# Gas-trap Capturing of Enzyme Inhibitors in Explosion Gas from the Pretreatment of Corn Stalk with Dilute-Sulfuric Acid Steam

Huanlei Yang, Jun Li,* Lihuan Mo, and Jun Xu

In order to reduce production costs, heated explosion gases generated from dilute-sulfuric acid catalytic steam explosion (SE) pretreatment in the pilot production were recovered to provide energy for subsequent steps and to supply heated water (gas condensate water) for the washing steps. However, organic compounds in the explosion gases accumulated in the circulating water during continuous production, which affected subsequent enzymatic hydrolysis steps. The aim of this work was to investigate the major organic components in SE pretreatment gaseous products, their formation mechanism, and their inhibitory effects on the subsequent enzymatic hydrolysis of pretreated corn stalk.

*Keywords: Explosion gas; Pretreatment; Enzymatic hydrolysis; Inhibitory effect; Corn stalk*

*Contact information: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China; *Corresponding author: ppjunli@scut.edu.cn*

## INTRODUCTION

Lignocellulosic biomass is a promising substrate for bioethanol conversion and value-added products, and it has received extensive attention due to its abundance, low cost, and environmental benefits (Vallejos *et al*. 2015). Cellulose and hemicellulose are the major structural biopolymers of lignocellulosic biomass and they can be enzymatically hydrolyzed to monosaccharides and then fermented to ethanol (Lynd 1989; Esteghlalian *et al*. 1997; Cotana *et al.* 2015). However, plant biomass has a complex plant cell wall structure that is reluctant to engage in enzymatic and microbial deconstruction (Pu *et al*. 2013; McCann and Carpita 2015). A pretreatment step is therefore necessary to overcome the recalcitrance of the biomass, which is achieved by altering cell wall structural features to create a more suitable structure for enzyme access (Ruiz *et al.* 2013).

SE pretreatment is one of the most suitable pretreatment methods for industrial production. SE pretreatment efficiently removes hemicellulose to create structural changes of the substrates and to expose cellulose (Zhou and Runge 2015), but it has a relatively high production cost. Thermal and water processing cycles are expected to be adopted to reduce production cost in the pilot production. An efficient energy cycle (Fig. 1) is created when the heated explosion gases that are generated from SE pretreatment are collected to provide the energy for subsequent steps through a heat exchanger. The aqueous condensates are utilized in the washing steps. This method is environmentally friendly, and it is an effective utilization of pretreatment exhaust. However, the organic compounds in gaseous products that accumulate into the circulating water are often ignored. The continuous production of the organic compounds has the potential to affect subsequent hydrolysis steps. Further study is needed to determine if the gaseous products, after they are condensed, can be released directly into the atmosphere. The hot gaseous products are

difficult to capture using a conventional gas collecting device during the sudden release of high pressure after SE pretreatment.

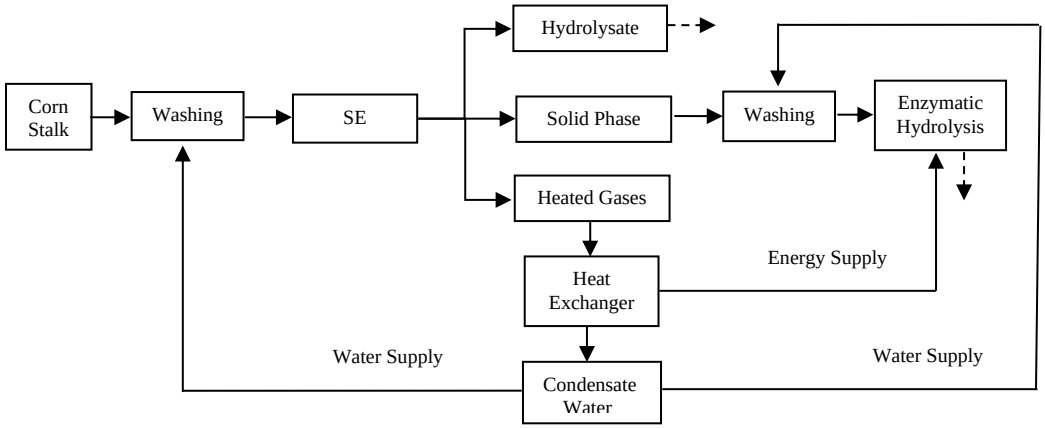


**Fig. 1.** A typical flow chart of the energy cycle for the SE pretreatment of corn stalk in the pilot production

There have been several studies published concerning the effects of lignocellulose-derived inhibitors on enzymatic hydrolysis (Monlau *et al*. 2014; Jonsson and Martin 2016). However, very few of these studies specifically have analyzed the inhibitors in gaseous products from SE pretreatment of corn stalk and their inhibitory effects on enzymatic hydrolysis. The aim of this work was to investigate the major organic components in SE pretreatment gaseous products, the formation source, and the resulting inhibitory effects on the enzymatic hydrolysis of pretreated corn stalk. Further understanding of the action mechanisms of these organic products was achieved through a simple kinetic model. Alternative approaches were raised and discussed in detail. Prior to these analyses, organic compounds in explosion gases were captured using a self-designed gas trap combined with a gas absorption instrument.

