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Inhibition Performance of Lignocellulose Degradation Products on Industrial Cellulase Enzymes During Cellulose Hydrolysis

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Abstract This study examined the inhibition performance by the major lignocellulose degradation products, including organic acids, furan derivatives, lignin derivatives, and ethanol, on a broadly used commercial cellulase enzyme Spezyme CP (Genencor International, Rochester, NY, USA) to cellulose hydrolysis at both the well-mixing state (shaking flask) and the static state (test tube). The cellulase activity in the cellulase complex of Spezyme CP was assumed to be one single "cellulase", and the apparent kinetic parameters of this cellulase enzyme were measured as an approximate index of the inhibitory effect to the industrial cellulase enzyme. The inhibition performance of these degradation products was compared and analyzed using the determined apparent kinetic parameters. All the degradation products strongly inhibit the cellulose hydrolysis by cellulase enzyme, and the inhibitions on cellulase were all competitive type. The order of the inhibition strength by the lignocellulose degradation products to cellulase is lignin derivatives > furan derivatives > organic acids > ethanol. This study gave a quantitative view to the enzymatic hydrolysis of lignocellulose under the inhibition performance of the lignocellulose degradation products and will help to understand the lignocellulose recalcitrance to enzyme hydrolysis.

Keywords Inhibition · Lignocellulose degradation products · Cellulase enzyme · Hydrolysis · Apparent inhibition parameters

Introduction

The major processing steps of bioconversion of lignocellulose for production of ethanol include pretreatment, enzymatic hydrolysis, microbial fermentation, and ethanol purifica-

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tion. Pretreatment is crucial to make the cellulose available for enzymatic hydrolysis by breaking the lignin seal, partially dissolving hemicellulose, and disrupting the crystalline structure of cellulose into the amorphous form [1–2]. Generally, these pretreatment operations result in a solid fraction consisting of amorphous cellulose and lignin and a liquid fraction (prehydrolysate) containing hemicellulose-derived monomeric sugars and lignocellulose degradation products. The degradation compounds in prehydrolysate inevitably influence the enzyme hydrolysis as the solid fraction is absorbed with prehydrolysate liquid up to 60–90% weight of the total solid fraction. These degradation products include organic acids such as acetic acid, formic acid, and levulinic acid, sugar degradation products such as furfural from xylose and 5-hydroxymethylfurfural (5-HMF), and lignin degradation products such as vanillin, syringaldehyde, and 4-hydroxybenzaldehyde [3–4]. The composition and concentration of the degradation products varies on the types of lignocellulose used, the chemistry, and the nature of the pretreatment process such as temperature, time, pressure, pH, redox conditions, and addition of catalysts [5]. Acetic acid, formic acid, and levulinic acid are the major organic acid components formed during the pretreatment. Acetic acid is released during the hydrolysis of hemicellulose in which the acetyl group of hemicellulose linked to the lignin was released and reacted in acid form; levulinic acid is the terminal product of oxidation of D-glucose and D-mannose; for formic acid, one is the terminal product of xylose oxidation, and another one is the byproduct of D-glucose and D-mannose oxidation to levulinic acid [5]. The major sugar-derived degradation products are furan compounds, including furfural from xylose and 5-HMF from glucose [6]. The lignin degradation products are phenol compounds such as vanillin, syringaldehyde, and 4-hydroxybenzaldehyde [7].

It has been well realized and carefully investigated that acetic acid, furan derivatives, and lignin derivatives are strong inhibitors to ethanol fermentation [8–11]. However, the inhibition on the enzymatic hydrolysis has not been clearly elucidated. Different or even controversial results were reported in the previous works. In a recent review [12], the inhibitory effect of the potent compounds in the lignocellulose processing was summarized: The product inhibitions by cellobiose and glucose were considered as the major inhibitions on the cellulase (cellobiohydrolase) and β-glucosidase, respectively; ethanol was regarded as a weaker inhibitor on the cellulase compared to glucose and cellobiose but it poses an important problem in simultaneous saccharification and fermentation (SSF) because of the accumulated high concentration of ethanol. Organic acids are potent inhibitors to cellulase enzyme but the results are rather diverse [12]. Although furan and phenol derivatives are generally considered as hydrolysis inhibitors to the cellulose enzymes [4, 13], the inhibitory performance was not clearly elucidated and characterized. Similar to furfural and 5-HMF, the phenol derivatives have been long recognized as the fermentation inhibitor, but its inhibitory performance on cellulase was not well characterized [11]. A clear elucidation of the inhibitory effect of the degradation products will contribute to a rational design of pretreatment technology to release less strong inhibitors and the reduction of cellulase usage in a modified process condition.

