to form a single combined mode on the insoluble pretreated corn stover materials, and the surface structure of insoluble materials is considered homogeneous. The adsorption experimental data were fit to the following Langmuir adsorption isotherm equation (Kuan, Lo, Chang, & Wang, 2000).

$$B = \frac{n[F]}{K + F} \tag{4}$$

where B is the concentration of cellulase absorbed on the pretreated corn stover substrate (mg/g substrate), $[F]$ the concentration of free cellulase in the hydrolyzate (μmol/L), K Langmuir equilibrium constant (mL/mg cellulase), and n maximum cellulase adsorption domains content of per g of pretreated substrate.

Applying Langmuir adsorption isotherm equation and Eq. (1), a mathematical equation can be deduced as follow:

$$\frac{[ES]}{[P_0]} = \frac{\alpha \times [S_0]}{K_1 + [S_0]} \tag{5}$$

When the concentration of pretreated corn stover substrate was constant, a mathematical equation between the intermediate complexes (ES) and free enzyme expressed by the following equation:

$$\frac{[ES]}{[S_0]} = \frac{ma\{[P] - [P_0](1-\alpha)\}}{K_2 + \{[P] - [P_0](1-\alpha)\}} \tag{6}$$

where α is the largest protein fraction of Cellulase protein combined with pretreated substrate, K_1 half saturation adsorption constant of substrate, K_2 half saturation adsorption constant of substrate of cellulose, and m maximum adsorption amount of cellulase by the pretreated substrate.

2.5. Statistical analysis

All experimental data were estimated in triplicate, and all statistical calculations were carried out using the statistical analysis software OriginLab 8.5 (OriginLab Corporation) and Matlab R2009a (The MathWorks, Inc.). The experimental results are reported as their replicate means ± SD, and significant levels were set at 0.05.

3. Results and discussion

3.1. Effect of substrate concentration on cellulase adsorption

Cellulase consists of three components is assumed to form a single combined effect on the hydrolysis of insoluble substrate, and the surface structure of insoluble substrate is considered homogeneous (Shen & Agblevor, 2008).

Cellulase adsorption isotherm on CO_2 laser pretreated corn stover was conducted by varying the amount of the pretreated corn stover substrate. The content of cellulase protein in the supernatant was determined as free cellulase in hydrolyzate. After CO_2 laser plasma pretreated, 5, 10, 15, 20, 25, 30 g/L corn stover substrate was aborpted during 30 min in the cellulase concentration $[P_0] = 0.4$ g/L at 50 °C about pH 5.0 in the air bath shaker under the speed of 160 r/min. Cellulase adsorption score $[ES]/[P_0]$ with the initial concentration of the pretreated straw cellulose $[S_0]$ curve was shown in Fig. 1, where $[ES]/[P_0]$ and $[S_0]$ was a very good hyperbolic relationship. The cellulase adsorption was assumed to take reciprocal on both sides of Eq. (5), as described in Eq. (7).

$$\frac{[P_0][S_0]}{[ES]} = \frac{K_1}{\alpha} + \frac{[S_0]}{\alpha} \tag{7}$$

where $[P_0][S_0]/[ES]$ and $[S_0]$ were shown a linear relationship, and the slope was $1/\alpha$, while the intercept was K_1. According to

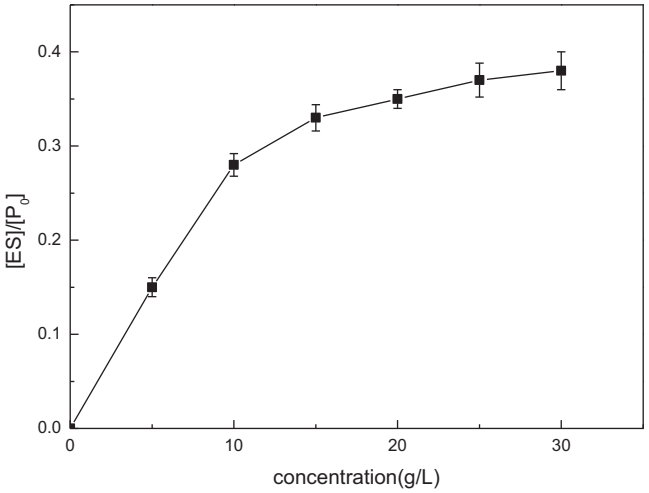

Fig. 1. The relationship of lignocellulose concentration and adsorbed cellulase.