## EXPERIMENTAL

### Materials and Reagents

Raw corn stalk obtained from northeast China was used as the feedstock. Commercial cellulase (Celluclast 1.5) with a filter paper activity of 127 FPU/mL and β-glucosidase (Novozyme 188) with an enzyme activity of 285 CBU/mL were obtained from Novozymes Biotechnology Co., Ltd. (Tianjin, China). All other chemical reagents used in this study were of analytical reagent grade.

### Capture of Explosion Gas

The dilute sulphuric-acid impregnation was performed by mixing 200 g of dry corn stalk with 5% (w/w) $H_2SO_4$ at room temperature for 10 min, resulting in a solid-to-liquid ratio of 1:2.5. Steam explosion pretreatments were carried out using a removable explosion device (BL-08, Prosperous Technology Co., Ltd., Beijing, China) at a pretreatment temperature of 180 °C for 20 min. To capture these gaseous effusions, a self-designed stainless gas trap (Fig. 2) was connected to a cyclone separator by a unidiameter with three direct links. The gas trap consisted of an intake pipe 40 mm in diameter (a), a

discharge valve (c) on the bottom of tank, and an export pipe, 20 mm in diameter, that was connected to the atmosphere by a ball valve (b), and two make-and-break coupling pipes (d, e) outside of the gas trap. The make-and-break pipes connected the gaseous sample to the gas sample absorption equipment. To prevent a sharp pressure increase during the accumulation of effusion gases, the air export (b) was kept open while the gaseous substances were being collected. When the discharge ball valve was opened, the gaseous effusion partially entered into the gas trap through the intake pipe, with a 60-degree dip angle to the inclined plane. Eventually, the gaseous sample was retained in the gas trap when the (a) valve and the (b) valve were shut off simultaneously at the end of explosion process.

## Absorption of NCG

The absorption of the non-condensable gas (NCG) was performed by tandem absorption bottles filled with dichloromethane ($CH_2Cl_2$). The gas sample that was in the gas trap was then extruded by nitrogen ($N_2$) and forced to enter the absorption plant, in which soluble organic fractions of the gaseous sample were absorbed into $CH_2Cl_2$. After the gaseous sample was completely absorbed, the $CH_2Cl_2$ solution was collected and concentrated under reduced pressure with a rotary evaporator.

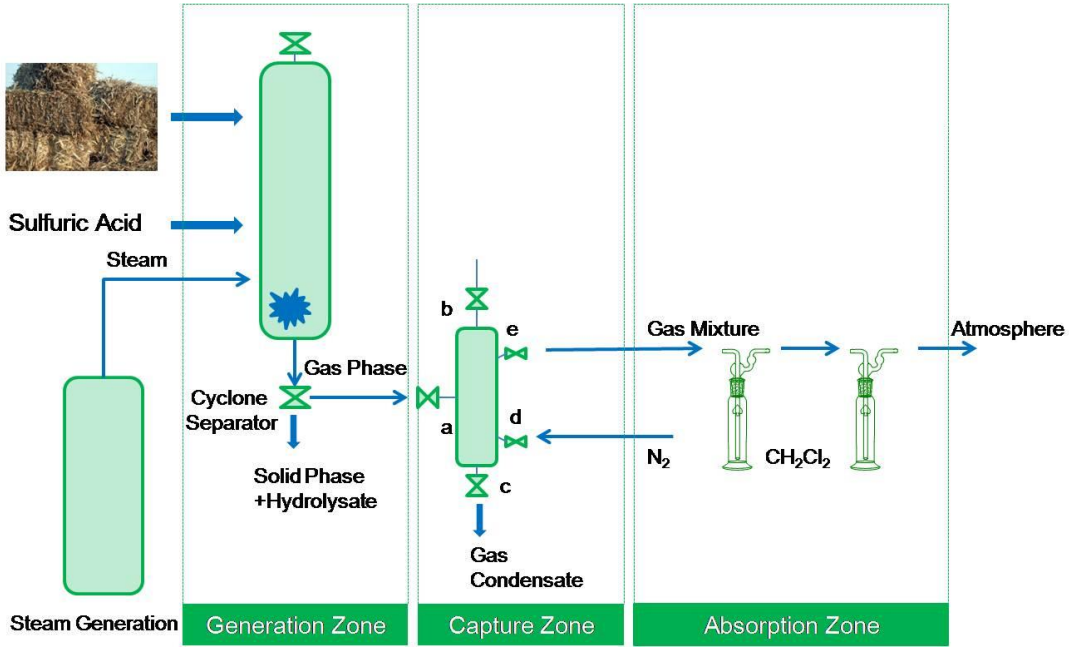


**Fig. 2.** Overview of SE pretreatment of corn stalk and the subsequent capture/absorption of explosion gas

## Extraction of CG

Condensate was collected from the discharge valve (Fig. 2c), and the condensable gas (CG) was extracted by $CH_2Cl_2$ with a ratio of 10:1. The extraction process was repeated three times to gain completely soluble organic fractions. The organic phase was then separated by a 250 mL separatory funnel. Finally, all extracted samples were combined and concentrated under reduced pressure with a rotary evaporator.

