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Characterization of Value-Added Non-Carbohydrate Compounds Solubilized during Acidic Hot Water Flowthrough Pretreatment of Poplar Wood

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Acidic hot water flowthrough pretreatment (AHWF) is an attractive approach primarily because of its high efficiency for sugar recovery. However, a significant portion of carbon content in the solubilized fractions from lignin depolymerization and monosugar degradation has been studied to a lesser degree. Herein, we investigated the solubilized non-carbohydrate products from a series of flowthrough pretreatments of poplar wood by water-only or very dilute acid (0.05% to 0.1% w/w, H_2SO_4) at different temperatures (220 to 280 °C) and flow rates (10 to 62.5 mL/min). Results revealed that tailoring reaction parameters (temperature) and operational parameters of reactor (flow rate) without adding expensive catalysts can selectively produce specific non-carbohydrate compounds. Up to 50.9% selectivity of vanillin and 45.0% selectivity of syringaldehyde were obtained at 240 °C for water-only treatment with flow rates of 25 mL/min and 62.5 mL/min, respectively. Lower temperature (*e.g.*, 220 °C) was favorable for the formation of coniferyl alcohol, with the highest selectivity of 36.2%. Higher temperature (*e.g.*, 280 °C) or lower flow rate (*e.g.*, 10 mL/min) led to the formation of varied other aromatic compounds and HMF. Adding very dilute acid (0.05% to 0.1% w/w, H_2SO_4) into the water-only system considerably enhanced the formation of HMF with up to 66.7% selectivity.

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INTRODUCTION

The daily growing demand for liquid transportation fuel, together with the diminishing reserves of petroleum because of the growth of worldwide population, has increased the demand to switch from petroleum to renewable energy. Lignocellulosic biomass, because of its high abundance in forest/agriculture residues and relatively low environmental footprint, has been considered as a promising alternative for biofuels and biochemical production (Wyman *et al.* 2005). However, the intrinsic recalcitrance of lignocellulosic biomass presents challenges for its utilization on an industrial scale (Mora-Pale *et al.* 2011; Foston and Ragauskas 2012).

Pretreatment is one of the most poweful tools for disrupting the lignin-carbohydrate matrix to fractionate and liquify lignocellulosic biomass into low-molecular weight

platform molecules that can be further converted into biofuels and other value-added products (Himmel *et al.* 2007). However, consumption of expensive/corrosive chemicals in most pretreatment strategies has prevented them from being implemented.

Very dilute acid or even water-only pretreatment of lignocellulosic biomass at elevated temperatures (>200 °C) is an attractive approach because of its economic viability, higher recovery of carbohydrates, low chemical consumption, and fewer safety concerns (Yang and Wyman 2008b; Tao *et al.* 2011; Yan *et al.* 2014). Passing liquid hot water with or without addition of very dilute acid (*e.g.*, 0.05% w/w H_2SO_4) through lignocellulosic biomass by a continuous reactor (*e.g.*, flowthrough reactor) at temperatures ranging from 200 to 290 °C can result in up to 98% hemicellulose (Yang and Wyman 2008a; Yan *et al.* 2014), and 86% cellulose (Yan *et al.* 2014, 2016b) being hydrolyzed into solubilized C5 and C6 sugar monomers and oligomers, which comprises the majority of sugar recovery from lignocellulosic biomass (Yan *et al.* 2014, 2016b).

However, a considerable portion of carbon content in the solubilized fraction has been investigated to a lesser degree. Non-carbohydrate products such as aromatics from lignin depolymerization and monosugar degradation products are commonly observed in pretreatment liquor but have not been studied. Up to 60% lignin was decomposed into solubulized aromatic low-molecular weight compounds when pretreating lignocellulosic biomass with hot water in flow/percolation systems at reaction temperatures ranging from 190 to 280 °C (Allen *et al.* 1996; Phaiboonsilpa *et al.* 2010). Dissolved lignin-derived aromatic compounds were observed in the form of varied structures, including vanillin, isoeuginol, coniferaldehyde, and coniferyl alcohol (Phaiboonsilpa *et al.* 2010). In addition, various monosugar degradation compounds such as HMF(5-hydroxymethylfurfural), furfural, and lactic acid were observed through continuous reaction systems (*e.g.*, flowthrough) at 230 to 280 °C, which accounted for up to 40% of C5 or C6 sugar content (Phaiboonsilpa *et al.* 2010; Lü and Saka 2012).

Lignin is the largest source of natural polymers with an aromatic skeleton and thus an important renewable resource for phenolic chemical production (Davin and Lewis 2003, 2005; Ralph *et al.* 2004; Laskar *et al.* 2010; Ma *et al.* 2015; Vasco *et al.* 2016). Lignin-derived low-molecular weight compounds such as vanillin, syringaldehyde, and coniferaldehyde have been well documented as important value-added chemicals. For example, vanillin is important for food and flavor additives; syringaldehyde is a component in dyes and a pharmaceutical precursor (Hill *et al.* 2007; Ma *et al.* 2014, 2016). Sugar degradation compounds such as HMF are considered to be important building blocks for producing various kinds of chemicals and fuels (Yan *et al.* 2013).

Although observation of non-carbohydrate products is common, the product's profile is usually random, and high selectivity has not been achieved yet. Some preliminary attempts, such as adding high concentrations of sodium hydroxide (Silva *et al.* 2009), calcium salts (Silva *et al.* 2009), and copper salts (Fache *et al.* 2016) to produce specific non-carbohydrate compounds from lignocellulosic biomass (*e.g.*, vanillin, syringaldehyde) have been carried out, but high chemical consumption makes these strategies less competitive. By reviewing the intrinsic kinetics of hot water pretreatment (Yan *et al.* 2016a), the authors realized that optimizing and tailoring reaction parameters (*e.g.*, temperature) and reactor operational parameters (*e.g.*, flow rate) can likely achieve the same goal to selectively produce these non-carbohydrate structures with much less chemical usage. However, there exists a large knowledge gap on how to optimize the reaction parameters and the key reaction mechanisms.