In this study, the inhibition performance of the major lignocellulose degradation products from dilute acid pretreatment on one of the most widely used cellulase enzyme, Spezyme CP (Genencor International, Rochester, NY, USA), was examined. Spezyme CP is a commercial cellulase enzyme exhibiting high activity of exoglucanase (cellobiohydrolases) and relatively high certain activities of endoglucanase, xylanase, and β-glucosidase [3]. The enzymatic hydrolysis performance under the inhibition was examined in the well-mixing state (shaking flask) and the static state (test tube).The apparent Michaelis–Menten constant and inhibition constant of the cellulase enzyme were measured by assuming the

cellulase Spezyme CP as a single "cellulase" enzyme. Then, the apparent kinetic constants of these degradation compounds were measured as an approximate index of the inhibitory effect. The inhibition of ethanol on the enzymatic hydrolysis was also studied in both the well-mixing state and the static state. The inhibition performance of these degradation products was compared and analyzed. This quantitative study gave an important view on the inhibition performance of the typical degradation products of lignocellulose on the enzymatic hydrolysis of lignocellulose.

Materials and Methods

Enzymes and Reagents

Two industrial cellulase enzymes used in this work were Spezyme CP from Genencor International and the cellobiase enzyme, Novozyme 188, from Novo Industrial A/S (purchased from Sigma-Aldrich, St Louis, MO, USA). Corn stover was harvested in fall 2006 from Jilin Province, China. The Whatman No. 1 filter paper was purchased from Whatman International Ltd (Maidstone, UK). Furfural and 5-HMF were from Acros Organics (NJ, USA). Levulinic acid was from Alfa Aesar (Ward Hill, MA, USA). Vanillin, 4-hydroxybenzaldehyde, sodium hydroxide, sodium acetate, 3,5-dinitrosalicylic acid, and cellobiose were all from Sinopharm Chemical Reagent (Shanghai, China). Glucose, sodium potassium tartrate, sodium citrate, citric acid monohydrate, formic acid, acetic acid, and phenol were purchased from other local chemical reagent companies in Shanghai, China. The Glucose Kit from Kexin Biotech Institute (Shanghai, China) was used for glucose concentration measurement.

Analysis and Enzyme Assays

The sugar and degradation compounds in the hydrolysate were analyzed by high-performance liquid chromatography (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with a Bio-rad Aminex HPX-87H column. The composition of the major components was shown in Table 1.

The activity of Spezyme CP was assayed using the method by National Renewable Energy Laboratory, USA [14]. One unit of filter paper cellulase unit (FPU) was defined as

Table 1 Composition of major components in enzymatic hydrolysate of lignocellulose.

Components	Concentrations (mg/mL)
Glucose	84.65
Xylose	36.39
Acetic acid	9.10
Levulinic acid	1.49
Formic acid	2.56
Furfural	0.32
5-Hydroxymethylfurfural (5-HMF)	1.01
Vanillin	0.061
4-Hydroxybenzaldehyde	0.103

the amount of enzyme which produces 2.0 mg of reducing sugar from 50 mg of filter paper in 1 h. The experiment was carried out in a reaction mixture containing 0.5 mL appropriately diluted Spezyme CP, 1.0 mL of 50 mM citrate buffer (pH 4.8) and 50 mg of Whatman No. 1 filter paper. The reaction solution was incubated at 50 °C for 1 h. Then, the concentration of the released reducing sugar was measured using 3,5-dinitrosalicylic acid (DNS) method.

The activity of the cellobiase Novozyme 188 was assayed in a reaction mixture containing 1.0 mL of 80 mM cellobiose solution in 50 mM citrate buffer at pH 4.8 and 1.0 mL of appropriately diluted enzyme solution [15]. The reaction solution was incubated at 50 °C for 10 min, and the reaction was terminated by boiling it in a water bath for 2 min. One unit of cellobiase activity (CBU) was defined as the amount of enzyme that forms 2 μmol glucose per minute from cellobiose. Then, the concentration of the released glucose was measured using Glucose Kit. The Kit uses glucose oxidase to convert glucose to gluconic acid and hydrogen peroxide; then, the hydrogen peroxide reacts with a chromogenic oxygen acceptor in the presence of peroxidase and *o*-dianisidine to form a colored chromogen. The Kit was used for determination of reaction rate of cellulose hydrolysis when furan and phenol degradation products were inhibitors.