## GC-MS Analysis

Soluble organic fractions in the solutions of $CH_2Cl_2$ were detected by a gas chromatograph-mass spectrometer (GC-MS) (7890A GC system/5975C MS Detector, Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-5 ms column (30 m × 250 μm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The interface temperature and the injection temperature were both set at 250 °C. The GC oven temperature began at 35 °C for 5 min, increased to 130 °C for 4 min at a heating rate of 25 °C/min, and then increased again to 240 °C for 10 min at a heating rate of 10 °C/min. The scan range of the mass spectrum was 33 to 500 m/z, and the mass spectra was identified with NIST 14 Library.

## Analysis of Sugars

The solid composition of raw and pretreated corn stalk was analyzed following the standard NREL method (Sluiter *et al.* 2008), and the results are presented in Table 1. The sugar concentrations were analyzed by an ion chromatography system (Dionex ICS-5000, Thermo Fisher Scientific, Waltham, MA, USA) equipped with electrochemical detection. The sugars were separated by a Dionex CarboPac PA20 column in a solution of 2 mmol/L NaOH at a flow rate of 0.5 mL/min at 30 °C.

**Table 1.** Solid Composition of the Investigated Raw and Pretreated Corn Stalk

| Constituent | Raw Corn Stalk (%) | Pretreated Corn Stalk (%) |
|---|---|---|
| Glucan | 28.0 | 31.7 |
| Xylan | 19.30 | 7.41 |
| Arabinan | 2.74 | 0.98 |
| Galactan | 0.88 | 0.29 |
| Mannan | 0.72 | ND |
| Lignin | 26.6 | 30.9 |
| Ash | 4.29 | 3.11 |
| Extractive | 4.06 | 7.0 |

## $^{13}$C NMR Analysis

Two lignins obtained from raw and SE pretreated corn stalk were prepared by ball milling, and they were purified by solvent extraction as described (Yang *et al.* 2016). $^{13}$C NMR spectra were performed on a Bruker AVANCE III HD 600 MHz spectrometer (Ettlingen, Germany). A total of 120 mg of the isolated lignin was dissolved in 0.6 mL of DMSO-$d_6$ (99.9 %). The spectra were recorded in FT mode at 100.6 Hz with a 90° pulse angle, 2 s relaxation delay, and 60000 scans.

## Enzymatic Hydrolysis Inhibition Assay

Integrated inhibitor compounds were used to evaluate the enzymatic hydrolysis experiments. For this purpose, the dry pretreated corn stalks were mixed with Celluclast 1.5L and Novozyme 188 in 50 mmol/L of pH 4.8 sodium citrate buffer and placed in several 150 mL flasks. The inhibitor compounds were then added into each flask in various concentrations, resulting in a total reaction volume of 100 mL. The enzymatic hydrolysis was conducted at 50 °C and 250 rpm for 48 h in a thermomixer shaker. Samples were taken at regular time intervals, and 1.0 mL of suspension was removed and heated to 98 °C for 2 min. The obtained samples were processed for 30 s in a micro-centrifuge. The supernatants were retained for sugar analysis using a YSI 2900 biochemistry analyzer (Xylem,

Tunbridge Wells, UK).

### Cellulase Activity Inhibition Assay

Cellulase activity inhibition assays were carried out with individual cellulase quantities (Celluclast 1.5L) in the presence of inhibitor compounds, which were placed in several 25 mL test tubes. The mixtures were incubated in a Thermomixer shaker at 50 °C and 250 rpm for 48 h.

At regular time intervals, 0.5 mL was taken from each mixture, and the enzyme activity was determined by published methods (Li *et al.* 2005). The generated glucose concentration in each sample was analyzed with an ion chromatography system. The protein content of cellulase was measured following the Bradford method. The inhibition ratio ($\eta$) pertaining to cellulase activity was found by determining the ratio of cellulase activity in the presence of the model compound at incubated time ($t$) in comparison to the initial enzyme activity, as shown in Eq. 1.

$$\eta = \frac{\text{Enzyme activity [t]}}{\text{Enzyme activity [t]}_0} \quad (1)$$

## RESULTS AND DISCUSSION

### Characterization of Explosion Gas Generated from the SE Pretreatment of Corn Stalk

The characterizations of gaseous and aqueous by-products in the captured explosion gas were identified using GC-MS. The obtained chromatograms are illustrated in Fig. 3, and their identities as well as the relative contents of the major organic compounds are listed in Tables 2 and 3. Table 2 shows that the tested non-condensable gas were mainly volatile compounds, which consisted of phenols, furans, alcohols and small amounts of hydrocarbons. Phenol ($C_6H_6O$) was largely retained in the gas samples and the percent concentration reached about 71.68%. Furan, 2-vinyl- ($C_6H_6O$), was formed by the acid-promoted breakage of a hemicellulose furan structure. Polyetherols consisted of octaethylene glycol ($C_{16}H_{34}O_9$) and heptaethylene glycol ($C_{14}H_{30}O_8$), which can be found naturally in plants. A small amount of alcohols, mainly benzyl alcohol, were formed by the thermal degradation of lignin. The remaining organic compounds after production resulted in excess exhaust.

