INDUSTRIAL CROPS AND PRODUCTS

Corn stover lignin is modified differently by acetic acid compared to sulfuric acid

Thibaut Mouthier[a], Maaike M. Appeldoorn[b], Herman Pel[b], Henk A. Schols[a], Harry Gruppen[a], Mirjam A. Kabel[a],*

[a] *Wageningen University and Research, Laboratory of Food Chemistry, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands*
[b] *DSM Biotechnology Center, Alexander Fleminglaan 1, 2613 AX Delft, The Netherlands*

ABSTRACT

In this study, two acid catalysts, acetic acid (HAc) and sulfuric acid (H_2SO_4), were compared in thermal pre-treatments of corn stover, in particular to assess the less understood fate of lignin. HAc-insoluble lignin, analyzed by pyrolysis GC–MS, showed decreasing levels (%) of Cα-oxidized (from 3.7 ± 0.2 to 1.8 ± 0.1), propenyl (from 2.5 ± 0.1 to 1.0 ± 0.1), vinyl-G (from 34.5 ± 1.8 to 28.4 ± 0.9), vinyl-S (from 4.2 ± 0.2 to 3.7 ± 0.1) and methylated (4.9 ± 0.04 to 2.8 ± 0.1) lignin units at increasing HAc amounts. Concurrently, unsubstituted and vinyl-H units increased (from 7.5 ± 0.5 to 11.3 ± 0.2 and from 40.5 ± 1.9 to 49.9 ± 0.9, respectively). Similar trends were seen for residual lignin in H_2SO_4 catalyzed pretreatments, al-though the composition differed from that of residual HAc-lignin. In particular, H_2SO_4-lignin showed slightly lower values (%) for unsubstituted (9.9 ± 0.2) and vinyl-H (45.7 ± 4.1) units, while Cα-oxidized (3.4 ± 0.4), propenyl (1.9 ± 0.1), vinyl-G (28.5 ± 0.9), vinyl-S (4.4 ± 0.6) and methylated (4.6 ± 0.2) lignin units re-mained higher compared to HAc-catalysis at similar pH values. Xylan yields and corresponding enzymatic conversions of the solids were similar regardless the type of acid. Our findings show that HAc in pretreatments decreased lignin complexity, possibly due to cleavage reactions, although subsequent recondensation reactions increased solid lignin yields, more than H_2SO_4, while removal of xylan and enzymatic conversion of solids were equal.

1. Introduction

Lignocellulosic biomass is considered as sustainable feedstock for the production of biochemicals or biofuels (Chen et al., 2017). A ma-jority of lignocellulosic by-products of the agro-industry are grass-like feedstocks, such as corn stover or wheat straw. These grasses are mainly constituted by the plant cell wall polysaccharides cellulose (30–50%w/w) and xylan (20–40%w/w), which together with the aromatic lignin polymer (5–25%w/w) form a complex network (Buranov and Mazza, 2008). Pretreatment of lignocellulosic biomass is required to open-up this complex network of polymers to give access to carbohydrate de-grading enzymes to release monosaccharides that can be further used in biomass valorization.

In commercial setups (i.e. POET-DSM in North America), majorly sulfuric acid is used as catalyst in thermal (above 160 °C) pretreatments of corn stover (Chen et al., 2017). Such acid pretreatment largely allows to dissolve hemicellulosic xylan, while cellulose and most of the lignin remains in the solids (Alvira et al., 2010; Kabel et al., 2007). This re-sidual carbohydrate fraction shows, as mentioned above, an improved enzymatic conversion (Hu and Ragauskas, 2012; Yang and Wyman, 2008). However, high pretreatment temperatures (above 160 °C) and residence time can lead to the formation of inhibitory by-products for further fermentation (Jonsson and Martin, 2016).

The amount of xylan dissolved has been reported to increase at decreased pH, which relates to an increased amount of xylan cleavages by acid generated H^+-ions in solution (Chen et al., 2017; Kabel et al., 2007). In contrast, lignin becomes only partly soluble during acid cat-alyzed thermal pretreatment and cellulose remains completely in the solid residue. In addition, during pretreatment dissolved lignin can again condensate and precipitate on the residual cellulosic fibers during cooling down (Pu et al., 2015). The latter has been shown to con-siderably decrease carbohydrate degradability due to either physical hindrance of enzymes by lignin to reach the cellulose, or by enzyme-protein adsorption to lignin avoiding that the enzyme reaches the

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cellulose (Hendriks and Zeeman, 2009). To overcome such lignin condensation and precipitation lignin should be degraded far enough to remain in the soluble, hence removable, fraction even upon cooling. Hereto, weak acids are reported to be more efficient than strong acids, although, so far only researched on dimeric lignin models. To be more specific, (Rahimi et al., 2014) have shown that weak acids, in particular formic and acetic acid, catalyze the cleavage of oxidized β-O-4 linked lignin dimers with a conversion yield of 61.2 w/w% into soluble low molecular-mass aromatics such as syringol, guaiacol and *p*-hydroxyphenol. In the same study, sulfuric acid was tested, but no cleavage of the oxidized dimer used was observed.

The lignin of grasses, like corn stover, is known to be rich in such β-O-4 linkages, which represents approximately 80% of the interunit linkages in grasses (del Rio et al., 2012; Rencoret et al., 2009). Other linkages are, for example, β-5 phenyl coumaran, β-β' pinoresinol, 5-5′ biphenyl and β-1 diaryl propane (Boerjan et al., 2003; Makela et al., 2015; Stewart et al., 2009). Moreover, in corn stover, acetic acid is intrinsically present as part of the xylan structure, hence, after it becomes released, it can possibly directly act on the lignin structures present (Van Dongen et al., 2011).

