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Aqueous-ammonia delignification of miscanthus followed by enzymatic hydrolysis to sugars

Zhongguo Liu [a,b,c], Sasisanker Padmanabhan [a,b], Kun Cheng [a], Philippe Schwyter [a,b], Markus Pauly [a], Alexis T. Bell [a,b], John M. Prausnitz [a,b,*]

[a] Energy Biosciences Institute, University of California at Berkeley, CA 94720-1462, USA
[b] Department of Chemical & Biomolecular Engineering, University of California at Berkeley, CA 94720-1462, USA
[c] Alan G. MacDiarmid Institute, College of Chemistry, Jilin University, Changchun 130012, PR China

HIGHLIGHTS

- We report the effect of ammonia concentration and pretreatment temperature on delignification of miscanthus.
- The 2D NMR spectra indicate that ammonia pretreatment leads to de-acetylation of the xylan-backbone.
- The β-O-4′ linked aryl ether linkages remain the most abundant linkage in pretreated miscanthus.
- Infrared spectra indicate the disappearance of ester-linkages after aqueous ammonia pretreatment.

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ABSTRACT

This work concerns the effect of aqueous ammonia pretreatment at four temperatures and at 10, 20 or 30 wt.% ammonia. After 1 h, more than 65% delignification is achieved at 150 or 180 °C for high and for low concentrations of ammonia. When the delignified miscanthus is enzymatically hydrolyzed for 96 h using cellulases and beta-glucosidase, conversion of the recovered solid to glucose is 53.4% and to xylose 70.0%. Additional glucose and xylose can be obtained from the ammonia-containing aqueous phase. Increased ammonia concentration leads to better conversion. Fourier-transform infrared and Two-dimensional ^{13}C–^{1}H Heteronuclear Single Quantum Coherence (HSQC) Nuclear Magnetic Resonance spectroscopy provide data for the composition of the pretreated miscanthus and for that of the liquid extract. These spectra indicate that pretreatment with ammonia leads to de-acetylation of the xylan-backbone. The β-O-4′ linked aryl ether remains the most abundant linkage in the pretreated miscanthus.

1. Introduction

For the production of a liquid biofuel, miscanthus, provides a promising lignocellulosic-biomass feedstock (Brosse et al., 2010; Villaverde et al., 2009). As a perennial grass, miscanthus offers several significant advantages: rapid growth (over 3 m tall) in poor soils with minimal water and fertilizer needs, and high lignocellulosic-based carbohydrates (Carroll and Somerville, 2009; De Vrije et al., 2002). After pretreatment, miscanthus can be hydrolyzed to glucose and xylose by chemical or biochemical methods (Dee and Bell, 2011; Murnen et al., 2007). These sugars are then fermented to alcohol. However, prior to hydrolysis, it is necessary to remove lignin to increase reagent accessibility to the recovered polysaccharide-enriched solid. To raise reactivity for hydrolysis, it is also desirable to decrease cellulose crystallinity (Blanch et al., 2011; El Hage et al., 2010; Wang et al., 2012b).

Numerous research groups have discussed the production of biofuels from biomass (Garlock et al., 2011; Gupta and Lee, 2010; Dale et al., 2007; Rollin et al., 2011; Yang and Wyman, 2008). Lee and coworkers (Gupta and Lee, 2010) and Dale and coworkers (Krishnan et al., 2010) have given attention to using ammonia for pretreatment to remove lignin and to swell the solid residue, thereby increasing the accessibility of reagents to carbohydrates for conversion to sugar (Garlock et al., 2011; Kang et al., 2012; Kim et al., 2009; Yoon et al., 1995). Kim et al. (2009) described an aqueous-ammonia pretreatment method for corn stover. They reported enzymatic digestibility of 85% and 78% for glucan and xylan using 15 wt.% ammonia at 60 °C for 12 h. Kang et al. (2012) described a similar low-temperature aqueous-ammonia

* Corresponding author. Address: Energy Biosciences Institute, University of California at Berkeley, CA 94720-1462, USA. Tel.: +1 510 642 3592; fax: +1 510 642 4778.
E-mail address: prausnit@cchem.berkeley.edu (J.M. Prausnitz).

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Table 1
Recoveries of individual components of miscanthus after pretreatment. All recoveries are for the recovered solid only, based on the native miscanthus.

Aqueous ammonia concentration (wt.%)	T (°C)	Solid recovery (wt.%)	Recoveries of individual components of miscanthus from the recovered solid after pretreatment (wt.%)			Delignification (wt.%)
			Cellulose	Hemicellulose	Lignin	
30	180	60 ± 0.3	87.7 ± 0.6	39.3 ± 0.5	23.1 ± 0.3	76.9
	150	62 ± 0.1	91.9 ± 0.4	47.2 ± 0.6	26.20.8	73.8
	120	71 ± 0.5	94.4 ± 0.9	69.2 ± 1.0	38.3 ± 1.2	61.7
	100	77 ± 0.2	94.4 ± 1.0	76.0 ± 0.8	50.3 ± 1.5	49.7
20	180	62 ± 0.2	91.4 ± 0.3	45.6 ± 0.4	28.6 ± 1.0	71.4
	150	65 ± 0.2	90.7 ± 0.6	54.2 ± 0.6	32.5 ± 0.3	67.5
	120	73 ± 0.4	88.8 ± 1.5	60.4 ± 1.2	44.9 ± 0.5	55.1
	100	75 ± 0.5	88.5 ± 1.8	65.4 ± 1.3	49.0 ± 0.9	51.0
10	180	64 ± 0.3	92.8 ± 0.7	52.0 ± 0.5	34.5 ± 1.2	65.5
	150	70 ± 0.2	92.9 ± 0.3	66.6 ± 0.4	37.7 ± 0.5	62.3
	120	75 ± 0.4	95.5 ± 0.4	73.2 ± 0.9	46.2 ± 0.8	53.8
	100	79 ± 0.1	95.9 ± 0.8	77.1 ± 1.1	57.7 ± 0.7	42.3

pretreatment process for rapeseed yielding 60.7% glucose. These previous results suggest that the use of aqueous ammonia for pretreatment is highly effective.