Table 3 details the major organic compounds in the pretreatment of condensable gas. Furfural is a main by-product and fermentation inhibitor after pretreatment of lignocellulosic biomass (Qureshi *et al.* 2012; Park *et al.* 2015), and its content reached approximately 31.49%. Phenols with the relative contents ranging from 0.89 to 13.65% mainly included phenol ($C_6H_6O$), phenol, 2-methoxy- ($C_7H_8O_2$), phenol, 2-methoxy-4-vinyl- ($C_9H_{10}O_2$), phenol, 4-ethyl-2-methoxy- ($C_9H_{12}O_2$), phenol, 3-methyl- ($C_7H_8O$), phenol, 2,6-dimethoxy- ($C_8H_{10}O_3$), and phenol, 2,4-bis(1,1-dimethylethyl)- ($C_{14}H_{22}O$). Apart from phenols, aromatic aldehydes including benzaldehyde, 3, 5 -dimethyl- ($C_9H_{10}O$), and benzaldehyde, 3-hydroxy-4-methoxy- ($C_8H_8O_3$) reached a relative content of 2.08 and 3.25%, respectively. In addition, a small amount of volatile organic acids were found in the form of acetic acid ($C_2H_4O_2$), Butanoic acid, and 4-hydroxy- ($C_4H_8O_3$).

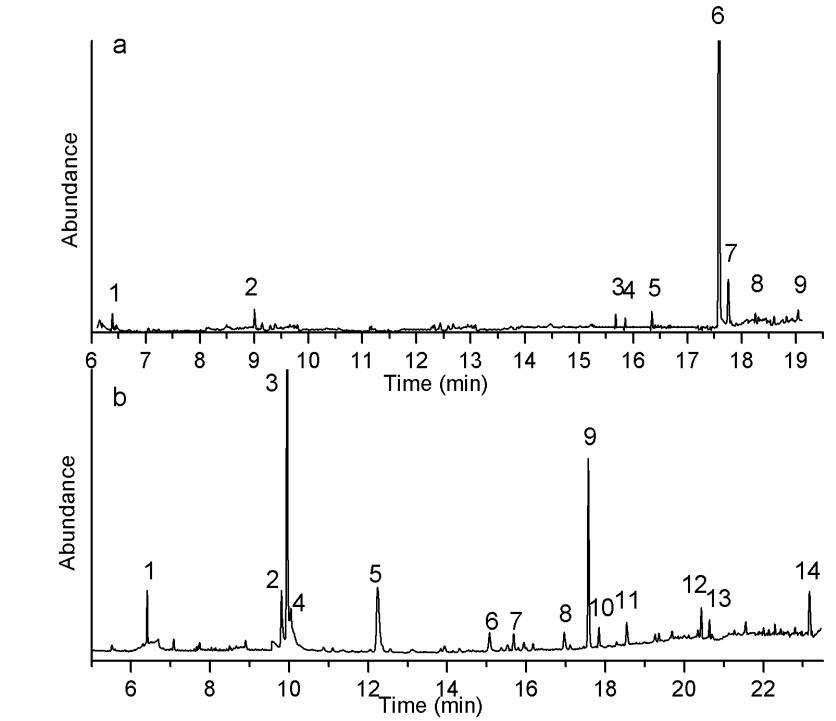


**Fig. 3.** GC-MS chromatograms of the extracted explosion gas and its condensate, (a): non-condensable gas; (b): condensable gas

**Table 2.** Major Organic Compounds in the SE Pretreatment of Non-Condensing Explosion Gas Identified by GC-MS

| No. | RT (min) | Compounds | Formula | Area (%) |
|---|---|---|---|---|
| 1 | 6.380 | Undecane | $C_{11}H_{24}$ | 0.86 |
| 2 | 9.010 | 2(5H)-furanone | $C_4H_4O_2$ | 1.33 |
| 3 | 15.674 | Phenol , 2-methoxy- | $C_7H_8O_2$ | 3.12 |
| 4 | 15.848 | Benzyl alcohol | $C_7H_8O$ | 2.13 |
| 5 | 16.350 | Phenol, 2-methoxy-4-methyl- | $C_8H_{10}O_2$ | 1.62 |
| 6 | 17.569 | Phenol | $C_6H_6O$ | 71.68 |
| 7 | 17.749 | Furan, 2-vinyl- | $C_6H_6O$ | 3.90 |
| 8 | 18.257 | Octaethylene glycol | $C_{16}H_{34}O_9$ | 1.19 |
| 9 | 19.041 | Heptaethylene glycol | $C_{14}H_{30}O_8$ | 1.70 |
| | Total | | | 87.53 |

## Formation of Major Organic Components during SE Pretreatment

During SE pretreatment, hemicellulose was degraded into monosaccharides that consisted mainly of xylose and arabinose. The monosaccharides underwent a series of secondary reactions to produce furfural and 5-HMF.

Acetic acid was generated in the next reaction through hydrolysis of the acetyl group in hemicellulosic compounds. The molecular mechanism of acidic hydrolysis to cellulose is the cleavage of the glycosidic bonds. Nevertheless, cellulose in SE pretreatment is difficult to degrade due to its linear crystalline structure consisting of β-1, 4-linked D-glucose units. Lignin is rarely included in solubilization during SE pretreatment; fragmented lignin compounds are observed as compared to that of alkali catalyzed pretreatments.

