# FRACTAL-LIKE KINETIC ANALYSIS ON THE ENZYMATIC HYDROLYSIS OF LIQUID HOT WATER, HYDROCHLORIC ACID AND SODIUM HYDROXIDE PRETREATED SUGARCANE BAGASSE

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LHW (liquid hot water), HCl (hydrochloric acid) and NaOH (sodium hydroxide) were used to pretreat sugarcane bagasse (SB), and composition assay showed that the carbohydrate content in NaOH pretreated SB got to 84.3% and lignin decreased to 3.4%, while the LHW and HCl pretreatments brought similar composition changes, where the carbohydrate content reached 61.2% and 66.6%, respectively, and the lignin content – about 30%. The subsequent enzymatic digestion yielded sugar (glucose and xylose) concentrations from LHW, HCl and NaOH pretreated SB of 12.0, 13.7 and 30.8 g/L, respectively. In order to improve the enzymatic digestion, Tween 80 and xylanase were added to LHW/HCl and NaOH pretreated SB, respectively. Results showed that the sugar concentration from the modified enzymatic digestion increased to 16.9, 17.7 and 33.7 g/L, respectively. Fractal-like kinetics also showed Tween 80 addition decreased the fractal dimension of the pretreated SB, and xylanase addition increased the constant rate during enzymatic hydrolysis.

***Keywords***: cellulase; xylanase; fractal-like kinetics; xylose; glucose; pretreatment

## INTRODUCTION

Conversion of lignocelluloses to reducing sugars, namely saccharification, has gained increasing attention due to its potential important value in bioenergy and biorefining.[1] Natural lignocelluloses have long evolved complex structural and recalcitrance mechanisms for resisting assault from the microbial and animal kingdoms. Existing lignocelluloses saccharification typically relies on a combination of chemical pre-treatment and enzymatic hydrolysis.

A pre-treatment step is usually conducted to reduce recalcitrance by depolymerizing carbohydrates and solubilizing lignin.[2,3] Many pretreatments had been proposed in the past decades, where thermochemical pre-treatment was recognized as a critical technology to make lignocelluloses become substrate with acceptable enzymatic digestibility.[4] Three typically used pretreatments, LHW (Liquid Hot Water), HCl (Hydrochloric Acid) and NaOH (Sodium Hydroxide) pretreatments, had made much progress, and were applied in some pilot and commercial scale plants.

Although these pretreatments have been developed for so many years, it is still difficult to deeply and theoretically evaluate their relative merits on a sound basis. Only simple and intuitive sugar concentration or conversion efficiency was compared in most past reports. As a result of an unclear enzymatic mechanism, a deeper understanding of the differences among pretreatment technologies partly depends on the development of enzymatic kinetics.

Therefore, in this study, we used a fractal-like kinetics model to investigate the effect of different pretreatments on the enzymatic hydrolysis of sugarcane bagasse. The fractal dimension of various pretreated substrates and the reaction rate

constant of the enzymes to the substrates were compared.

## EXPERIMENTAL

### Materials

Xylanase powder was purchased from Sigma-Aldrich (Shanghai, China). The activity was $4.39\times10^5$ U/g (a unit of xylanase activity was defined as the amount of enzyme producing 1 μmol of xylose per minute).

Liquid cellulase was provided by KDN BIOTECH GROUP (Qingdao, China). The activity was 152 FPU/ml (FPU is the activity unit of cellulase when filter paper is used as substrate), strictly assayed by the description of the Commission on Biotechnology of the International Union of Pure and Applied Chemistry. Besides, liquid cellulase also contained 756 U/ml xylanase activity, which could be ignored compared to the above mentioned commercial xylanase.

Sugarcane bagasse (SB), the solid residues left after juice extraction, was supplied by Guangxi Fenghao Sugar Co., Ltd. (Chongzuo, China). It was milled and screened to 8-18 mesh and dried at 105 $^{o}$C to constant weight (4 h). The chemical composition of the raw material (on a dry weight basis) was 45.2% glucan, 23.6% xylan, 2.1% arabinan, 21.3% acid-insoluble lignin, 3.9% extractives and 3.4% ash.

Tween 80 was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjing, China). All other reagents were of the highest purity available.