This study intends to provide comprehensive characterization and comparison of non-carbohydrate product profiles from AHWF of poplar wood at various representative conditions. Gas chromatography mass spectrometry (GC-MS) was employed to separate and investigate the structures of low-molecular weight degradation products to elucidate the key reaction mechanisms. The outcomes from this study are expected to fill the knowledge gap of characterization of non-carbohydrate products from AHWF and to extend utilization of AHWF to new territories for value-added non-carbohydrate compound production.

EXPERIMENTAL

Feedstock

Poplar wood was acquired from Hebei, PR China. It contains 47.2% cellulose, 18.7% xylan, and 22.3% lignin, which was determined by standard National Renewable Energy Laboratory Analytical Procedures (Sluiter *et al.* 2008).

The materials were ground to particles using a laboratory mill and then were collected to pass between 20-mesh and 40-mesh sieves to obtain particles over a size range of 0.425 to 0.850 mm for experiments.

AHWF Experiment

The AHWF reactor used in this work was 1.2 cm i.d.× 15 cm length with an internal volume of 17.0 mL, including two gasket filters (316 stainless-steel, average pore size 5 μm) inside it. This unit was constructed of 316 stainless-steel. A 0.3 cm stainless-steel thermocouple was installed at the outlet of the reactor to monitor temperature. Stainless-steel tubing (316) was used as a preheating coil (0.6 cm o.d. × 0.1 cm wall) and to connect the reactor with other system components, as well the cooling coil (0.3 cm o.d. × 0.1 cm wall). A high-pressure pump with a flow rate range of 0 to 100 mL/min, a pressure gauge (pressure range 0 to 10 MPa), and a back-pressure regulator were used to control flow through the system. All reactor parts were obtained from Keteng Co., Suzhou, Jiangsu, China.

To operate the flowthrough unit, 1 g of substrate was loaded into the reactor, which was then connected to the system. Distilled water or 0.05-0.1(w/w) sulfuric acid was continuously pumped through the loaded biomass in the reactor at the targeted reaction conditions. The reactors were heated to reaction temperatures at 220 °C, 240 °C, 260 °C, and 280 °C for 6 min in an oil bath. The corresponding flow rates applied in this series of experiments were 10 mL/min, 25 mL/min, and 62.5 mL/min. The water consumption based on the aforementioned flow rates and reaction time (6 min) was 60 mL, 150 mL, and 375 mL, respectively. After the reaction, the pretreated liquids passed a cooling tube immersed in water for quenching to room temperature within around 1 min.

Analytical Methods

The collected samples were extracted with dichloromethane then analyzed with an Agilent gas chromatograph mass spectrometer (GC–MS; GC, Agilent 7890A; MS, Agilent5975C, Agilent Technologies, Santa, CA, USA) equipped with a DB-5MS column (30 m × 250 μm × 0.25 μm, Agilent Technologies, Santa, CA, USA). The oven temperature was programmed from 45 to 250 °C at a ramping rate of 5 °C/min. Both the initial and final temperatures were held for 5 min. The flow rate of the carrier gas (helium) was 1.3 mL/min.

The calculation of the selectivity of each compound was based on the percentage of peak areas determined from GC-MS chromatograms.

RESULTS AND DISCUSSION

A series of AHWF experiments under temperatures ranging from 220 to 280 °C at flow rates of 10 to 62.5 mL/min, with water-only or very dilute sulfuric acid (0.05 w/w - 0.1 w/w, H_2SO_4) was applied to pretreat poplar wood for generating dissolved non-carbohydrate compounds. The effects of reaction temperature, flow rate, and the addition of very dilute acid on the selectivity of specific dissolved non-carbohydrate compounds decomposed from poplar wood were investigated.