**Table 3.** Major Organic Compounds in the SE Pretreatment of Explosion Gas Condensate Identified by GC-MS

| No. | RT (min) | Compounds | Formula | Area (%) |
|---|---|---|---|---|
| 1 | 6.407 | Undecane | $C_{11}H_{24}$ | 0.86 |
| 2 | 9.806 | Acetic acid | $C_2H_4O_2$ | 5.28 |
| 3 | 9.945 | Furfural | $C_5H_4O_2$ | 31.49 |
| 4 | 10.042 | 3-furaldehyde | $C_5H_4O_2$ | 7.86 |
| 5 | 12.238 | Butanoic acid, 4-hydroxy- | $C_4H_8O_3$ | 10.72 |
| 6 | 15.068 | Benzaldehyde, 3,5-dimethyl- | $C_9H_{10}O$ | 2.08 |
| 7 | 15.681 | Phenol, 2-methoxy- | $C_7H_8O_2$ | 1.36 |
| 8 | 16.959 | Phenol, 2-methoxy-4- vinyl- | $C_9H_{10}O_2$ | 1.56 |
| 9 | 17.571 | Phenol | $C_6H_6O$ | 13.65 |
| 10 | 17.839 | Phenol, 4-ethyl-2-methoxy- | $C_9H_{12}O_2$ | 1.31 |
| 11 | 18.537 | Phenol, 3-methyl- | $C_7H_8O$ | 1.70 |
| 12 | 20.428 | Phenol, 2,6-dimethoxy- | $C_8H_{10}O_3$ | 1.75 |
| 13 | 20.632 | Phenol, 2,4-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 0.89 |
| 14 | 23.166 | Benzaldehyde, 3-hydroxy-4-methoxy- | $C_8H_8O_3$ | 3.25 |
| | Total | | | 83.76 |

Figure 4 displays the $^{13}C$ spectra of the isolated lignin prepared before and after the pretreatment of corn stalk.

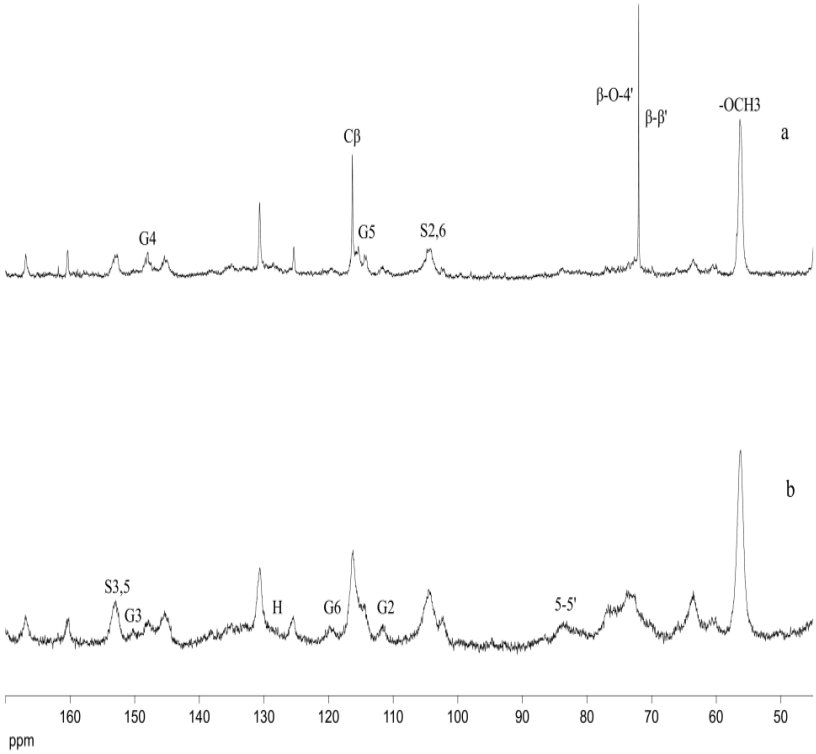


**Fig. 4.** $^{13}C$ NMR spectra of the isolated lignin prepared from before and after pretreatment of corn stalk, and (a) is pretreated lignin; (b) is raw lignin

The strong signal at 56.5 ppm is assigned to methoxyl (-$OCH_3$). Following the SE pretreatment, there were decreased signals at 104.3 ppm ($C_2/C_6$ in S), 152.7 ppm ($C_3/C_5$ in

S), 111.4 ppm ($C_2$ in G), 115.4 ppm ($C_5$ in G), 119.7 ppm ($C_6$ in G), 148.0 ppm ($C_3$ in G), and 128.6 ppm ($C_2$/$C_6$ in H). The decrease indicated considerable fragmentation in the lignin structure. Under acidic conditions, the degradation of lignin resulted predominantly in fragmentation by acidolysis of β-O-4' linkages and polymerization by acid-catalyzed condensation between the aromatic $C_6$ or $C_5$ and a carbonium ion, normally located at the $C_\alpha$ of the side chain (Li *et al*. 2007, 2009). In the initial stages of acidic pretreatment, the carbonium ions were formed by the cleavage of the α-hydroxyl and α-ether groups, and the abundant corresponding aromatic monomers were released. Moreover, the intermediary carbonium ions reacted with the weakly nucleophilic positions of other phenylpropane units to form stable carbon-carbon bonds. The narrowing signals near 72.8 ppm (β-O-4') and 84.3 ppm (β-β') indicated that there was side-chain cleavage and acid-catalyzed lignin condensation.

