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# Evaluation of soluble fraction and enzymatic residual fraction of dilute dry acid, ethylenediamine, and steam explosion pretreated corn stover on the enzymatic hydrolysis of cellulose

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## HIGHLIGHTS

- Enzymatic hydrolysis of different pretreated CS are compared.
- Soluble and enzymatic residual fraction are prepared to examine the inhibition.
- Inhibitions are compared between soluble fraction and enzymatic residual fraction.

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## ABSTRACT

This study is aimed to examine the inhibition of soluble fraction (SF) and enzymatic residual fraction (ERF) in dry dilute acid (DDA), ethylenediamine (EDA) and steam explosion (SE) pretreated corn stover (CS) on the enzymatic digestibility of cellulose. SF of DDA, EDA and SE pretreated CS has high xylose, soluble lignin and xylo-oligomer content, respectively. SF of EDA pretreated CS leads to the highest inhibition, followed by SE and DDA pretreated CS. Inhibition of ERF of DDA and SE pretreated CS is higher than that of EDA pretreated CS. The inhibition degree ($A_0/A$) of SF is 1.76 and 1.21 times to that of ERF for EDA and SE pretreated CS, respectively. The inhibition degree of ERF is 1.05 times to that of SF in DDA pretreated CS. The quantitative analysis shows that SF of EDA pretreated CS, SF and ERF of SE pretreated CS cause significant inhibition during enzymatic hydrolysis.



## 1. Introduction

Lignocellulosic biomass is considered as a renewable and sustainable feedstock to produce biofuels or chemicals (Zhong et al., 2010). Enzymatic hydrolysis of heteropolysaccharide to fermentable sugar is a feasible and effective route in biomass biorefinery (Li et al., 2010). Pretreatment of biomass is an essential step to achieve high sugar yields. Pretreatment brings about the compositional or structural changes and makes cellulose more digestible (Liu et al., 2015). Pretreatment affects the operation and yields of downstream processes, which ultimately determines the efficiency of the whole process (Garlock et al., 2011).

Pretreatment process generates amounts of soluble or insoluble materials, which inhibit the enzymatic digestibility of cellulose, especially in the enzymatic hydrolysis with high solid loading (Zhong et al., 2009; Qin et al., 2013). Soluble phenolics derived from lignin are generally produced after most pretreatment processes (Du et al., 2010). Phenolics are more detrimental to enzyme than soluble sugars, furan derivatives or organic acids at the comparative concentrations (Kim et al., 2011; Qin et al., 2013). Other work on phenolics inhibition also found that cellulase is more susceptible to be inhibited than $\beta$-glucosidase (Ximenes et al., 2011). Oligosaccharides were identified as strong cellulase inhibitors from the liquid fraction of the pretreated biomass (Kont et al., 2013; Xue et al., 2015). Xylo-oligomers were found more inhibitory than monomeric sugars, cellobiose or xylan for equal amounts (Qing et al., 2010). Xylo-oligomers and arabino-oligomers were shown to have high recalcitrance to enzyme activities of cellulase, $\beta$-glucosidase, hemicellulase and pectinase (Xue et al., 2015).

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Inhibition degree of soluble materials was found to depend on their concentrations with linear correlation (Holtzapple et al., 1990). The inhibition of insoluble materials is mainly from the enzymatic residual lignin. Lignin inhibition caused by non-productive enzyme adsorption has been deeply studied in past years (Berlin et al., 2006; Nakagame et al., 2010; Rahikainen et al., 2013; Guo et al., 2014). The adsorption of enzymes on lignin differs for enzyme species, lignin properties and pretreatment methods. Lignin inhibits cellulases most, followed by xylanases and $\beta$-glucosidase (Berlin et al., 2006; Guo et al., 2014).

Dry-to-dry pretreatment process is drawing more and more attention due to its operation convenience and thrift of water usage and energy consumption. Dry dilute acid pretreatment (DDA) is an improved technology of dilute acid pretreatment, which pretreats the biomass at high solid-to-liquid ratio (He et al., 2014a,b). No waste water is generated after DDA, and hence the pretreated materials with high solids content can be utilized directly in subsequent enzymatic hydrolysis and fermentation without washing or solid–liquid separation (Zhang et al., 2011; Zhu et al., 2015). Similar to dilute acid pretreatment (Hsu et al., 2010; Weiss et al., 2010; Noureddini and Byun, 2010), DDA degrades most of hemicellulose to xylose and by-products. Ethylenediamine pretreatment (EDA) is a relatively new alkaline pretreatment method and it can be operated at ambient pressure and high solid-to-liquid ratio without water addition (Qin et al., 2015). EDA pretreatment transforms cellulose crystal form, breaks the ether bonds in lignin and hemicellulose, and re-localizes lignin, thus drastically improves cellulose conversion in enzymatic hydrolysis. Both DDA and EDA are dry-to-dry processes. Steam explosion pretreatment (SE) is an extensively studied process in which the lignocellulosic biomass is heated by high-pressure saturated steam, followed by an explosive decompression (Oliveira et al., 2013; Cotana et al., 2014). SE partially solubilizes hemicellulose, but preserves most of cellulose and lignin in solid (Liu et al., 2014). All these three pretreatment methods can lead to high sugar yield in enzymatic hydrolysis process. However, the chemical properties of biomass pretreated by DDA and EDA have not been fully characterized yet. In addition, the inhibition of soluble and insoluble materials in the pretreated biomass on the cellulose conversion has not been compared.