Impact of Reaction Temperature on Product Distribution

Figure 1 shows the selectivity distribution of non-carbohydrate compounds dissolved from poplar wood with water-only at temperatures ranging from 220 to 280 °C at a flow rate of 25 mL/min for 6 min. Such reaction temperature ranges, as reported in previous studies (Allen *et al.* 1996; Yang and Wyman 2008a; Phaiboonsilpa *et al.* 2010), can result in up to 100% hemicellulose and 60% lignin being degraded into soluble form. Among the dissolved non-carbohydrate products, lignin-derived aromatic compounds were the predominant products in GC-MS, with little sugar degradation compounds detected. This agreed with the fact that the majority of polysaccharide degradation products were sugar monomers and oligomers without undergoing further degradation. At 220 °C, coniferyl alcohol and syringaldehyde presented higher selectivity, at 36.2% and 32.6%, respectively. As reaction temperature was raised to 240 °C, vanillin, with 50.9% selectivity, became the major product among the dissolved non-carbohydrate compounds. As shown in Scheme 1, the formation of coniferyl alcohol under diluted acidic condition was the result of acidic cleavage of the β-O-4 bonds of lignin, while vanillin and syringaldehyde were considered to be formed through further oxidative cleavage at the $C_α$-$C_β$ position of the monolignol units coniferyl alcohol (G) and sinapyl alcohol (S), respectively (Laskar *et al.* 2013). In this regard, elevating the reaction temperature during hot water pretreatment appeared to enhance the oxidation cleavage of monolignol units, especially for the G unit, which was a benefit for vanillin production. However, as reaction temperatures continuously increased to 260 and 280 °C, the selectivity of vanillin decreased because of the emergence of minor by-products such as sinapaldehyde, 2-methoxy-4-propylphenol, acetovanillone, and 2,6-dimethoxyphenol, which developed from coniferyl alcohol (G) or sinapyl alcohol (S) through complicated side-chain removal or transformation (Zakzeski *et al.* 2010; Ma *et al.* 2015) at high temperatures. The exact mechanisms for the formation of these compounds needs further investigation. All these compounds were observed with less than 5% selectivity. Apart from the aromatic structure of compounds, the sugar dehydration product HMF was formed at relatively high temperatures (260 and 280 °C) as well. Because cellulose decomposes considerably into glucose at temperatures above 240 °C (Yan *et al.* 2014), HMF was recognized to form through glucose dehydration when reaction temperatures were further increased. The selectivity of HMF obtained at 260 and 280 °C was 6.2% and 38.7%, respectively. That is to say, the elevated temperature (*e.g.*, 280 °C) encouraged the formation of sugar degradation compounds such as HMF while reducing the selectivity for vanillin production.
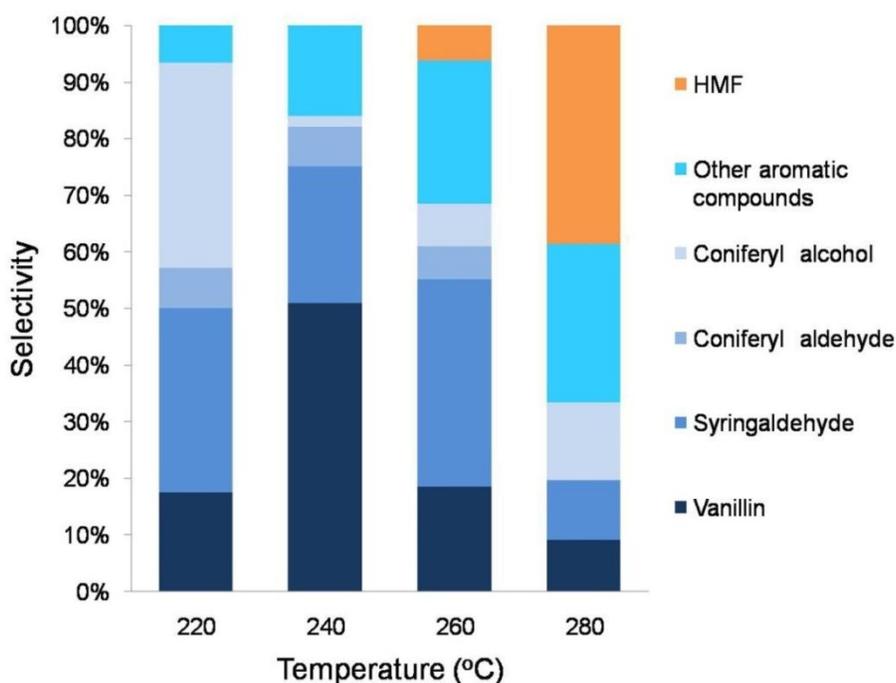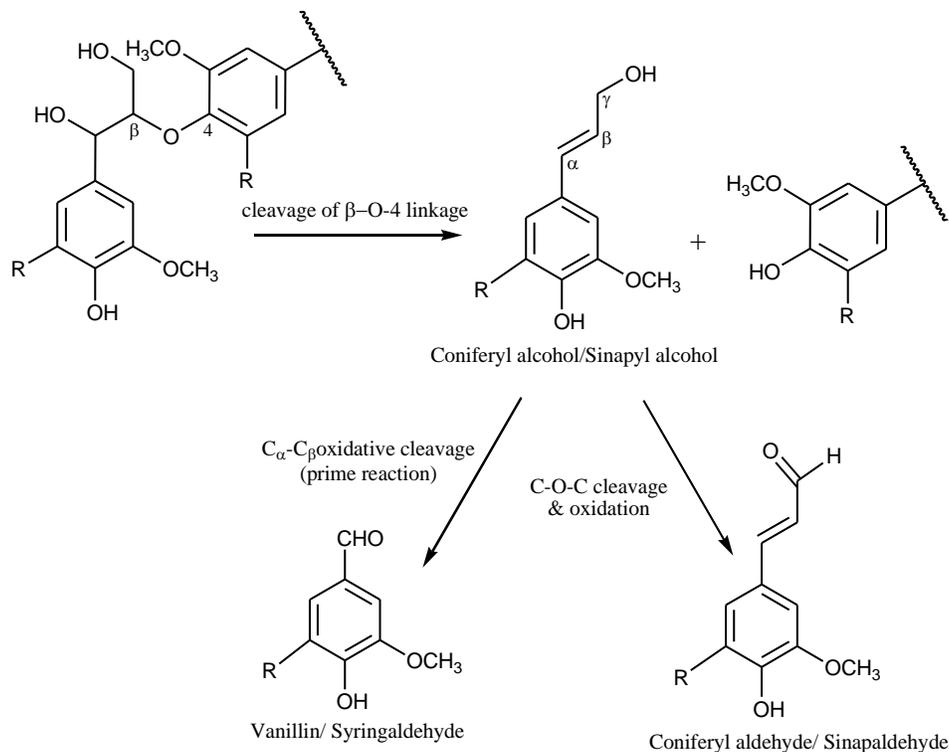
Fig. 1. Distribution of non-carbohydrate compounds dissolved from poplar wood with flowthrough pretreatment at various temperatures (220, 240, 260, and 280 °C). Conditions: 25 mL/min, 6 min, water-only.