Dilute Acid Pretreatment and Enzyme Hydrolysis

Corn stover was milled and fractionated through a sieve with the pore diameter of 5 mm. The chipped corn stover was presoaked with 2.5% sulfuric acid for 1.5 h at ambient temperature. The hot steam (3 MPa) was jetted into the 2.5 L pretreatment reactor and kept jetting for 5 min at 180 °C. The pretreated corn stover was separated into a solid fraction and a liquid prehydrolysate through a stainless mesh under the pressure in the pretreatment reactor. The separation stopped when the pressure released to the atmospheric pressure.

The enzymatic hydrolysis (saccharification) experiment in the well-mixing state was carried out in 100-mL shaking flask containing 20 mL liquid at 200 rpm orbit shaking rate. The reaction conditions were 50 °C and pH 4.8 for 60 min, 1 g of filter paper (Whatman No. 1), 6 FPU of cellulase (Spezyme CP), and 90 CBU of cellobiase (Novozyme 188).

The saccharification experiment in the static state (kinetic experiment) was carried out at 50 °C in 50 mM citrate buffer, pH 4.8 for 60 min under various substrate additions (filter paper). The substrate cellulose (filter paper) ranged from 10 to 70 mg; furfural, 1.6 to 6.7 mg/mL; 5-HMF, 3.3 to 13.3 mg/mL; vanillin, 0.13 to 1.33 mg/mL; 4-hydroxybenzaldehyde, 0.13 to 1.33 mg/mL; ethanol, 10 to 60 mg/mL. The reactions were terminated in boiling water for 2 min. Aliquots were suitably diluted with water and assayed for total reducing sugars or glucose concentration.

Kinetics Parameters Estimation

The apparent kinetic parameters were obtained under the hypothesis that the industrial cellulase enzyme Spezyme CP, which is a complex composed of multiple cellulase components, was treated as one single cellulase enzyme. Similarly, the industrial cellobiase Novozyme 188, which is also composed of multiple enzyme components, was also treated as one single "cellobiase" enzyme. The Linewaver–Burk reciprocal to initial rate of the sugar formation were plotted for determination of the apparent parameters, including the maximum reaction rate V_{max}, the apparent Michaelis–Menten constant K_M, and inhibition constant K_I. In the case of competitive inhibition, the inhibitor binds to the same site on the enzyme as the substrate does, forming a dead-end enzyme–inhibitor complex. The rate

expression can be written as Eq. 1, and the equation is used as the working equation for the determination of kinetic parameters.

$$\frac{1}{v} = \frac{1}{V_{\max}} + \frac{K_{\mathrm{M}}}{V_{\max}}\left(1 + \frac{I}{K_{\mathrm{I}}}\right)\frac{1}{S} \tag{1}$$

Results

Reaction Rate Determination with the Existence of Lignocellulose Degradation Products

The enzymatic hydrolysis of cellulose includes the two consequent reactions: First, the cellulose is hydrolyzed into cellobiose by cellulase enzyme; then, the produced cellobiose is converted to glucose by cellobiase. In this work, DNS method was used for the determination of the initial reaction rate of the cellulase catalyzed hydrolysis of cellulose. When furan and phenol derivatives were used as inhibitors, the Glucose Kit method was applied with the sufficient cellobiase (Novozyme 188) addition because the two compounds react with DNS reagents and interfere the colorimetric measurement strongly. The glucose oxidase enzyme shows a very high degree of specificity for glucose determination, while it was not interfered by furan and phenol derivatives (data not shown). An experiment was designed to determine the appropriate enzyme ratio of cellobiase to cellulase to make the conversion of cellobiose to glucose not the rate-limiting step from cellulose to glucose.

Figure 1a showed that the glucose releasing rate increased with increasing ratio of Novozyme 188 (in CBU unit) to Spezyme CP (in FPU unit) and was almost constant when the ratio was greater than 20. The existence of the furfural, 5-HMF, and vanillin in the experimental concentration range did not change the effect of the ratio (data was not shown). Figure 1b and c showed the cases with the existence of certain concentration of 4-hydroxybenzaldehyde and ethanol, respectively. The similar glucose releasing tendency was found in the two cases. The glucose releasing rate was almost constant when the ratio of Novozyme 188 (CBU) to Spezyme CP (FPU) was greater than 30 with the existence of 4-hydroxybenzaldehyde and ethanol, respectively. Therefore, the enzyme ratio of Novozyme 188 (CBU) to Spezyme CP (FPU) was set to 20 except the cases with the existence of 4-hydroxybenzaldehyde and ethanol, which were 30.