### Pretreatments

#### *NaOH pretreatment*

SB and NaOH solution (0.18%, w/v) were mixed at a L/S ratio of 1:6 and temperature of 110 $^{o}$C for 60 min in a rotating cooker.

#### *HCl pretreatment*

For the HCl pretreatment, the SB was hydrolysed in a flow-through reactor at 130 $^{o}$C for 10 min.[5] The HCl concentration was 1.25% (v/v) and the solid/liquid ratio was 1:8.

#### *LHW pretreatment*

Details of the LHW pretreatment are described elsewhere.[6,7] SB and water was put into the reactor at L/S ratio of 1:20, which was sealed and heated to the reaction temperature with the magnetic agitator operating at 500 rpm for 20 min. The reaction pressure was controlled by the addition of nitrogen.

### Enzymatic hydrolysis

Before the enzymatic hydrolysis, the HCl and NaOH pretreated SB samples were washed with distilled water until the washing liquid presented neutral pH. Our previous work[8] indicated that the washing step did not lead to a further increase in the final sugar recovery for the LHW pretreatment. Thereby, the LHW pretreated SB was directly used for enzymatic hydrolysis without any washing.

The enzymatic hydrolysis was carried out at a temperature of 50 $^{o}$C and pH 4.8 for 72 h. The solid/liquid ratio, the rotational speed and the cellulase loading were as follows: 1:20 (w:v), 100 rpm, and 15 FPU/g solid, respectively. Other experiment conditions are presented in Table 1.

### Analytical method

#### *Sugar assay*

Glucose and xylose contents were determined by the HPLC Waters 2695 system consisting of a Waters 600E system controller, a Waters 717 automatic sampler, a Waters 2414 differential refractometer and a Shodex sugar SP-0810 column. The mobile phase consisted of distilled water at a flow rate of 0.6 mL/min. The column temperature was 80 $^{o}$C. The injected sample volume was 10 μL. Standard samples and hydrolysed samples were filtrated by a 0.45 μm filter before analysis.

#### *Composition assay*

Glucan, xylan, and Klason lignin contents in raw and pretreated SB were measured according to the analytical procedures provided by the National Renewable Energy Laboratory (NREL).

Table 1
Experimental design of enzymatic hydrolysis of differently pretreated SB

| Trial | Pretreatment | Enzyme | Surfactant |
|---|---|---|---|
| a | LHW | Cellulase | No |
| b | HCl | Cellulase | No |
| c | NaOH | Cellulase | No |
| d | LHW | Cellulase | Tween 80 |
| e | HCl | Cellulase | Tween 80 |
| f | NaOH | Cellulase+xylanase | No |

**Model development**

The model was developed based on the followed assumptions:

(1) Cellulase and xylanase consisting of several components were assumed to have a single combined effect on the hydrolysis of the insoluble substrate;

(2) The rate constant was always assumed to be a power function of time;[9]

(3) The hydrolysis was considered as a pseudo first-order reaction;

(4) Volume variation of the hydrolysis system was ignored.

As we deduced before,[9] the produced glucose concentration could be expressed by the following equation:

$$[G]=\frac{[S_0]C_G}{0.9}\left[1-\exp\left(-\frac{0.9k_G}{1-h_G}t^{1-h_G}\right)\right] \quad (1)$$

where [G] represents the glucose concentration, $[S_0]$ is the initial substrate concentration, $C_G$ is the glucan content in the substrate, $k_G$ is the rate constant of glucan hydrolysis and $h_G$ is the glucan dimension.

For xylan hydrolysis, the produced xylose concentration could be expressed by the following equation:

$$[X]=\frac{[S_0]C_X}{0.88}\left[1-\exp\left(-\frac{0.88k_X}{1-h_X}t^{1-h_X}\right)\right] \quad (2)$$

where [X] represents the xylose concentration, $[S_0]$ is the initial substrate concentration, $C_X$ is the xylan content in the substrate, $k_X$ is the rate constant of xylan hydrolysis and $h_X$ is the xylan dimension.