Scheme 1. The possible degradation pattern of lignin in acidic hot water conditions (*e.g.*, 240 °C, water-only). R: OCH_3/ H

Impact of Flow Rate on Product Distribution

The distribution of non-carbohydrate compounds was also investigated at various flow rates. The effects of flow rate on the selectivity of non-carbohydrate products dissolved from poplar wood degradation are shown in Fig. 2. At 240 °C, higher flow rates of 25 mL/min and 62.5 mL/min resulted in fewer kinds of aromatic compounds, therefore leading to higher selectivity of vanillin and syringaldehyde. Particularly, at 62.5 mL/min, the selectivity of vanillin and syringaldehyde was 46.1% and 45.0%, respectively, with merely 8.9% other aromatic compounds detected. In comparison, the lower flow rate of 10 mL/min led to other aromatic compounds (12.7% selectivity), including 2-methoxy-4-propylphenol, 2,6-dimethoxyphenol, and phenol. These compounds were considered to be derived from the further transformation of C-C linkages on the propanyl side-chains of G and S (Zakzeski *et al.* 2010; Ma *et al.* 2015). It was supposed that the lower flow rate increased the exposure of released monolignols (*e.g.*, G and S) in hot water at high temperatures (*e.g.*, 240 °C), providing higher energy for more complicated C-C transformation reactions. This hypothesis in turn suggests that the acidic cleavage of ether linkages of lignin and the following oxidative cleavage reaction that resulted in vanillin and syringaldehyde occurred faster than other reactions. Apart from aromatic compounds, 18.7% HMF selectivity was obtained at a flow rate of 10 mL/min, indicating that the precursor of HMF, *i.e.*, glucose, was susceptible to dehydration if exposed in hot water for a relative long retention time because of the lower flow rate. This corresponds with previous studies (Yan *et al.* 2016a), which reported that glucose degraded rapidly once hydrolyzed from cellulose in hot water conditions.
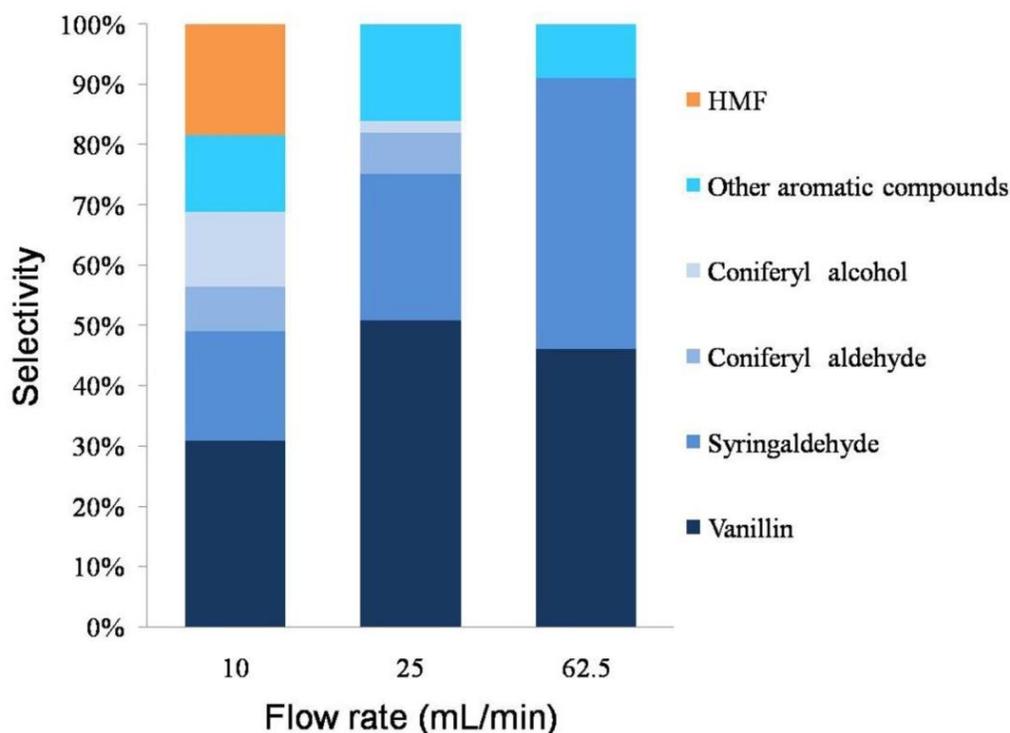

Fig. 2. Distribution of non-carbohydrate compounds dissolved from poplar wood with flowthrough pretreatment at various flow rates (10, 25, and 62.5 mL/min). Conditions: 240 °C, 6 min, water-only.