Hydrolysis in the Well-Mixing State (Shaking Flask) Under the Inhibition of Lignocellulose Degradation Products

The hydrolysis of cellulose by Spezyme CP in the well-mixing state under the inhibition of lignocellulose degradation products was performed by taking into account of mass and heat transfer in a strongly shaking flask (200 rpm). The glucose concentration was assayed with Glucose Kit method. The results were shown in Fig. 2. Figure 2a–d showed the inhibition performances of organic acids (formic acid, acetic acid, and levulinic acid), furan derivatives (furfural and 5-HMF), lignin derivatives (vanillin and 4-hydroxybenzaldehyde), and ethanol, respectively. The control experiments were set at same conditions but without the addition of the above degradation products. The concentration of all the inhibitors were arranged based on that of the dilute acid pretreated hydrolysate as shown in Table 1. The concentration of ethanol was arranged according to the maximum ethanol concentration after the consequent fermentation process. All the inhibitors and ethanol showed significant competitive inhibitory effect to Spezyme CP.

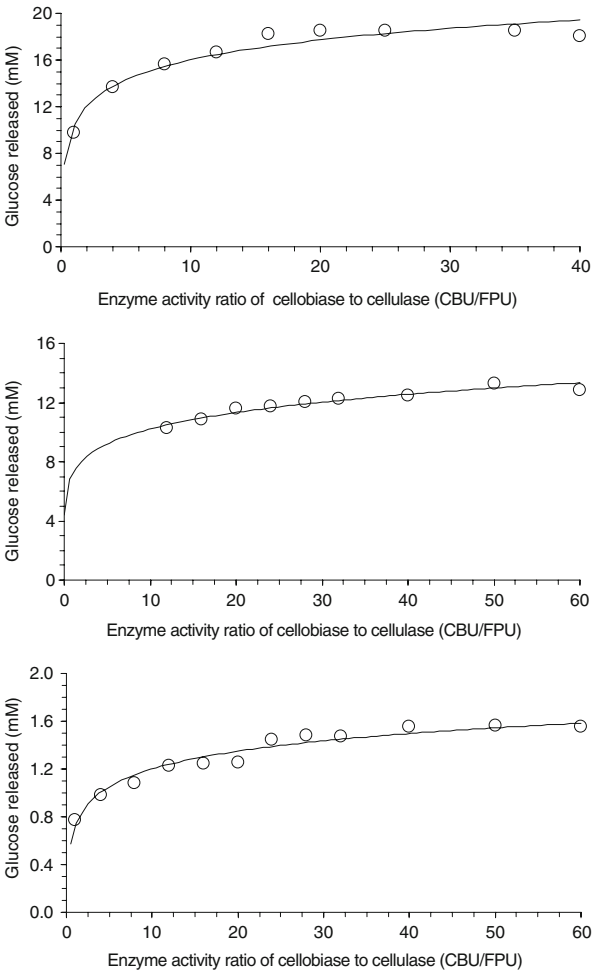

Fig. 1 Effect of the enzyme activity ratio of cellobiase (Novozyme 188) to cellulase (Spezyme CP) on hydrolysis reaction rate indicated by glucose released. The experiments were carried out in a glass tube with 1.5 mL solution at 50 °C, pH 4.8, filter paper (Whatman No. 1) addition of 10 mg, and Spezyme CP at 0.255 FPU/mL. **a**–**c** The experiments under the conditions without inhibitor addition, with the addition of 1.33 mg/mL 4-hydroxybenzaldehyde, and with the addition of 100 mg/mL ethanol, respectively

Hydrolysis in the Static State (Glass Tube) Under the Inhibition of Organic Acids

The hydrolysis of cellulose by Spezyme CP in the static state under the inhibition of lignocellulose degradation products was performed in a glass tube as described above for the determination of the apparent inhibition parameters of cellulase enzyme (Spezyme CP). The cellulase complex in Spezyme CP, including various exoglucanase (cellobiohydrolases) and endoglucanase, was assumed to be one single cellulase enzyme for the merit of quantitative assessment of the inhibition performance among these lignocellulose degradation products. The experiment was carried out in a glass tube as described in the "Materials and methods" section.