## RESULTS AND DISCUSSION

**Composition variation after different pretreatments**

Table 2 lists the composition variation between raw and differently pretreated SB samples. All the pretreatments drastically changed the composition of SB. The glucan content increased significantly after the three pretreatments, the LHW pretreated SB exhibiting the highest glucan content. For xylan and lignin content variation, the HCl pretreatment yielded almost identical results with those of the LHW pretreatment, where xylan and lignin content changed to about 5% and 30%, respectively. In contrast, the NaOH pretreatment sharply decreased the lignin content (to less than 4%), while little variation occurred in the xylan content.[10]

Table 2
Composition comparison of raw, NaOH, HCl and LHW pretreated SB

| Sugarcane bagasse | Glucan (%) | Xylan (%) | Klason lignin (%) |
|---|---|---|---|
| Raw | 45.2 | 23.6 | 21.3 |
| NaOH | 56.9 | 27.4 | 3.4 |
| HCl | 55.9 | 5.3 | 30.9 |
| LHW | 61.7 | 4.7 | 30.9 |

**Enzymatic hydrolysis of different pretreated SB**

Table 3 lists the glucose and xylose concentration produced under different conditions. Different pretreatments resulted in different sugar concentration production, but little difference was observed between the LHW and HCl pretreated SB samples. Lignin, as the major barrier to enzymatic accessibility, was removed effectively by the NaOH pretreatment, which led to the highest enzymatic digestibility. As a result of xylan retaining caused by the NaOH pretreatment, apparently higher xylose concentration was also obtained from the enzymatic hydrolysis of NaOH pretreated SB.[11]

For each enzymatic hydrolysis condition, the hydrolysis rate reached to the maximum value at the initial stage and gradually decreased as time elapsed. Over 50% sugar was produced within the first 24 h. The slowdown of the hydrolytic rate could be caused by enzyme deactivation, lignin against enzyme adsorption and substrate recalcitrance.[12]

**Fractal-like kinetic equations based fit**

The experimental data of Table 3 were fitted to Eqs. (1) and (2) by nonlinear regression analysis. Fig. 1 shows the plot of the fit by Eqs. (1) and (2) against the experimental values. Both glucose concentration and xylose concentration were fitted very well by the two equations. Table 4 presents the values of the rate constant and fractal dimension determined from the fit.

Table 3
Glucose and xylose concentration from enzymatic hydrolysis of differently pretreated SB under different conditions

| Time | a | b | c | d | e | f |
|---|---|---|---|---|---|---|
| | Glucose concentration | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 2.460 | 2.969 | 2.831 | 3.227 | 4.327 | 3.705 |
| 7 | 3.912 | 5.131 | 5.211 | 5.402 | 7.169 | 6.311 |
| 12 | 5.516 | 6.686 | 7.675 | 7.347 | 9.816 | 8.843 |
| 24 | 7.270 | 8.955 | 12.303 | 9.894 | 12.362 | 13.299 |
| 30 | 8.069 | 9.894 | 14.213 | 10.796 | 13.171 | 15.278 |
| 36 | 8.736 | 10.361 | 15.703 | 11.612 | 13.482 | 16.532 |
| 48 | 9.363 | 11.465 | 17.523 | 12.953 | 14.495 | 18.916 |
| 60 | 10.363 | 12.039 | 19.660 | 13.849 | 15.386 | 20.747 |
| 72.5 | 10.756 | 12.855 | 20.195 | 15.040 | 16.815 | 22.812 |
| | Xylose concentration | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0.551 | 0.331 | 2.080 | 0.677 | 0.412 | 1.976 |
| 7 | 0.714 | 0.430 | 3.870 | 0.924 | 0.566 | 3.674 |
| 12 | 0.895 | 0.472 | 5.164 | 1.132 | 0.690 | 5.004 |
| 24 | 1.076 | 0.575 | 7.236 | 1.374 | 0.787 | 6.944 |
| 30 | 1.149 | 0.591 | 8.100 | 1.477 | 0.813 | 7.776 |
| 36 | 1.249 | 0.616 | 8.747 | 1.568 | 0.792 | 8.302 |
| 48 | 1.294 | 0.655 | 9.427 | 1.687 | 0.787 | 9.272 |
| 60 | 1.396 | 0.630 | 10.496 | 1.806 | 0.819 | 10.118 |
| 72.5 | 1.228 | 0.842 | 10.634 | 1.934 | 0.872 | 10.912 |