Impact of System Acidity on Product Distribution

AHWF benefits from the acidic characteristic of water because of its dissociation into hydronium ions at high temperatures (*e.g.*, 240 °C), which enhances the degradation of lignocellulosic biomass into sugar and non-carbohydrate compounds (Yan *et al.* 2016a). To evaluate the impact of acidity on the selectivity of non-carbohydrate products, 0.05% (w/w) and 0.1% (w/w) H_2SO_4 were added into the hot water flowthrough system. It was intriguing that the acidity of the system had more impact on sugar degradation products than lignin depolymerization. As shown in Fig. 3, 0.05% (w/w) H_2SO_4 flowthrough pretreatment at 240 °C led to 66.7% selectivity of HMF and 15.2% selectivity of furyl hydroxymethyl ketone, respectively, which was drastically different from the water-only conditions at the identical temperature, where no sugar degradations such as HMF were observed. The reaction mechanism regarding the formation of HMF and its isomer furyl hydroxymethyl ketone in very dilute acid conditions (*e.g.*, 0.05% (w/w) H_2SO_4) is proposed in Scheme 2. It can be anticipated that the proton would interact with the oxygen atom of the hemiacetal portion of glucose and the closest hydroxyl group. This leads to the formation of an enediol intermediate and the subsequent isomer of glucose, *i.e.*, fructose. Fructose is easily dehydrated into HMF through C2 and C5 hydroxy group intermolecular closure, with a consequent loss of three water molecules. In addition to HMF, its isomer furyl hydroxymethyl ketone was observed with 15.2% selectivity. The formation of this isomer can be extrapolated as a C3 carbonyl and C6 hydroxy group intermolecular closure, but not the C2 and C5 ring closure. It was interesting that the HMF selectivity decreased to 61.0% when sulfuric acid concentration increased to 0.1% (w/w). The reduced selectivity of HMF could be attributed to the formation of humins, which were observed by Patil and Lund (2011) as the condensed insoluble product of HMF.
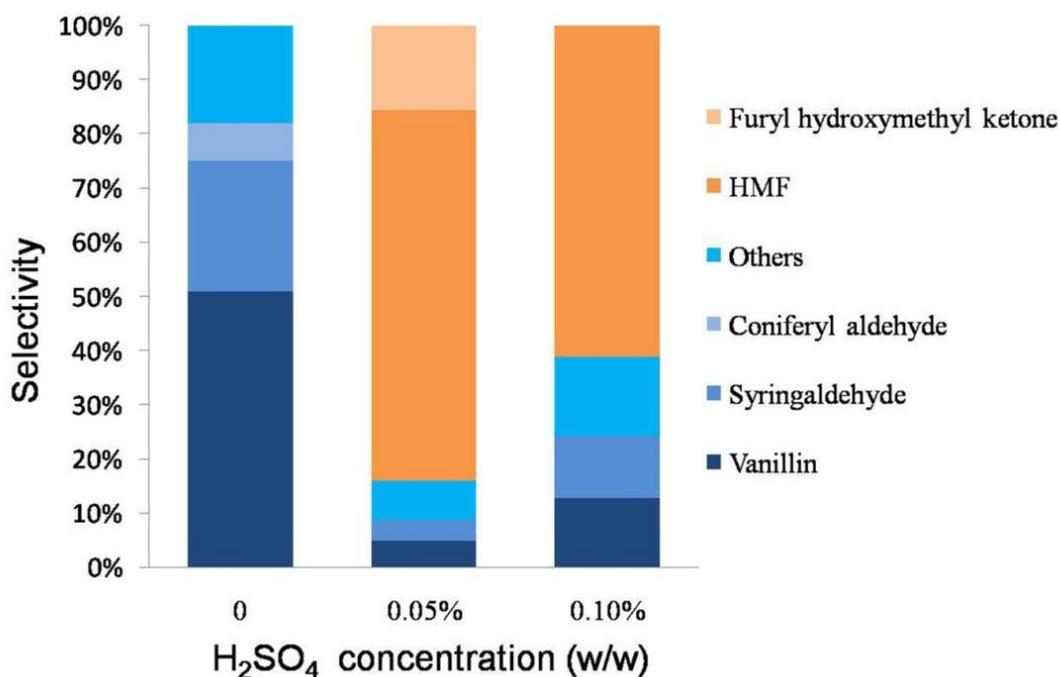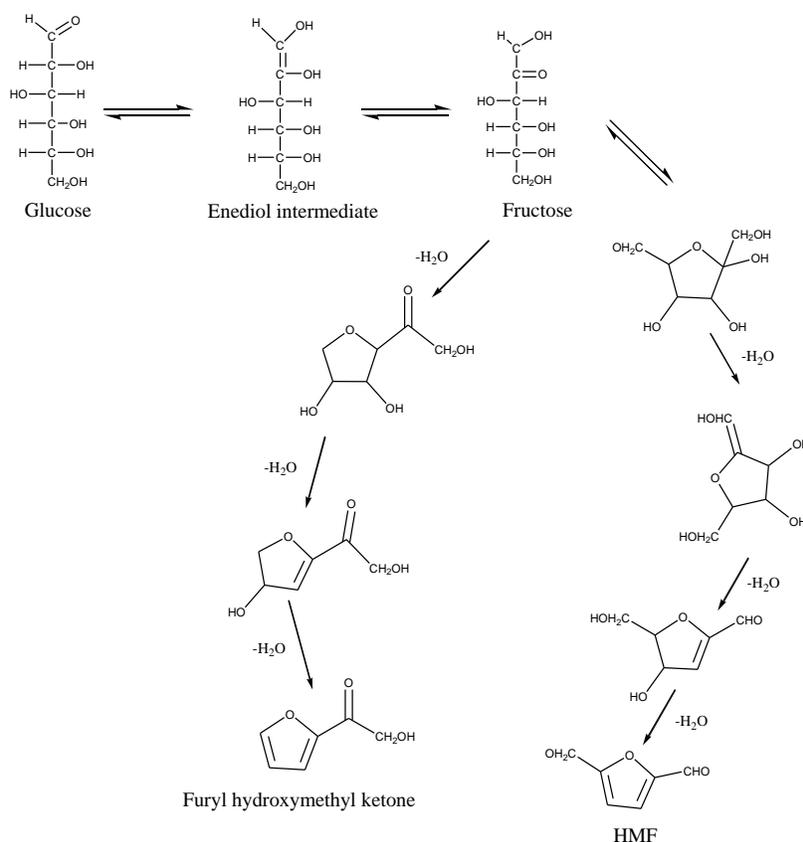

Fig. 3. Distribution of non-carbohydrate compounds dissolved from poplar wood with flowthrough pretreatment at various H_2SO_4 acid concentrations (0, 0.05% (w/w), 0.1% (w/w). Conditions: 240 °C, 25 mL/min, 6 min.
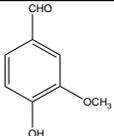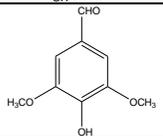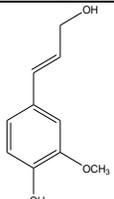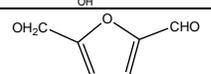
Scheme 2. The proposed reaction mechanism of glucose degradation in acidic hot water conditions (*e.g.*, 240 °C, 0.05% w/w H_2SO_4)