The initial rates of the hydrolysis under the inhibition of organic acids were determined in the concentration range for formic acid of 0 to 25 mg/mL, acetate acid of 0 to 30 mg/mL, and levulinic acid of 0 to 25 mg/mL, respectively. Since the organic acid does not react with DNS reagent, the DNS method was used for the initial rate determination. The Linewaver–Burk double-reciprocal plots of the initial reaction rate against the cellulose substrate loading were shown in Fig. 3. Figure 3a–c indicates the inhibition by formic acid, acetic

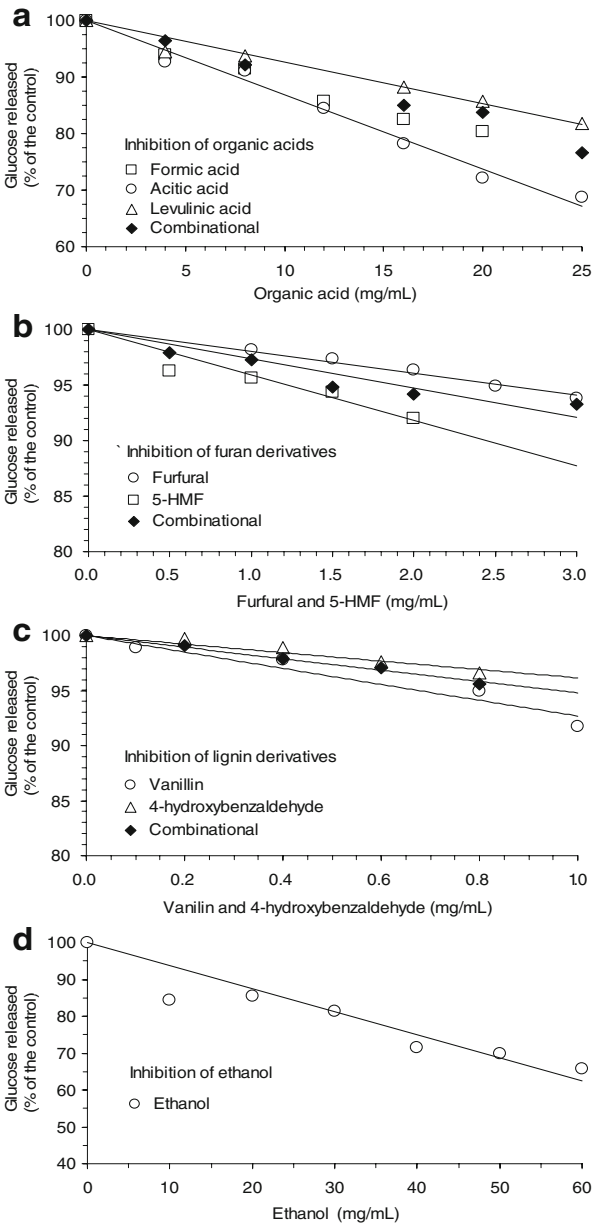

Fig. 2 Inhibition performance on hydrolysis rate under the well-mixing state. The reaction was carried out in 100 mL shaking flask containing 20 mL liquid at 200 rpm orbit shaking rate. Reaction conditions: 50 °C, pH 4.8, 60 min, 1 g filter paper (Whatman No. 1), 6 FPU of cellulase (Spezyme CP), and 90 CBU of cellobiase (Novozyme 188). The "control" data indicate the hydrolysis rate without the addition of inhibitors; the "combinational" data indicate the results under several inhibitors (the figures showed the total concentration of all inhibitors). **a**–**d** The inhibition performances of organic acids, furan derivatives, lignin derivatives, and ethanol, respectively

acid, and levullinic acid, respectively. The determined apparent parameters K_M, K_I, and V_{max} values were shown in Table 2.

Hydrolysis in the Static State (Glass Tube) Under the Inhibition of Furan Derivatives, Lignin Derivatives, and Ethanol

The initial hydrolysis rate under the inhibition of the two furan derivatives, furfural and 5-HMF, on Spezyme CP were determined using the method described in the "Reaction Rate