## Influence of Inhibitors on Enzymatic Hydrolysis of Pretreated Corn Stalk

The influence of the major organic components in explosion gas on enzymatic hydrolysis was evaluated with pretreated corn stalk, and the solid composition of the investigated corn stalk was reported in Table 1. To better reproduce the compounds from the explosion gas, an inhibitor model was prepared by mixing the major organic compounds at a certain proportion based on the CG composition. The major compositions of the prepared inhibitor model are presented in Table 4. Subsequently, the inhibitor model concentrations evaluated in this study fell in the range that was estimated for samples from the pretreatment gas condensate, where the releases of 5 to 10 % (2 to 6 g/L) of degradation by-products as gaseous products were expected. Additionally, these inhibitory chemicals were constantly accumulated by the water cycle in the continuous production, so a broad concentration range (2 to 25 g/L) of inhibitor model was estimated. Enzymatic hydrolysis was carried out with the inhibitor model at the different concentrations, solid loadings, and enzyme dosages.

**Table 4.** Major Composition of the Prepared Inhibitor Model Used in Enzymatic Hydrolysis Inhibitory Assay

| Composition | Ratio (%, v/v) |
|---|---|
| Furfural | 44.17 |
| Acetic acid | 7.40 |
| 3-furaldehyde | 11.03 |
| Phenol | 19.15 |
| Phenol , 2-methoxy- | 1.91 |
| Phenol , 2-methoxy-4-vinyl- | 1.83 |
| Phenol , 4-ethyl-2-methoxy- | 1.96 |
| Phenol , 3-methyl- | 2.38 |
| Phenol , 2, 6-dimethoxy- | 2.45 |
| Benzaldehyde , 3, 5-dimethyl- | 2.92 |
| Benzaldehyde , 3-hydroxy-4-methoxy- | 4.56 |

The inhibitory impacts of the model compound on the enzymatic hydrolysis of pretreated corn stalk at the concentrations of 2, 5, 10, 15, and 25 g/L are represented in Fig. 5. The hydrolysis was very fast in the first 8 h, and the glucose concentration was at nearly 60% of the maximum concentrations for the blank control. A glucose concentration of 4.80 g/L was obtained after 48 h of enzymatic hydrolysis. The glucose formation continually decreased as the dosage of inhibitor model increased. A notable decease occurred when the

inhibitor model concentration increased to 25 g/L, and the glucose concentration was reported at just 0.69 g/L after 48 h, which indicated that the enzymatic hydrolysis was extremely inhibited by the inhibitor model. In addition, end-product inhibition was observed in this series of tests, which indicated that the activity of β-glucosidase was suppressed by the investigated inhibitors. Ximenes *et al.* (2011) showed that lignin-derived compounds, mainly phenols, severely inhibit the activity of cellulase, and particularly, the activity of β-glucosidase from *Trichoderma reesei.*

Interestingly enough, when the inhibitor model was at a concentration of 2 g/L, it did not show obvious inhibition for enzymatic hydrolysis. However, glucose formation was observed in the initial stage of the reaction. These observations suggested that when the inhibitor model was at low concentrations, it increased the permeability of the substrate at the beginning of assays, which resulted in easier access for enzymes, even though there was a relatively small amount of damage. Cantarella *et al.* (2004) showed that the addition of 2 g/L furfural and 5-HMF does not display significant inhibitory effects on enzymatic hydrolysis. Comparatively, acetic acid and furans have little inhibition of the enzyme. Kim *et al.* (2011) showed that the presence of furfurals at 4 g/L and the presence of acetic acid at concentrations as high as 13 g/L were not obviously inhibitory to cellulase. In fact, the predominant role in the inhibitor model was in phenols rather than the carbohydrate by-products or organic acids.

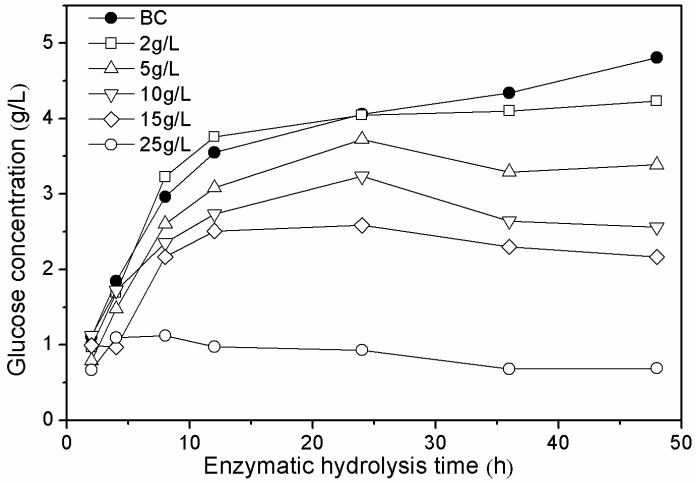


**Fig. 5.** Inhibitors influence on the enzymatic hydrolysis of pretreated corn stalk at the different concentrations of inhibitor model. BC is a blank control of pretreated corn stalk without inhibitors. The inhibitor model concentrations are 2, 5, 10, 15 and 25 g/L, respectively. The enzyme dosages of cellulase and β-glucosidase are 60 FPU/g and 120 CBU/g, respectively.