Denotations a, b, c, d, e, f are explained in Table 1

Table 4
Rate constant and fractal dimension from the fit based on Eqs. (1) and (2)

| Trial | $k_G$ | $h_G$ | $k_X$ | $h_X$ |
|---|---|---|---|---|
| a | 0.0272 | 0.527 | 0.0659 | 0.660 |
| b | 0.0384 | 0.528 | 0.0270 | 0.717 |
| c | 0.0379 | 0.260 | 0.0601 | 0.375 |
| d | 0.0369 | 0.486 | 0.0896 | 0.544 |
| e | 0.0567 | 0.547 | 0.0339 | 0.783 |
| f | 0.0434 | 0.262 | 0.0554 | 0.354 |

For cellulase-catalyzed hydrolysis, the LHW pretreated SB had the smallest $k_G$. The smaller the $k_G$, the more difficult was the contact between cellulase and the substrate. So, the accessibility of cellulase to the HCl pretreated SB was the highest, while the NaOH and HCl pretreated SB presented similar accessibility to cellulase. However, the glucose concentration produced from the pretreated SB was not identical with $k_G$. This was because the substrate fractal dimension $h_G$ was a more dominant factor for affecting the cellulase-catalyzed reaction. The LHW and HCl pretreated SB had a similar $h_G$ value, while the $h_G$ of the NaOH pretreated SB was smaller. The value of $h_G$ was between 0 and 1. The larger the $h_G$, the more difficult was the digestion of the substrate.[13] When $h_X$ is 0, the reaction becomes homogenous. The highest and lowest glucose concentration was produced from the NaOH and LHW pretreated SB, respectively. The HCl pretreatment brought a slightly higher glucose concentration than the LHW pretreatment.

For xylanase-catalyzed hydrolysis, the HCl pretreated SB had the smallest $k_X$. The smaller the $k_X$, the more difficult was the contact between xylanase and the substrate.[12] Thus, the accessibility of xylanase to the HCl pretreated SB was the lowest,

and the accessibility of xylanase to the LHW pretreated SB was slightly superior to that of xylanase to the NaOH pretreated SB. However, the highest xylose concentration was produced from the NaOH pretreated SB. This was because the $h_X$ of the NaOH pretreated SB was the smallest. Most xylan was removed by the LHW and HCl pretreatments, while the NaOH pretreatment retained most xylan.