Optimization of Treatment Conditions

AHWF provides an effective approach to selectively generate non-carbohydrate compounds from lignocellulosic biomass. Table 1 compares and summarizes the highest selectivity of the major non-carbohydrate compounds dissolved from poplar wood after flowthrough pretreatment with water-only or very dilute sulfuric acid (0.05% H_2SO_4). The corresponding GC-MS spectra are shown in Fig. 4. Among the tested conditions, pretreatment carried out at 240 °C and 25 mL/min with water-only resulted in the highest vanillin selectivity among the tested conditions (50.9%). A higher flow rate of 62.5 mL/min at an identical temperature led to the highest syringaldehyde selectivity of 45.0%. Based on this, it would be plausible that the formation of syringaldehyde from its precursor sinapyl alcohol was faster than vanillin. Thus, the minor amount of sinapyl alcohol observed in the tested conditions could be attributed to its rapid oxidative cleavage to syringaldehyde.

The obtained vanillin and syringaldehyde could be purified through molecular imprinting technology or organic solvent extraction (Mohamad Ibrahim *et al.* 2009; Mbotchak *et al.* 2015), and applied as food additives and pharmaceutical precursors (Eckert *et al.* 2007). The highest selectivity of coniferyl alcohol was 36.2% at 220 °C, indicating that such a monolignol unit was more stable instead of undergoing subsequent oxidative cleavage reactions at relatively lower temperatures. As previously mentioned, HMF, the glucose dehydrated compound, reached its highest selectivity of 66.7% after pretreating the poplar wood with 0.05%(w/w) H_2SO_4 at a temperature of 240 °C and flow rate of 25 mL/min.

Table 1. The Highest Selectivity of the Major Non-Carbohydrate Compounds Dissolved from Poplar Wood after AHWF

Compounds	Conditions	Selectivity	Structure	Formula
Vanillin	240 °C, 25mL/min, water-only	50.9%		$C_8H_8O_3$
Syringaldehyde	240 °C, 62.5mL/min, water-only	45.0%		$C_9H_{10}O_4$
Coniferyl alcohol	220 °C, 25mL/min, water-only	36.2%		$C_{10}H_{12}O_3$
HMF	240 °C, 25mL/min, 0.05%H_2SO_4	66.7%		$C_6H_6O_3$
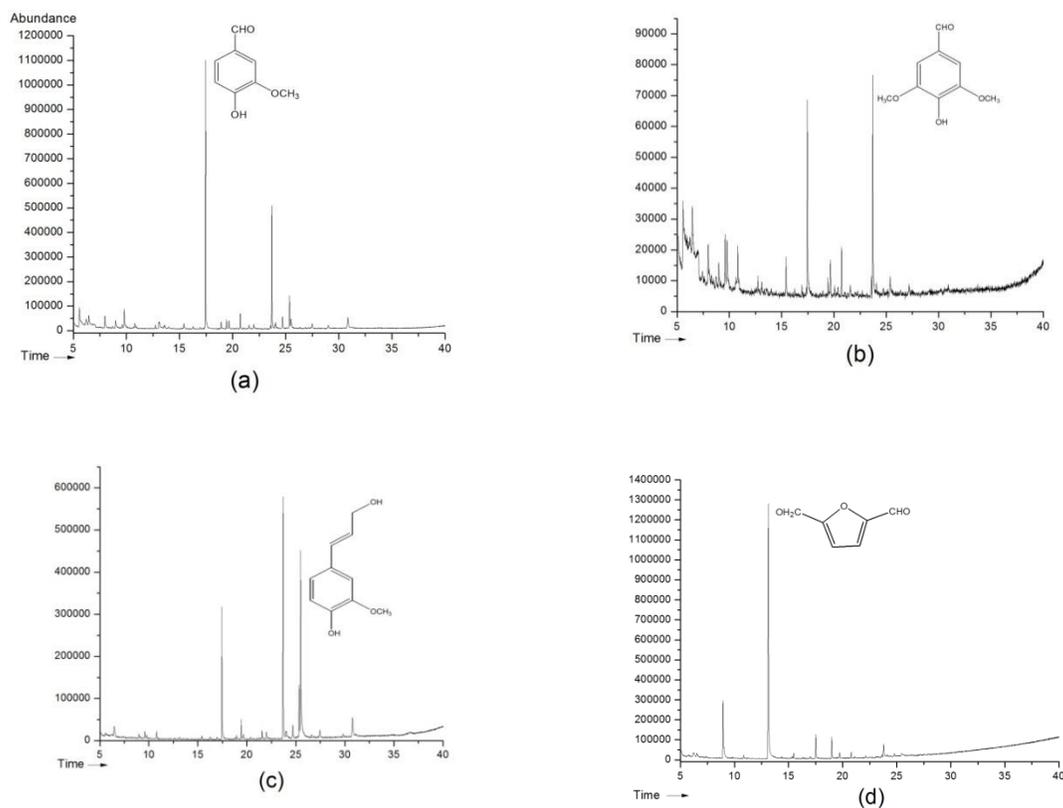
Fig. 4. The profile of major non-carbohydrate compounds dissolved from poplar wood after AHWF: (a) 240 °C, 25 mL/min, water-only; (b) 240 °C, 62.5 mL/min, water-only; (c) 220 °C, 25 mL/min, water-only; (d) 240 °C, 25 mL/min, 0.05% H_2SO_4

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On the contrary, the addition of sulfuric acid in turn alleviated the selectivity of aromatic compounds. The HMF as an important platform intermediate for the production of the biofuel dimethylfuran (DMF) and other molecules (Rosatella *et al.* 2011) could be extracted with low boiling point solvent tetrahydrofuran (THF) or diethyl ether for purification (Wang *et al.* 2013).