In this study, we prepare the soluble fraction (SF) and the enzymatic residual fraction (ERF) from corn stover (CS) of DDA, EDA and SE, and present the chemical compositions of them. Besides, we investigate the relationship between the amounts of these two parts (SF and ERF) and their inhibitory effects. Previous studies often compared the effect of one component (soluble content or insoluble lignin) on cellulose conversion between different pretreated biomass. Here we compare the effect of SF and ERF to find out the main cause of inhibition in each pretreated corn stover (PCS).

## 2. Methods

### 2.1. Materials

CS harvested from Tianjin (China) was air-dried and milled. The moisture content of the milled CS was 5%. The particles between 20 and 80 meshes were collected and stored in air-tight containers prior to pretreatment. Moisture content and composition analysis of CS were determined according to the Laboratory Analysis Protocol (LAP) of the National Renewable Energy Laboratory (NREL). The dry matter of CS composed of 30.5% glucan, 19.8% xylan and 16.5% acid-insoluble lignin (AIL).

Pure cellulose, Avicel PH-101, was purchased from Sigma–Aldrich (MO, US). Commercial cellulase Accellerase 1500™ (89 mg protein/mL, 77 FPU/mL) and hemicellulase Multifect Xylanase™ (42 mg protein/mL) were gifted by Genencor (NY, US).

### 2.2. Pretreatment

All pretreatment processes were carried out according to previous experiences. An appropriate condition of each pretreatment was applied in order to achieve a relative high sugar yield. The pretreatment conditions were summarized in Table 1.

Dry dilute acid pretreatment (DDA) was conducted by the collaboration partners from East China University of Science and Technology as described previously (He et al., 2014a,b). In brief, 2100 g of the presoaked CS (1400 g of dry CS plus 700 g of dilute acid solution) was fed into a 20-L stainless cylinder reactor with a helical ribbon stirrer. The desired temperature (175 °C) was reached by replenishing saturated steam and the condition was maintained for 3 min. To stop the pretreatment operation, the steam supply was switched off and the steam inside the reactor was quickly released from the outlet of the reactor. The pretreated CS solid was taken out directly from the bottom of the reactor, air-dried and stored in −20 °C.

Steam explosion pretreatment (SE) was conducted in a 15-L reactor system as previous description (Liu et al., 2013a,b). During pretreatment, 150 g CS (dry matter) was top-loaded into the reactor. Steam was filled into the reactor until the temperature reached 200 °C (1.8 MPa). After 5 min of exposure to the saturated steam, CS was exploded into the reception chamber by the ball-valve. After pretreatment, the pretreated CS was separated from the liquid fraction by vacuum filtration. The pretreated solid was air-dried and stored in −20 °C.

Ethylenediamine pretreatment (EDA) was conducted in a vacuum drying oven (Qin et al., 2015). 100 g CS was mixed with 100 g ethylenediamine on a stainless tray. The mixture was then held in the vacuum oven at 130 °C for 10 min. After the holding time, vacuum pump was opened to vent ethylenediamine until the residual ethylenediamine was less than 5% (wt) in pretreated CS.

All PCS was used without any washing or detoxification process. The compositions of PCS were listed in Table 2.

### 2.3. Enzymatic hydrolysis of PCS

The enzymatic hydrolysis (EH) was conducted at 1% or 6% glucan loading with a 20-mL reaction volume in a 100-mL Erlenmeyer flask. Cellulase and hemicellulase loading were 15 and 10 mg protein/g glucan, respectively. Sodium azide (0.2%, w/v) was used to inhibit microbial contamination. Citrate buffer (5 mM) was used to keep solution pH = 4.8. Flasks were incubated at 50 °C in a shaker at 150 rpm. Samples were withdrawn and subjected to sugar analysis by HPLC with Aminex HPX-87H column as described previously (Qin et al., 2012). Glucose yield and xylose yield are calculated as following equation:

**Table 1**
Pretreatment conditions.

| Pretreatment conditions | Pretreatment | | |
|---|---|---|---|
| | DDA | EDA | SE |
| Chemicals | Sulfuric acid | Ethylenediamine | / |
| Loadings | 2.5% wt | 99% wt | / |
| Liquid to solid ratio | 1:2 | 1:1 | / |
| Temperature (°C) | 175 | 130 | 200 |
| Time (min) | 3 | 10 | 5 |