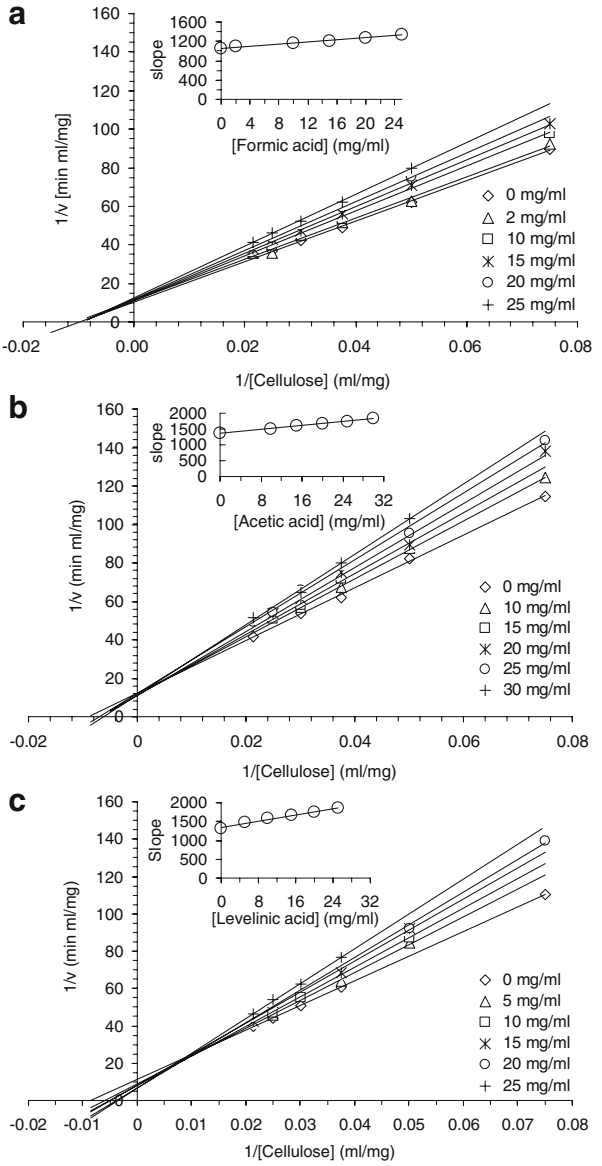

Fig. 3 Determination of the apparent inhibition parameters for organic acids under the static state. **a**–**c** The double-reciprocal plot of the initial rate with respect to the substrate concentration under the inhibition of formic acid, acetic acid, and levulinic acid, respectively. The experiments were carried out in a glass tube with 1.5 mL solution at 50 °C, pH 4.8, 10 to 70 mg filter paper (Whatman No. 1), 0.255 FPU/mL of cellulose (Spezyme CP). Plots of slope versus inhibitors concentrations are shown in the *insets*

Determination with the Existence of Lignocellulose Degradation Products" section in the furfural concentration range of 1.6 to 6.7 mg/mL and 5-HMF of 3.3 to 13.3 mg/mL. The released glucose was measured using Glucose Kit method. The glucose release was measured using Glucose Kit method. The Linewaver–Burk double-reciprocal plots of the initial reaction rate against the cellulose substrate loading under the different furfural and 5-HMF concentrations were shown in Fig. 4. The determined apparent parameters K_M, K_I, and V_{max} were shown in Table 2.

The initial rate of hydrolysis inhibited by the two lignin derivatives, vanillin and 4-hydroxybenzaldehyde, on Spezyme CP was determined using the same method in the

Table 2 Apparent kinetic parameters of the hypothesized cellulase in Spezyme CP.

Inhibitors	K_M (mg/mL)	K_I (mg/mL)	K_M /K_I	V_{max} (mg/min mL)
Formic acid	111.7	96.8	1.15	0.082
Acetic acid	111.7	88.7	1.26	0.082
Levulinic acid	110	109.5	1.01	0.082
Furfural	72.8	25.8	2.82	0.145
5-HMF	72.8	8.37	8.70	0.140
Vanillin	72.8	4.57	15.9	0.145
4-Hydroxybenzaldehyde	72.8	6.84	10.6	0.145
Ethanol	72.8	70.0	1.04	0.145

Conditions: 50 °C, pH 4.8, filter paper substrate (Whatman No. 1) at the static state

concentrations range of 0.13 to 1.33 mg/mL for both vanillin and 4-hydroxybenzaldehyde. The released glucose was measured using Glucose Kit method. The Linewaver–Burk double-reciprocal plots of the initial reaction rate against the cellulose substrate loading under the different vanillin and 4-hydroxybenzaldehyde concentration were shown in Fig. 5. The determined apparent parameters K_M, K_I, and V_{max} were shown in Table 2.

The initial rate of ethanol inhibited hydrolysis on Spezyme CP was determined using the same method in the ethanol concentration range of 10 to 60 mg/mL. The released glucose