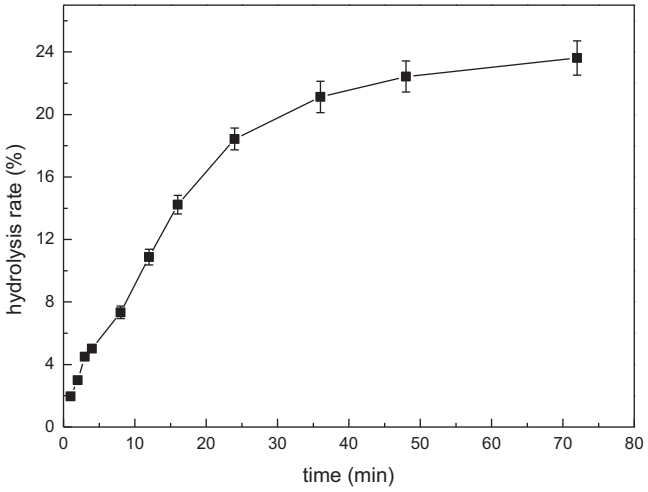

Fig. 2. The relationship of laser plasma catalytic corn stover lignocellulose enzymatic hydrolysis time and enzyme hydrolysis rate.

the experimental results, a linear relationship relevant equation was obtained: $y = 25.63 + 2.261x$, and the correlation coefficient was 0.989. Cellulase adsorption experiments measured cellulase the maximum adsorption protein fraction combined with the pretreated corn stover cellulose fiber concentration value α approximately 0.4423. The results showed that the effective combination of cellulase protein and the pretreated corn stover cellulose fibers was 44.23% of the total of the enzyme protein, while the remaining 55.77% of cellulase protein and the pretreated corn stover cellulose fibers could not be effectively combined. These unbound protein maybe include non-enzyme proteins or inactivation denatured cellulase protein.

3.2. Effect of cellulase concentration on cellulase adsorption

Cellulase adsorption isotherm on CO_2 laser pretreated corn stover was conducted by varying the amount of cellulase protein. Under the constant conditions of the initial concentration of the pretreated corn stover cellulose fibers, the adsorption of different concentrations of cellulase protein and cellulose fibers reached equilibrium, the adsorpted cellulase protein content of the fibers [*ES*] and the solution of free enzyme protein amount [*P*] relationship should obey Eq. (6). The cellulase protein adsorption was assumed to take reciprocal on both sides of Eq. (6), as described in Eq. (8).

$$\frac{[S_0]}{[ES]}\{P - P_0(1-\alpha)\} = \frac{K_2}{m} + \frac{P - P_0(1-\alpha)}{m} \quad (8)$$

where $[S_0]\{P - P_0(1-\alpha)\}/[ES]$ and $\{P - P_0\,(1-\alpha)\}$ were shown the linear relationship, and the slope was $1/m$, while the intercept was K_2/m.

After CO_2 laser plasma pretreated, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 g/L cellulase protein was adorpted by corn stover substrate during 30 min in the initial substrate concentration $[P_0] = 40$ g/L at 50 °C about pH 5.0 in the air bath shaker under the speed of 160 r/min. According to cellulase adsorption results, a linear relationship relevant equation between $[S_0]\{P - P_0(1-\alpha)\}/[ES]$ and $\{P - P_0(1-\alpha)\}$ was obtained: $y = 9.71 + 8.97x$, and the correlation coefficient is 0.9861. The results shown that the maximum cellulase adsorption m (g enzyme/g cellulose fibers) by the unit weight of the pretreated corn stover cellulose fibers could reach 0.111.