The inhibitory effects on enzyme hydrolysis from aromatic compounds are mainly due to deactivation of the enzyme. Enzyme deactivation is dependent on the incubation time; normally, it can be adapted to a first order model as described in Eq. 2,

$$\ln \eta = -kt \quad (2)$$

where ($\eta$) is the inhibition ratio of cellulase activity, ($k$) is the rate constant, and ($t$) is the incubated time in hours.

Figure 6 shows that linear relations between incubated time and inhibition ratio of cellulase activity were seen in all inhibitor loadings ranging from 2 to 25 g/L, and that the

rate constant (k) was considerably increased when the dosage of model compound reached 25 g/L, which indicated the loss of cellulase activities in the presence of a higher dosages of inhibitors. The related experiment was performed by Sattler *et al.* (1989), in which the effect of vanillin on cellulase activity was investigated, finding that vanillin decreased cellulase activity without causing a loss of enzymes. Ximenes *et al.* (2011) showed that the aromatic compounds, such as sinapic, vanillin, and syringaldehyde, were capable of causing both deactivation and reversible loss of cellulase activities. Interestingly enough, the latter is consistent with the results obtained in the present study.

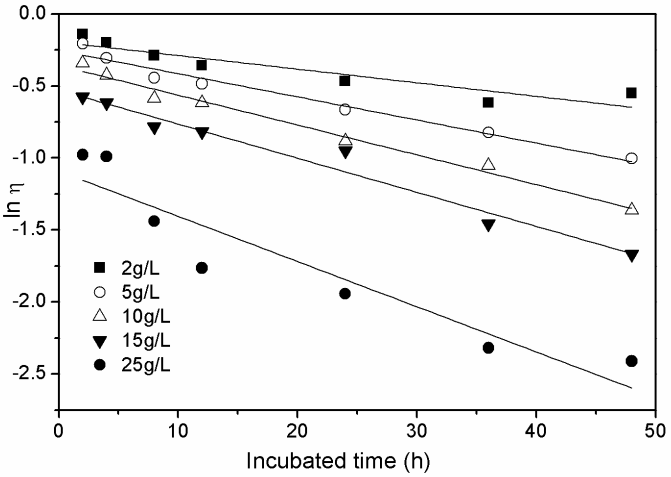


**Fig. 6.** Inhibitory influence of model compound on cellulase activity performed with individual cellulase in the presence of different dosages of model compound at regular time intervals

The inhibitory effects of phenolic compounds on enzymes are due in part to the protein precipitation interactions between them (Haslam 1974). The largest factor contributing to the inhibitor efficiency in enzymes is the inhibitor-to-enzyme dosage ratio. Therefore, further experiments were conducted to investigate the inhibitors influence on cellulase activities at different enzyme dosages. As shown in Fig. 7, there was a linear decrease in the inhibition ratios in cellulase activities as inhibitor/cellulase dosage ratios increased, which indicated that the inhibitory effect of model compound on cellulase activity is dependent on inhibitor-to-cellulase dosage ratio.

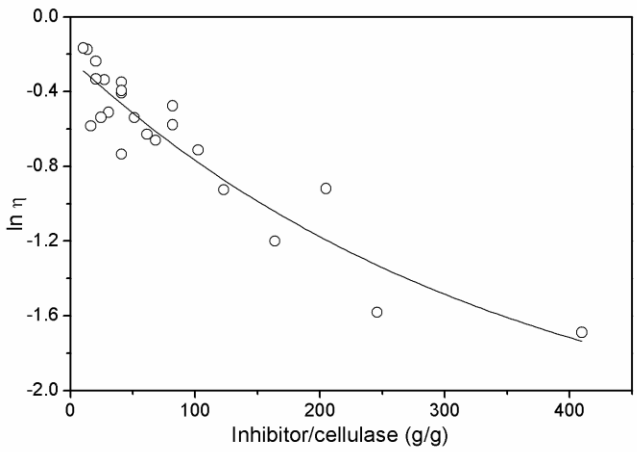


**Fig. 7.** Inhibitory influence as a function of inhibitor-to-cellulase dosage ratio (g/g) on cellulase activities

Related experiments were performed by Cantarella *et al.* (2004), who observed that increasing the enzyme dosage prevented enzyme inactivation in the presence of inhibitory compounds; however, there was also a decrease in the specific reaction rate (Malgas *et al.* 2016). Apart from these chemical damages to enzyme, the aromatic compounds can also occupy the enzyme active sites and hinder the contact between enzymes and substrates. Thus, the increase of inhibitor/cellulase dosage ratios indicated that more of the active sites of cellulase were exposed to cellulose, leading to higher cellulase activities.