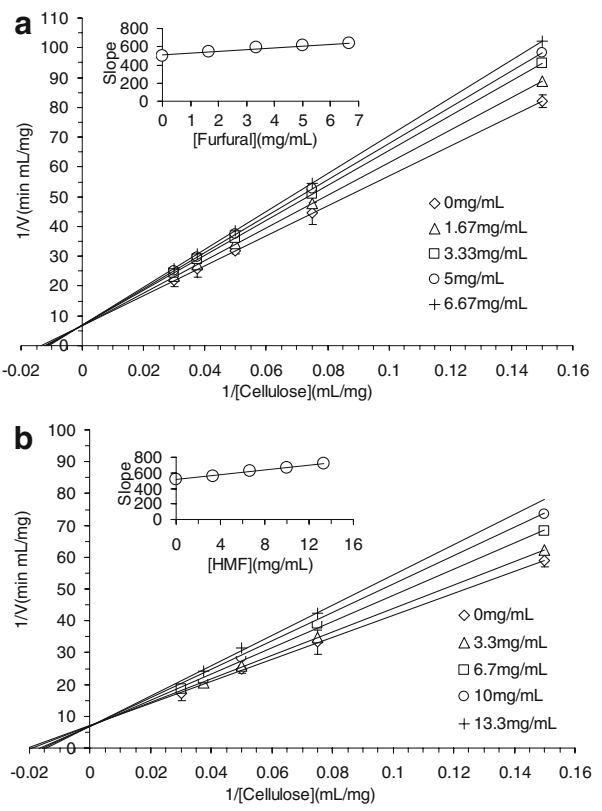

Fig. 4 Determination of the apparent inhibition parameters for furan derivatives under the static state. **a** and **b** The double-reciprocal plot of the initial rate with respect to the substrate concentration under the inhibition of furfural and 5-HMF, respectively. The experiments were carried out in a glass tube with 1.5 mL solution at 50 °C, pH 4.8, 10 to 50 mg of filter paper (Whatman No. 1), and 0.255 FPU/mL of cellulase (Spezyme CP). Plots of slope versus inhibitors concentrations are shown in the *insets*

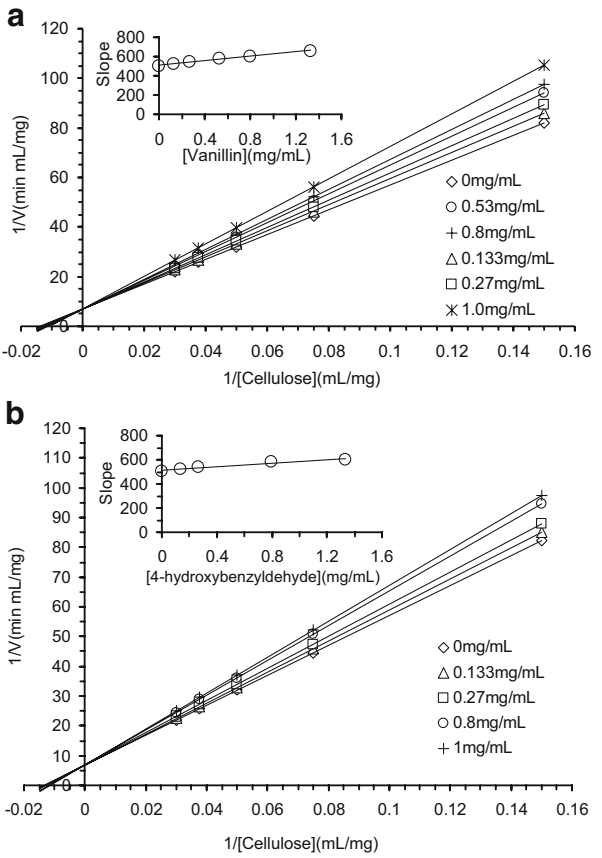

Fig. 5 Determination of the apparent inhibition parameters for lignin derivatives under the static state. **a** and **b** The double-reciprocal plot of the initial rate with respect to the substrate concentration under the inhibition of vanillin and 4-hydroxybenzyldehyde, respectively. The experiments were carried out in a glass tube with 1.5 mL solution at 50 °C, pH 4.8, 10 to 50 mg of filter paper (Whatman No. 1), 0.255 FPU/mL of cellulose (Spezyme CP). Plots of slope versus inhibitors concentrations are shown in the *insets*

was measured using Glucose Kit method. The Linewaver–Burk double-reciprocal plots of the initial reaction rate against the cellulose substrate loading under the different ethanol concentration were shown in Fig. 6. The determined apparent parameters K_M, K_I, and V_{max} were shown in Table 2.

Discussion

The degradation products inhibit the enzymatic hydrolysis by reducing the enzyme activities and inactivating the enzymes, reversibly or irreversibly, which makes more enzyme usage to obtain efficient cellulose conversion. In this work, the lignocellulose degradation products were represented by three groups of compounds based on the degradation pathway analysis. The inhibitors include three organic acids: formic acid, acetic acid, and levulinic acid; two furan derivatives, furfural and 5-HMF; and two lignin derivatives, vanillin and 4-hydroxybenzaldehyde. The inhibition performance of the degradation products as well as ethanol as an end product in the lignocellulose processing for production of fuel ethanol was investigated. First, the hydrolysis by Spezyme CP was examined in the well-mixing state to have the direct observation of the inhibition