3.3. Enzymatic hydrolysis of pretreated corn stover

When adding a certain cellulase concentration to the CO_2 laser pretreated cellulose substrates for enzymatic hydrolysis, the relationship between the enzymatic hydrolysis rate or hydrolysis time and reducing sugars fermentable was shown in Fig. 2. The results indicated that the CO_2 laser pretreated cellulose substrates is composed by a number of different ingredients for the degree of difficulty enzymatic hydrolysis. The first 3 h of the enzymatic hydrolysis reaction was fast, and rised exponentially relationship, while after 3–24 h, the enzyme hydrolysis rate gradually slowed down, and increased linearly relations. After 24 h enzymatic hydrolysis, the rate of hydrolysis more slowly, and basically stabilized status. No longer conducted a detailed study of the enzymatic hydrolysis rate after 24 h of enzymatic hydrolysis. A linear relationship between the enzymatic hydrolysis rate and enzyme hydrolysis reaction 3–24 h experimental time was obtained by the statistical analysis. A linear relationship relevant equation between the enzymatic hydrolysis rate and enzyme hydrolysis time was: $y = 0.8384x$, and R^2 value was 0.9257. Therefore, this study mainly focused on the initial enzymatic hydrolysis for 3 h.

3.4. Relationship between enzymatic rate and enzymatic time

CO_2 laser plasma pretreated lignocellulose was obtained with the initial concentration $[S_0]$ 20 g/L (see Table 1) in boiling water bath for 30 min, and then cooled to room temperature. The main purpose is to kill most of the microorganisms. The results showed that the straw cellulose fibers of enzymatic hydrolysis rate would increase faster than the enzymatic hydrolysis rate of the enzymatic reaction early fiber with increase of CO_2 laser plasma preatreatment time. However, when the enzymatic reactiontime beyond a certain range, the pretreated corn stover cellulose enzymatic hydrolysis rate of increase would occur gradually decreased, while the cellulose fiber enzymatic hydrolysis rate would increase as improving the amount of cellulase. The results indicated that the fiber hydrolysis rate of the enzymatic reaction and the amount of enzyme was closely interrelated in the cellulase reaction processing.

The natural logarithm $\ln(100[S_t]/[S_0])$ of the pretreated corn stover lignocellulose fibers in the residual and CO_2 laser plasma pretreatment time t was plotted. Table 2 also showed a good linear relationship between $\ln(100[S_t]/[S_0])$ and CO_2 laser pretreatment time t. This linear relationship was analyzed.

Van Dyker and Brandt et al. proposed that cellulose enzymatic reaction could be described with quasi-one reaction of the use of a series of parallel addition. Under the present experimental conditions, the relationship between CO_2 laser pretreated corn stover

Table 1
Effect of the enzymatic hydrolysis rate of corn stover with the enzymolysis time and enzyme dosage.

Enzyme concentration (mg/mL)	Hydrolysis rate%							
	0	15 min	30 min	45 min	60 min	90 min	120 min	180 min
0.2	0	0.028	0.263	0.593	0.710	0.951	1.765	2.815
0.4	0	0.075	3.625	0.993	1.242	1.478	2.396	3.653
0.6	0	0.334	0.739	1.167	1.742	1.605	2.688	3.879
0.8	0	0.362	0.946	1.478	1.756	1.723	2.744	3.992
1.0	0	0.433	1.205	1.765	1.939	2.062	2.843	4.416
1.2	0	0.517	1.341	1.784	1.996	2.194	3.027	4.557

Table 2
The relationship between $\ln(100[S_t]/[S_0])$ and pretreatment time (t) under different cellulase dosage.

Enzyme concentration (mg/mL)	Linear equation	Correlation coefficient	F
0.2	$y = 4.6059 - 0.0001x$	0.9826	6
0.4	$y = 4.603 - 0.0002x$	0.9828	6
0.6	$y = 4.6059 - 0.0003x$	0.9877	6
0.8	$y = 4.6004 - 0.0002x$	0.9825	6
1.0	$y = 4.5991 - 0.0002x$	0.9821	6
1.2	$y = 4.5977 - 0.0002x$	0.9919	6

cellulose fiber enzymatic hydrolysis residual and enzymatic time can be expressed as

$$\frac{[S_t]}{[S_0]} = e^{-kt} \tag{9}$$

where t is the cellulase enzyme reaction time (min), $[S_t]$ straw cellulose cellulose theoretical residue amount for cellulase enzyme reaction time t(g/L), $[S_0]$ the initial concentration of the fiber (g/L), K is the reaction rate constant (min^{-1}).