Based on the above described literature findings, in our study, it was hypothesized that a weak acid such as acetic acid not only cleaves oxidized dimers, but also polymeric lignin via the cleavage of oxidized β-O-4 linkages. Hence, in this research, the effect of acetic and sulfuric acid was studied on the corn stover lignin structure during thermal pretreatments ranging in severity and pH. In addition, all dry matter and carbohydrate mass balances were evaluated, as well as water insoluble carbohydrate degradability using a (hemi-)cellulolytic enzyme cocktail.

2. Material and methods

2.1. Materials

Dried corn stover (98%w/w) was provided by DSM (Delft, The Netherlands), and milled (< 1 mm) (Retsch Mill MM 2000, Retsch, Haan, Germany). The corn stover was milled using consecutive 6, 4, 2 and 1 mm sieve. The corn stover was finally sieved through a 1 mm sieve.

2.2. Acetic acid and sulfuric acid pretreatment

Milled corn stover was subjected to acid catalyzed hydrothermal pretreatment under defined conditions as shown in Table 1 using a 1L-Parr reactor (Moline, Illinois, USA). Each pretreatment was carried out with 37.5 g of corn stover to which 500 g of distilled water combined with acid was added. The reactor was stirred (100 rpm) during the total pretreatment time, including heating up and cooling down. The Parr reactor was heated with help of a heating mantle. Heating up time was 20 min till 160 °C was reached after which the treatment time was set (Table 1). At the end of the treatment time the Parr reactor was cooled within 40 min (10 min to reach 80 °C) with help of temperature controlled oil (set at −10 °C prior to the experiment), which was leaded through a metal spiral within the pretreated corn stover mixture. A typical heating up and cooling down profile is presented in Appendix A.

2.3. Separation of water soluble from water insoluble material

Pretreated corn stover samples were directly neutralized using 1 M NaOH and centrifuged (10,000g, 15 min). Supernatants were collected and immediately frozen. Residues were washed 5 times with distilled water and each time centrifuged (10,000g, 15 min). Part of the washed residues was freeze dried for further analysis and part was kept as wet water unsoluble solids (WUS) for direct enzymatic hydrolysis (see 2.4). Freeze dried WUS was subjected to neutral sugar content and composition, uronic acid content, Klason lignin content and pyrolysis GC–MS.

2.4. Enzyme hydrolysis of wet water unsoluble solids pretreated corn stover

Wet WUS (not subjected to freeze drying (see 2.3)) (10 mg/mL dry matter) was suspended in 50 mM citrate buffer (pH 4.5). Incubations were started by the addition of 2% w/w DSM enzyme cocktail containing cellulases and hemicellulases (protein/dry matter (dm); protein concentration 52 mg/mL) (DSM, Delft, The Netherlands). Samples were incubated for 5, 24 and 48 h at 60 °C in a head over tail rotating device. Enzymes were inactivated by adding 50 μL of concentrated hydrochloric acid prior to centrifugation (10,000g, 5 min) of which was pretested to result in a pH lower than 2. The supernatants were collected and subjected to mono and oligo-saccharides analysis as discussed in 2.5.5. All enzyme hydrolyses were performed in duplicate.

2.5. Analytical methods

2.5.1. Neutral sugar content and composition

The neutral sugar content and composition was determined in duplicate according to (Englyst and Cummings, 1984), using inositol as an internal standard. Samples were treated with 72% (w/w) H_2SO_4 (1 h, 30 °C) followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100 °C and the constituent sugars released were analyzed as their alditol acetates using gas chromatography (Focus-GC, ThermoScientific, Waltham, MA, USA). The column used was DB-225 (15 m × 0.53 mm id × 1 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). The initial column temperature was 180 °C with 2 min holding time. The temperature was then increased to 210 °C with a ramp of 2 °C/min, followed by 5 min

Table 1
Acetic and sulfuric acid (HAc and H_2SO_4) pretreatment conditions of corn stover, including temperature (°C), treatment time (min), amount of acid added and resulting combined severity factor (CSF). Sample codes are given for each pretreated corn stover material. The pH prior to (soaked for 1 h at 20 °C) and after pretreatment are indicated as well as the amount of free acetic acid present after pretreatment (standard deviations are all lower than 0.01).

Sample code	Temperature (°C)	Treatment time (min)	HAc (w/w%)[a]	H_2SO_4 (w/w%)[a]	CSF[b]	pH prior	pH after	Free HAc after (% w/w)[c]
0-0	160	60	0	0	−0.63	5.85	4.17	1.9
5-0	160	60	5	0	−0.23	3.92	3.77	7.3
15-0	160	60	15	0	0.12	3.51	3.42	17.4
30-0	160	60	30	0	0.33	3.23	3.21	32.6
0-1.2	160	60	0	1.2	0.40	3.08	3.14	2.5
0-2	160	60	0	2	1.15	2.24	2.39	2.6
5-1.2	160	60	5	1.2	0.52	2.88	3.02	7.5
5-2	160	60	5	2	1.16	2.24	2.38	7.7

[a] Expressed per 100 g dry matter corn stover as g HAc added or g H_2SO_4 added or both.
[b] Combined severity factor (according to Abatzoglou et al., 1992). CSF = log R_0 − pH = t. exp ((T − 100)/14.75) − pH
[c] Expressed per 100 g dry matter corn stover as g free HAc present after pretreatment in the mixture.