CONCLUSIONS

1. Poplar wood pretreated with water-only at temperatures ranging from 220 to 280 °C with flow rates of 10 to 62.5 mL/min resulted in varied dissolved compounds, predominately in the aromatic form of vanillin, syringaldehyde, and coniferyl alcohol.

2. It is intriguing to find that tuning the reaction conditions could tailor the products profile. At 240 °C, water-only flowthrough pretreatment resulted in up to 50.9% selectivity of vanillin and 45.0% selectivity of syringaldehyde. The acidic cleavage of predominant β-O-4 bonds of lignin into coniferyl alcohol (G) and sinapyl alcohol (S) followed by subsequent oxidative cleavage at C_α-$C_\beta$position of these monolignol units were the possible prime degradation patterns of lignin in acidic hot water conditions, thereby generating vanillin and syringaldehyde. The formation of syringaldehyde was plausibly faster than vanillin at an identical temperature. Decreasing the reaction temperature to 220 °C was beneficial for the stability of coniferyl alcohol, which was obtained at up to 36.2% selectivity. Increasing temperature to 260 and 280 °C or reducing the flow rate to 10 mL/min led to other aromatic compounds such as 2-methoxy-4-propylphenol, acetovanillone, and 2,6-dimethoxyphenol, as well as HMF.

3. The addition of very dilute acid (0.05% to 0.1% w/w, H_2SO_4) drastically increased the selectivity of HMF up to 66.7%. The isomer of HMF, furyl hydroxymethyl ketone, was observed in 0.05% (w/w) H_2SO_4conditions as well and considered to be derived from C3carbonyl and C6 hydroxy group intermolecular closure during the glucose dehydration process.

4. Results showed that AHWF could provide the potential to selectively produce value-added solubilized non-carbohydrate products such as vanillin, syringaldehyde, and HMF in addition to the conventional sugars.

ACKNOWLEDGMENTS

REFERENCES CITED

Allen, S. G., Kam, L. C., Zemann, A. J., and Antal, J. M. J. (1996). "Fractionation of sugar cane with hot, compressed, liquid water," *Ind. Eng. Chem. Res*. 35(8), 2709-2715.DOI: 10.1021/ie950594s

Davin, L. B., and Lewis, N. G. (2003). "An historical perspective on lignan biosynthesis: Monolignol, allylphenol and hydroxycinnamic acid coupling and downstream metabolism," *Phytochem. Rev*. 2(3), 257-288. DOI: 10.1023/B:PHYT.0000046175.83729.b5

Davin, L. B., and Lewis, N. G. (2005). "Dirigent phenoxy radical coupling: Advances and challenges," *Curr. Opin. Biotechnol*. 16(4), 398-406. DOI:10.1016/j.copbio.2005.06.010

Fache, M., Boutevin, B., and Caillol, S. (2016). "Vanillin production from lignin and its use as a renewable chemical," *ACS Sust. Chem. Eng*. 4(1), 35-46. DOI:10.1021/acssuschemeng.5b01344

Foston, M., and Ragauskas, A. J. (2012). "Biomass characterization: Recent progress in understanding biomass recalcitrance," *Ind. Biotechnol*. 8(4), 191-208. DOI:10.1089/ind.2012.0015

Hill, E. M., Broering, J. M., Hallett, J. P., Bommarius, A. S., Liotta, C. L., and Eckert, C. A. (2007). "Coupling chiral homogeneous biocatalytic reactions with benign heterogeneous separation," *Green Chem*. 9(8), 888-893. DOI: 10.1039/B701409K

Himmel, M. E., Ding, S., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., and Foust, T. D. (2007). "Biomass recalcitrance: Engineering plants and enzymes for biofuels production," *Science* 135(5813), 804-807. DOI:10.1126/science.1137016

Lü, X., and Saka, S. (2012). "New insights on monosaccharides' isomerization, dehydration and fragmentation in hot-compressed water," *J. Supercrit. Fluids*. 61, 146-156. DOI: 10.1016/j.supflu.2011.09.005

Laskar, D. D., Corea, O. R., Patten, A. M., Kang, C., and Davin, L. B. (2010). "Vascular plant lignification: Biochemical/structural biology considerations of upstream aromatic amino acid and monolignol pathways," in: *Comprehensive Natural Products Chemistry II*, C. H. Wong and P. G. Wang (eds.), Elsevier, Oxford, UK, pp. 541-604. DOI: 10.1016/B978-008045382-8.00647-X

Laskar, D. D., Zeng, J., Yan, L., Chen, S., and Yang, B. (2013). "Characterization of lignin derived from water-only flowthrough pretreatment of *Miscanthus*," *Ind. Crops. Prod*. 50, 391-399. DOI: org/10.1016/j.indcrop.2013.08.002.