Based on the Eq. (3), Eq. (9) can be changed to Eq. (10) after a number of mathematical transformation.

$$\frac{[Y]}{[S_0]} = (1 - e^{-kt}) \tag{10}$$

$[Y]/[S_0]$ represents the initial amount of the cellulose fiber-reactive cellulose enzymatic hydrolysis score (g/g) at the enzyme reaction time (t). If both sides of Eq. (9) take the natural logarithm, Eq. (9) is also changed to

$$\ln\left(100\frac{[S_t]}{[S_0]}\right) = \ln 100 - kt \tag{11}$$

The Eq. (11) shows that $\ln(100[S_t]/[S_0])$ and the enzymatic hydrolysis time (t) obey a linear relationship, which is coordinated with Table 2. The results showed enzymatic kinetics Eq. (9) was fully established with the different cellulase concentration. The rate constant k of Enzymatic hydrolysis in the pretreated corn stover solution cellulose could be speculated by the slope of the linear equation. The results was shown in Table 3. Table 3 shows that the enzymatic hydrolysis rate constant k value would improve with the concentration of the cellulase enzyme $[P_0]$ increasing. However, under low concentration conditions $[P_0] < 0.6$ mg/L, the effect of the concentration of the enzyme protein on the reaction rate k was significant. When the enzyme protein concentration kept increasing, ($[P_0] > 0.6$ mg/L), the influence of the cellulase to the reaction rate was gradually lowered and reached a stable phase. This phenomenon may be due to the pretreated corn stover cellulose must be firstly combined to form the complex (ES) in the cellulase enzyme hydrolysis processing. However, the ratio surface area (S) and capable of forming a complex number of binding sites (n) for the cellulase was certain, so the amount of combined enzyme protein was increased to a certain concentration, the increase of the combined cellulase enzyme protein could not make the complex the more obvious changes in concentration (ES), which showed no significant increase of the pretreated corn stover enzymatic reaction rate (k). Therefore, the excessive concentration of the enzyme protein in the hydrolysis reaction was not economical. When the cellulase concentration increased from 0.2 mg/mL to 1.2 mg/mL, the cellulose enzymatic reaction rate k only increased by 2 times.

Table 3
Corn stover lignocellulose enzymatic hydrolysis reaction rate constant (k).

Enzyme concentration (mg/mL)	Reaction rate constant k (10^{-4} min^{-1})
0.2	1
0.4	2
0.6	3
0.8	2
1.0	2
1.2	2

3.5. Relationship between enzymatic rate and cellulase concentration

Table 1 shows the relationship of pretreated corn stover enzymatic reaction rate and enzyme concentration. Acorrding to Table 1, the relationship between the enzymatic hydrolysis rate of CO_2 laser pretreated corn stover cellulose ($[Y]/[S_0] \times 100\%$) and the cellulase protein concentration $[P_0]$ was shown in Fig. 3. Fig. 3 indicates CO_2 laser pretreated corn stover cellulose enzymatic rate will increase with the concentration of cellulase protein $[P_0]$ improving in the enzymatic reaction processing. However, when the concentration of cellulase proteins reaches a certain concentration, enzymatic hydrolysis rate of the pretreated corn stover cellulose would not significant increase (Sathitsuksanoh, Zhu, Ho, Bai, & Zhang, 2010).

According to the cellulase enzymatic reaction model, The enzymatic hydrolysis rate or total reducing sugar product formation rate

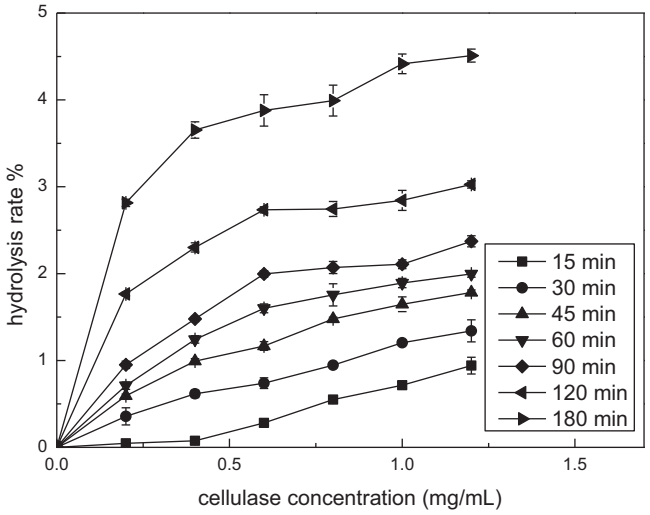

Fig. 3. Effect of cellulase concentration on corn stover lignocellulose enzymatic hydrolysis reaction rate constant (k).