There are only few reports of aqueous-ammonia pretreatment of miscanthus. Here, we examine aqueous-ammonia pretreatment of miscanthus. We studied the effect of ammonia concentration (10, 20 and 30 wt.% ammonia) at four temperatures (100, 120, 150 and 180 °C). Following delignification, we studied the composition of the resulting liquid extract and the residual solid using the standard methods developed by the National Renewable Energy Laboratory (NREL) (Templeton et al., 2010).

In addition to composition analysis, we use ATR-IR and 2D-HSQC-NMR spectroscopy for the structural characterization of pretreated miscanthus. Several recent articles have highlighted the advantages of using spectroscopy to gather information regarding the structure and chemical composition of pretreated biomass (Samuel et al., 2011; Villaverde et al., 2009; Wang et al., 2012a). In particular, several recent reports suggest the potential advantages of 2D HSQC for lignin structural, as a changes direct and accurate approach for the characterization of lignin, in miscanthus following pretreatment (Foston et al., 2011; Brosse et al., 2010; Kim and Ralph, 2010).

2. Experimental section

2.1. Materials

Miscanthus from the University of Illinois at Urbana-Champaign was milled to 4-mm particles using a Retsch grinder. The moisture content was 6.5 wt.%. On a water-free basis, miscanthus contains approximately: 42.0% cellulose, 25.0% hemicellulose, 27.0% lignin, 4.1% ash, and 4.0% others (pectin, lipid, salts). Aqueous ammonia (30 wt.%) was obtained from EMD Chemicals Inc., NJ, USA. Cellulase (Celluclast 1.5 L Product # C2730 – 50 mL) and beta-glucosidase (Novo188 Product # C6105 – 50 mL) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Activity of β-glucosidase (Novozyme 188 from Novo Inc., Lot No. 11K1088) was >700 cellobiase unit (CBU)/g. A CBU is defined as the quantity of enzyme that convents 1 μmol of cellobiose to 2 μmol of glucose per milliliter per minute. Enzyme loading was 20 FPU/g of glucan (Cellulase and beta-glucosidase), according to the procedure of Ghose et al. One FPU is defined as the quantity of enzyme that releases 1 μmol of glucose per milliliter per minute. All chemicals were analytical grade. Milli-Q Nano-pure water was used for washing and for diluting aqueous 30 wt.% ammonia solution to 10 or 20 wt.%.

2.2. Pretreatment process using aqueous ammonia

Miscanthus (dry, 1 g) and aqueous ammonia (10 g, 10, 20, or 30 wt.%) were added to a batch pressure reactor (4740 HP/HT Pressure Vessels, 25 mL, Moline Parr Instruments). The experiments were conducted without agitation. The solid-to-liquid ratio was 1:10. Two separate experiments were carried out under the same conditions, one for composition analysis of pretreated miscanthus using the NREL method, and the other for subsequent enzyme hydrolysis to sugar.

The pressure reactors were submerged for one hour in a preheated oil bath at the desired temperature (100, 120, 150 or 180 °C). Approximately 15–20 min were required to reach the desired temperature. The pressure inside the reactor was in the range 5–15 bar, depending on the concentration of ammonia.

After 1 h, the suspension was cooled to 70 °C. Ammonia gas was slowly released. The suspension was then filtered to separate the recovered solid from the aqueous phase that contains residual ammonia. The recovered solid was washed several times with Milli-Q pure water to remove traces of ammonia until the pH was 6–7. The solid was dried at 105 °C for 12 h. After drying, the weight of the recovered miscanthus was obtained using a Mettler-Toledo precision balance (±0.0001 g). Each experiment was done in triplicate. errors (%) are provided in Table 1.

For enzymatic hydrolysis, recovered miscanthus without drying was used because drying reduces enzyme accessibility. Keeping the solid wet avoids "hornification" of cellulose (Borrega and Karenlampi, 2011; Wang et al., 2012b).

2.3. Enzymatic hydrolysis of recovered miscanthus

Enzyme hydrolysis was performed for both native and recovered miscanthus following the NREL enzyme-protocol (Selig et al., 2008). Commercial enzymes were used, as indicated earlier.

Enzymatic hydrolysis was performed at 2-wt.% initial miscanthus loading in 0.05 M sodium citrate buffer (pH = 4.8). The flasks were incubated at 50 °C in a shaker at 150 shakes per minute for 96 h. Small samples were withdrawn at several time intervals (1,3,7, 24, 48, 72, and 96 h) and diluted with nano-pure water to measure glucose and xylose concentrations using HPLC.

2.4. Composition analysis of the recovered miscanthus

Analyses of the native miscanthus material and the recovered solids were performed using a standard NREL Analytical Procedure (Sluiter et al., 2010b) as briefly outlined below.