Ma, R., Guo, M., and Zhang, X. (2014). "Selective conversion of biorefinery lignin into dicarboxylic acids," *ChemSusChem* 7(2), 412-415. DOI:10.1002/cssc.201300964

Ma, R., Xu, Y., and Zhang, X. (2015). "Catalytic oxidation of biorefinery lignin to value-added chemicals to support sustainable biofuel production," *ChemSusChem* 8(1), 24-51. DOI: 10.1002/cssc.201402503

Ma, R., Guo, M., Lin, K. T., Hebert, V. R., Zhang, J., Wolcott, M. P., Quintero, M., Ramasamy, K. K., Chen, X., and Zhang, X. (2016). "Peracetic acid depolymerization of biorefinery lignin for production of selective monomeric phenoliccompounds," *Chem-Eur. J*. 22(31), 10884-10891.DOI: 10.1002/chem.201600546

Mora-Pale, M., Meli, L., Doherty, T. V., Linhardt, R. J., and Dordick, J. S. (2011). "Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass," *Biotechnol. Bioeng*. 108(6), 1229-1245.DOI:10.1002/bit.23108

Mohamad Ibrahim, M. N., Sipaut, C. S., and Mohamad Yusof, N. N. (2009). "Purification of vanillin by a molecular imprinting polymer technique," *Sep. Purif. Technol.* 66(3), 450-456. DOI: 10.1016/j.seppur.2009.02.010

Mbotchak, L., Morvan, C. L., Duong, K. L., Rousseau, B., Tessier, M., and Fradet, A. (2015). "Purification, structural characterization, and modification of organosolv wheat straw lignin," *J. Agr. Food Chem.* 63(21), 5178-5188.
DOI: 10.1021/acs.jafc.5b02071

Patil, S. K. R., and Lund, C. R. F. (2011). "Formation and growth of humins via aldol addition and condensation during acid-catalyzed conversion of 5-hydroxymethylfurfural," *Energ. Fuels.* 25(10), 4745-4755. DOI:10.1021/ef2010157

Phaiboonsilpa, N., Yamauchi, K., Lu, X., and Saka, S. (2010). "Two-step hydrolysis of Japanese cedar as treated by semi-flow hot-compressed water," *J. Wood. Sci.* 56(4), 331-338. DOI: 10.1007/s10086-009-1099-0

Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P. F., Marita, J. M., Hatfield, R. D., Ralph, S. A., and Christensen, J. H. (2004). "Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids," *Phytochem. Rev.* 3(1), 29-60. DOI: 10.1023/B:PHYT.0000047809.65444.a4

Rosatella, A. A., Simeonov, S. P., Frade, R. F. M., and Afonso, C. A. M. (2011). "5-Hydroxymethylfurfural (HMF) as a building block platform: Biological properties, synthesis and synthetic applications," *Green Chem.*13, 754-793.
DOI: 10.1039/C0GC00401D

Silva, E. A. B., Zabkova, M., Araújo, J. D., Cateto, C. A., Barreiro, M. F., Belgacem, M. N., and Rodrigues, A. E. (2009). "An integrated process to produce vanillin and lignin-based polyurethanes from kraft lignin," *Chem. Eng. Res. Des.* 87(9), 1276-1292. DOI: 10.1016/j.cherd.2009.05.008

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). "Determination of structural carbohydrates and lignin in biomass," *National Renewable Energy Laboratory Analytical Procedure*.

Tao, L., Aden, A., Elander, R. T., Pallapolu, V. R., Lee, Y. Y., Garlock, R. J., Balan, V., Dale, B. E., Kim, Y., Mosier, N. S., *et al*. (2011). "Process and technoeconomic analysis of leading pretreatment technologies for lignocellulosic ethanol production using switchgrass," *Bioresour. Technol.* 102(24), 11105-11114. DOI: 10.1016/j.biortech.2011.07.051

Vasco, C. A., Ma, R., Quintero, M., Guo, M., Geleynse, S., Ramasamy, K. K., Wolcott, M., and Zhang, X. (2016). "Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): A source of lignin for valorization," *Green Chem.* 18(19), 5133-5141. DOI: 10.1039/C6GC01007E

Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., and Lee, Y. Y. (2005)." Coordinated development of leading biomass pretreatment technologies," *Bioresour. Technol*. 96(18), 1959-1966. DOI: 10.1016/j.biortech.2005.01.010

Wang, J., Ren, J., Liu, X., Lu, G., and Wang, Y. (2013). " High yield production and purification of 5-hydroxymethylfurfural," *AIChE J.* 59(7), 2558-2566.
DOI: 10.1002/aic.14019

Yan, L., Laskar, D. D., Lee, S. J., and Yang, B. (2013). "Aqueous phase catalytic conversion of agarose to 5-hydroxymethylfurfural by metal chlorides," *RSC Adv.* 3(46), 24090-24098. DOI: 10.1039/C3RA43293A

Yan, L., Zhang, L., and Yang, B. (2014). "Enhancement of total sugar and lignin yields through dissolution of poplar wood by hot water and dilute acid flowthrough pretreatment," *Biotechnol. Biofuels* 7, 76.DOI: 10.1186/1754-6834-7-76

Yan, L., Ma, R., Li, L., and Fu, J. (2016a). "Hot water pretreatment of lignocellulosic biomass: An effective and environmentally friendly approach to enhance biofuel production," *Chem. Eng. Tech.*39(10), 1759-1770.DOI: 10.1002/ceat.201600394

Yan, L., Pu, Y., Bowden, M., Ragauskas, A. J., and Yang, B. (2016b). "Physiochemical characterization of lignocellulosic biomass dissolution by flowthrough pretreatment," *ACS Sust. Chem. Eng.* 4(1), 219-227. DOI:10.1021/acssuschemeng.5b01021

Yang, B., and Wyman, C. E. (2008a). "Characterization of the degree of polymerization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water," *Bioresour. Technol.* 99(13), 5756-5762. DOI: 10.1016/j.biortech.2007.10.054

Yang, B., and Wyman, C. E. (2008b). "Pretreatment: The key to unlocking low-cost cellulosic ethanol," *Biofuels. Bioprod. Biorefin.* 2(1), 26-40. DOI:10.1002/bbb.49

Zakzeski, J., Bruijnincx, P. C., Jongerius, A. L. and Weckhuysen, B. M. (2010). "The catalytic valorization of lignin for the production of renewable chemicals," *Chem. Rev.* 110(6), 3552-3599. DOI: 10.1021/cr900354u