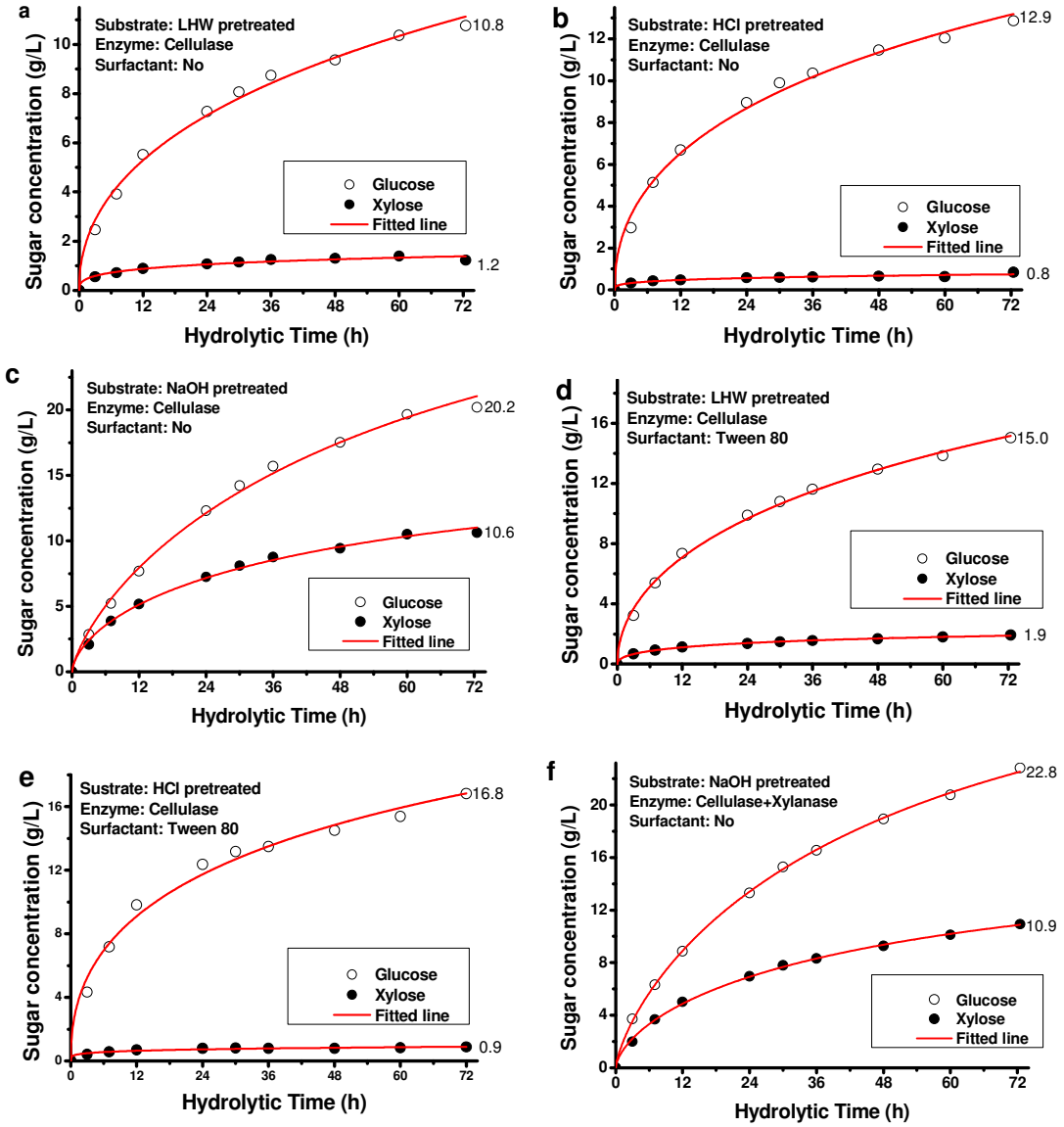


Figure 1: Glucose and xylose concentrations fitted by Eqs. (1) and (2) versus experimental values

Although the $h_X$ value of the LHW pretreated SB was slightly lower than that of the HCl pretreated SB, the LHW pretreatment brought higher xylose concentration. This was because the rate constant $k_X$ was a more dominant factor for affecting the xylanase-catalyzed reaction.

In order to improve the enzymatic digestion, some measures were further taken. Xylanase was added because of the high xylan content in the NaOH pretreated SB, while Tween 80 was added to decrease ineffective adsorption to cellulase because of the high lignin content in the LHW and HCl pretreated SB samples. Fig. 1 (c, d and e) indicate that the glucose and xylose concentration was significantly improved. In order to further explain the phenomenon, the rate constant and the fractal

dimension were compared.

For the HCl and LHW pretreated SB, Tween 80 addition resulted in a sharp decrease in the rate constant, while the fractal dimension was a little altered. Although the reaction system did not become more homogeneous, the mass transfer resistance contact between the enzyme and the substrate was smaller. Thus, the glucose and xylose concentration produced was increased a lot via Tween 80 addition. For the NaOH pretreated SB, xylanase addition brought a little change in the rate constant and the fractal constant. So, the increase in the glucose and xylose concentration was not obvious. This is probably explained by the fact the crude cellulase used in the study contains enough xylanase activity. Extra xylanase addition to hydrolyze the NaOH pretreated SB was unnecessary.

## CONCLUSION

LHW, HCl and NaOH pretreatments were compared in terms of composition analysis, enzymatic digestion and kinetics. The NaOH pretreated SB exhibited the highest carbohydrate content and the lowest lignin content, as well as the highest glucose and xylose concentration upon enzymatic hydrolysis. Fractal kinetics analysis also showed that the NaOH pretreated SB was the easiest to enzymatically digest. The LHW and HCl pretreatments brought similar composition variation and sugar concentration.

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