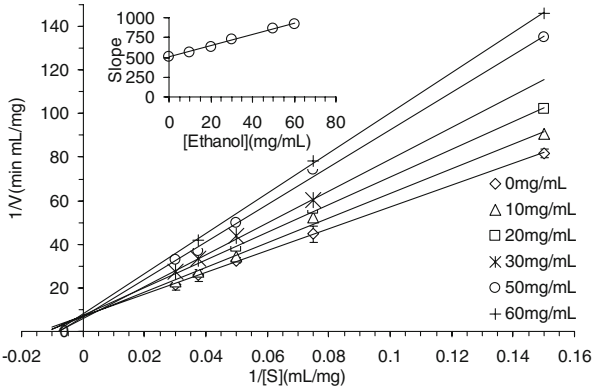

Fig. 6 Determination of the apparent inhibition parameters for ethanol under the static state. The double-reciprocal plot of the initial rate with respect to the substrate concentration under the ethanol inhibition. The experiments were carried out in a glass tube with 1.5 mL solution at 50 °C, pH 4.8, 10 to 50 mg of filter paper (Whatman No. 1), 0.255 FPU/mL of cellulose (Spezyme CP). Plots of slope versus inhibitors concentrations are shown in the *insets*

performance under the full spectrum of degradation products. Following the observation an "apparent" inhibition parameter concept was proposed by assuming the cellulase complex of Spzyme CP as one single cellulase, and only the cellulase function in Spezyme CP was assigned to this "single enzyme". The kinetic parameters were determined under the single cellulase hypothesis by measuring the initial rate of the hydrolysis under the inhibition of different degradation products. The Michaelis–Menten equation was used to be valid for modeling the cellulose hydrolysis [15]. Then, in this way, the obtained apparent "kinetic parameters", including the maximum velocity, Michaelis constant, and inhibition constant, could be used to assess the inhibition performance effectively.

The inhibition experiment in the well-mixing state gave a direct observation of degradation products on cellulase enzyme. Formic acid (25 mg/mL) only caused a 25% reduction of glucose concentration. For furfural and 5-HMF, glucose formation was reduced by 5% and 10%, respectively, at 3 mg/mL of inhibitor concentration. For lignin derivatives, the glucose formation reduced 7% and 2% when vanillin and 4-hydroxybenzyldehyde were inhibitors, respectively, at the concentration of 1 mg/mL concentration. Ethanol (60 mg/mL) reduced the glucose formation to 65% in the first 1 h of reaction.

The apparent kinetic experiment in the static state, as shown in Figs. 3, 4, 5, 6, showed that all the degradation products strongly inhibit the cellulose hydrolysis by cellulase enzyme, and the inhibitions on cellulase were all competitive type. The K_M/K_I value can be an approximate index to adjust the inhibitory effect to cellulase enzyme. The greater the K_M/K_I value, the stronger the inhibitory effect. The K_M/K_I values for the three organic acids are ranged from 1.01 to 1.26. Levulinic acid has the largest K_M/K_I value among the three acids, indicating levulinic acid is the stronger inhibitor, followed by acetic acid and formic acid consequently. The K_M/K_I values for furfural and 5-HMF are 2.82 and 8.70, indicating that the furan derivatives are much stronger inhibitors to cellulase than organic acids, and 5-HMF was the stronger inhibitor than furfural. The K_M/K_I values for vanillin and 4-hydroxybenzaldehyde are 15.9 and 10.6, indicating that lignin derivatives are the strongest inhibitors to cellulase among the three degradation products, and the inhibition of vanillin is stronger than that of 4-hydroxybenzaldehyde. The K_M/K_I value for ethanol is only 1.04, which is smaller than all three types of degradation products.

Although the order of the inhibition strength by the lignocellulose degradation products to cellulase is lignin derivatives > furan derivatives > organic acids > ethanol, the accumulated concentration of inhibitors in the practical lignocellulose processing is in the reverse order. Accumulated high ethanol concentration up to 5–10% is becoming the

priority of the processing target for the purpose of energy conservation in the final distillation step to get the high purified fuel ethanol. The organic acid concentration (1–3%) is the inevitable product of lignocellulose even at the modest condition, while in the furan and lignin derivatives, the concentration is very low as shown in Table 1. Therefore, the inhibition effect in the realistic hydrolysis by cellulase enzyme should consider both the intrinsic inhibition properties of the individual inhibitors and the inhibitor concentration.

This quantitative study gave an important view on the enzymatic hydrolysis of lignocellulose on the inhibition performance of the typical degradation products of lignocellulose. The results contribute to a rational design of pretreatment technology and lignocellulose hydrolysis process and also help to understand the lignocellulose recalcitrance to enzyme hydrolysis.

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