Table 4
The relationship of $([Y]/[S_0])^{-1}$ and $[P_0]^{-1}$ after different enzymatic reaction time.

Time (min)	Linear equation	Correlation coefficient	F
15	$y = 2.4618x + 0.9463$	0.9957	7
30	$y = 0.5128x + 0.379$	0.9921	7
45	$y = 0.2679x + 0.3529$	0.9953	7
60	$y = 0.2199x + 0.2915$	0.9937	7
90	$y = 0.1509x + 0.2937$	0.9898	7
120	$y = 0.0557x + 0.2886$	0.9892	7
180	$y = 0.0307x + 0.2017$	0.9806	7

Table 5
Enzymatic hydrolysis maximum reaction rate constant k' of lignocellulosic.

Time (min)	Maximum hydrolysis rate	K'
15	1.057	2.602
30	2.639	1.353
45	2.834	0.759
60	3.431	0.754
90	3.405	0.514
120	3.465	0.193
180	4.958	0.152

was mainly determined by the concentration of the complex (ES), as described in Eq. (12):

$$\frac{-d[S_t]}{dt} = \frac{d[Y]}{dt} = k_2[ES] \tag{12}$$

where $[ES]$ is the complex formation between the cellulase and fiber concentration (g/mL), k_2 is the reaction rate constant (min^{-1}).

The reaction rate constant is proportional to the complex concentration $[ES]$ as shown in Eq. (12). However, the results showed that the concentration of formed complex $[ES]$ and cellulase enzyme protein concentration $[E]$ existed hyperbolic relationship in cellulose adsorption processing. Accordingly, it was convenient to define the maximal concentration of formed complex $[ES]$ and cellulase protein concentration $[E]$ as Eq. (13) showed:

$$[ES] = \frac{[ES_{max}][E]}{K_2 + [E]} \tag{13}$$

where $[ES_{max}]$ represents the highest concentration of the pretreated corn stover cellulose adsorption enzyme protein complex formed (g/L) at the adsorption equilibrium, $[E]$ means the cellulase free enzyme protein concentration in the solution (g/L) at the adsorption equilibrium, K_2 represents the adsorption constant (g/L) in the half-saturation of the cellulase protein.

Thus, according to the proportional relationship between the enzymatic reaction rate and the complex concentration $[ES]$, the amount of enzymatic hydrolysis $[Y]$ and the initial concentration of the enzyme $[P_0]$ significant was shown hyperbolic relationship in Eq. (14):

$$\frac{[Y]}{[S_0]} = \frac{[Y_{max}]}{[S_0]} \cdot \frac{[P_0]}{K' + [P_0]} \tag{14}$$

where $[Y_{max}]$ represents when the concentration of enzyme protein $[P_0]$ is nearly infinite and enzymatic reaction time was carried out to t, the maximum enzymatic amount was obtained by the pretreated corn stover cellulose (g/L), while K' is cellulase enzyme half-maximum enzymatic rate constants, representing the desired cellulase enzyme dosage when the enzymatic amount reach half of the maximum enzymatic amount.

To determine the maximum enzymatic amount Y_{max} and half-maximum enzymatic rate constants K', a linearized form of Eq. (14) was used:

$$\left(\frac{[Y]}{[S_0]}\right)^{-1} = \frac{K'[S_0]}{[Y_{max}]} \cdot \frac{[P_0]^{-1} + [S_0]}{[Y_{max}]} \tag{15}$$

where the slope is $K'[S_0]/[Y_{max}]$, the intercept is $[S_0]/[Y_{max}]$. Eq. (15) shows that $([Y]/[S_0])^{-1}$ and $[P_0]^{-1}$ had a good linear relationship.