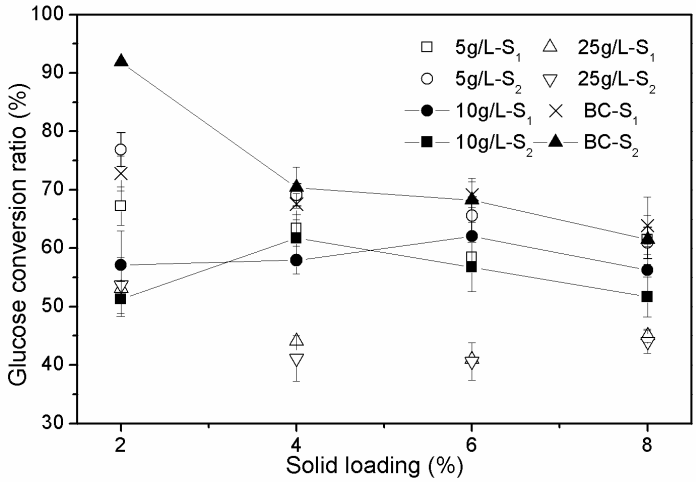


**Fig. 8.** Enzymatic hydrolysis of pretreated corn stalk loaded with various concentrations of inhibitor model at high solid loadings. S1: blank control with a 60 FPU/g cellulase dosage. S2: blank control with a 100 FPU/g cellulase dosage. The β- glucosidase-to-cellulase dosage ratio is 2:1.

Another series of hydrolysis tests was performed with different solid loadings (2 to 8%) in the presence of different concentrations of the inhibitor model ranging from 0 to 25g/L. Experiments BC-S1 and BC-S2, as shown in Fig. 8, were loaded with 60 and 100 FPU/g of cellulase dosages with a maximum glucose conversion ratio of 72.78 and 91.90%, and obtained at just 2% solid loadings. As shown in the blank control, the glucose formation continuously decreased as solid loadings increased from 2 to 8%. The experiments performed with different concentrations of inhibitor model showed unusual trends, particularly at 10 g/L when cellulase dosage was kept at 60 FPU/g and loaded with increasing solid contents (2, 4, 6, and 8%). The conversion rates of glucose, after 48 h, were 57.15, 58.96, 62.05, and 56.28%, respectively. This can be seen in the similar trend exhibited by the inhibitor model at 10 g/L with 100 FPU/g of cellulase dosage. It is therefore evident that by increasing substrate loadings while not exceeding 10 g/L, the inhibitor's effect on enzymatic hydrolysis can be alleviated. This is due to the increase of solid loadings directly resulting in the decreased inhibitor/cellulase dosage ratios.

As exhibited in Fig. 9, $C/C_0$ is the ratio of the glucose conversion rate in the presence of the model compound, in comparison to the blank control. Figure 9 demonstrates that the decrease in inhibitor/cellulase dosage ratios in the reaction medium alleviated competitive reactions. This was caused by introducing the inhibitor model to the active sites of cellulase, which consequently led to a decrease in glucose formation. However, the nonlinear relation curve, owing to the changes in medium transfer between enzymes and substrates, occurred as changes of solid loadings in enzymatic hydrolysis. When the concentration of the inhibitors exceeded 10 g/L, a detoxification step prior to the

water cycle was necessary to remove inhibitory chemicals in the continuous production. In order to alleviate the inhibitor's impact on enzymatic hydrolysis and on subsequent fermentation, the use of membrane filtration, activated carbon adsorption, and photo-catalytic oxidation of the circulating water prior to enzymatic hydrolysis should be incorporated.

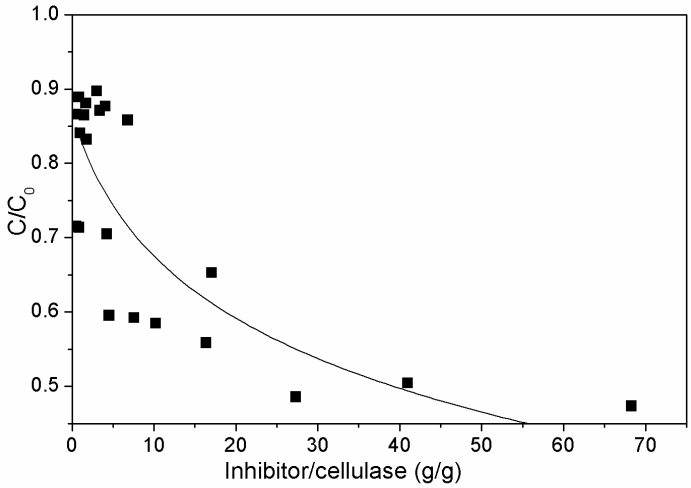


**Fig. 9.** Inhibitory influence as a function of inhibitor-to-cellulase dosage ratios (g/g) on glucose conversion ratios performed at high solid loadings

## CONCLUSIONS

1. Organic compounds in the pretreatment of explosion gas mainly included furans, phenols, aromatic aldehydes, and of volatile organic acids.
2. $^{13}C$ NMR analysis showed the degradation of lignin and the release of the corresponding aromatic monomers during SE pretreatment.
3. The presence of the organic compounds, at the investigated concentration range of 5 to 25 g/L, competitively inhibited the conversion of glucan to glucose by a time-dependent mode.
4. Cellulase inhibitory assays of the inhibitor model displayed a deactivation effect in cellulase activity.
5. Further experiments suggested that by not exceeding 10 g/L, the inhibitors impact on enzymatic hydrolysis in production could be controlled at a tolerable level by increasing enzyme dosages, or by appropriately adjusting substrate loadings.

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