Table 4 represents the curvilinear relation between $([Y]/[S_0])^{-1}$ and $[P_0]^{-1}$ at different enzymatic reaction time, and Table 4 shows that $([Y]/[S_0])^{-1}$ and $[P_0]^{-1}$ had a good linear relationship. As shown in Table 4, to CO_2 laser pretreated corn stover cellulose at 15–180 min enzymatic hydrolysis time, $([Y]/[S_0])^{-1}$ and $[P_0]^{-1}$ exists a good linear relationship. The results showed that Eq. 15 was entirely reasonable at this enzymatic reaction stage. Acorrding to Table 4, it was possible to calculate the maximum enzymatic rate $[Y_{max}]$ and the constant K', The results were shown in Table 5.

Table 5 shows that the maximum enzymatic rate and enzymatic reaction time was closely interrelated. The enzymatic reaction time was the longer, and the maximum enzymatic reaction rate was higher. Therefore, the relationship between the maximum enzymatic reaction rate and the enzymatic reaction time acted in accordance with Eq. (10). Another expression of the process is obtained on the ground of Eq. (10), as shown in Eq. (16).

$$\frac{[Y_{max}]}{[S_0]} = 1 - e^{k't} \tag{16}$$

where k' is enzymatic hydrolysis rate constant (min^{-1}) at infinite concentration of the cellulase enzyme protein,. The experimental results were shown in Table 5, and then $k' = 0.0002\ min^{-1}$ can be evaluated by the non-linear least squares method.

Maximum enzymatic hydrolysis rate $[Y_{max}]$ represented the pretreated corn stover cellulose theoretically could get the greatest degree of cellulase enzyme hydrolysis in a certain range of time. Under the same conditions for enzymatic hydrolysis, $[Y_{max}]$ value could infer the ability of the cellulase enzymatic hydrolysis of cellulose or enzymatic vitality, and estimate the degree of the hydrolysis. From this perspective, the maximum enzymatic hydrolysis $[Y_{max}]$ was the important parameters of the enzymatic hydrolysis of the pretreated corn stover cellulose. $[Y_{max}]$ reflected potential law of the cellulase enzymatic hydrolysis of cellulose and the characteristics of the enzymatic reaction.

In addition, as shown in Table, half maximum enzymatic reaction constant K' is an independent parameter in determining the pretreated corn stover cellulose enzymatic hydrolysis. K' is mainly determine by the ratio of surface area of the cellulose and adsorption site of the cellulase enzyme, which determines the maximum adsorption amount of cellulase enzyme (m). Combining Eq. (14) and Eq. (16), the relationship between the amount of enzymatic hydrolysis of cellulose $[Y]$ at different enzymatic reaction time (t) and cellulase protein concentration $[P_0]$ obeyed the basic dynamic Eq. (17).

$$\frac{[Y]}{[S_0]} = \frac{[P_0]}{K' + [P_0] \cdot (1 - e^{k't})} \tag{17}$$

K' values and k' values can be obtained through experiment and the series of formulas, substituting into the Eq. (17). The enzymatic kinetic equation of the cellulase enzyme hydrolysis of CO_2 laser pretreated corn stover cellulose was rearranged as the following form:

$$\frac{[Y]}{[S_0]} = \frac{[P_0]}{0.150 + [P_0] \cdot (1 - e^{-0.0002't})} \tag{18}$$

Therefore, CO_2 laser pretreated corn stover cellulose enzymatic reaction process could be effectively forecasted by Eq. (18), which was an effective enzymatic kinetic equation.

4. Conclusions

In the cellulase adsorption of the pretreated corn stover cellulose experimental, the results showed that the specific surface area of CO_2 laser pretreated corn stover cellulose and cellulase binding

sites to form the structure of the combination complex was supposedly fixed. Therefore, in the enzymatic reaction process, if the cellulase concentration reached a certain amount, keeping increase the cellulase concentration would not make the cellulase and the pretreated corn stover cellulose combine, and the results showed the enzymatic reaction rate did not increase any more. High cellulase protein concentration, both from the point of view of the enzymatic reaction rate and the economic cost, is unwise to choose in CO_2 laser pretreated corn stover cellulose enzymatic hydrolysis process.

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