ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Biomass Fractionation after Denaturing Cell Walls by Glycerol Thermal Processing

Wei Zhang,[†,‡] Justin R. Barone,[†,§] and Scott Renneckar*[,†,‡,⊥]

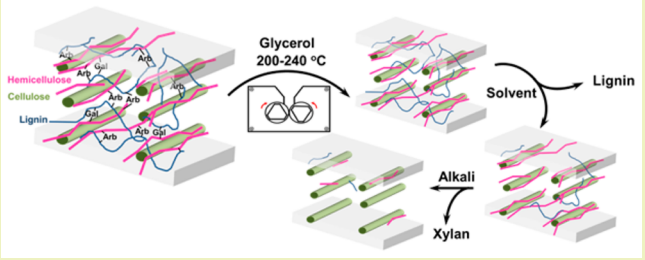
[†]Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, Virginia 24061, United States

[‡]Department of Sustainable Biomaterials, Virginia Tech, Blacksburg, Virginia 24061, United States

[§]Department of Biological Systems Engineering, Virginia Tech, Blacksburg, Virginia 24061, United States

[⊥]Department of Wood Science, The University of British Columbia, Vancouver, British Columbia V6T1Z4, Canada

ABSTRACT: Denaturing biopolymers allows the transformation of highly organized natural structures into industrially relevant materials such as extruded starch or gelatin. This approach was used to fractionate wood into its biopolymer constituents by treating wood particles in polymer processing equipment at high temperatures in the presence of anhydrous glycerol. Nine severities were studied to assess the impact of time and temperature during processing. After processing, the biomass was stripped of its lignin and xylan by subsequent extractions without addition of added catalysts, leaving a relatively pure cellulose fraction, 84% glucan, as found in chemical pulps. Additionally, 41% of the lignin and 68% of the xylan was recovered in a dry powdered form. The hemicellulose side-chain carbohydrates such as arabinose and galactose were water extracted, while the majority of the mannan remained with the cellulose fiber. High temperature processing for short times in a benign solvent provides significant disruption of the cell wall, while anhydrous glycerol prevents significant degradation of the majority of the biopolymers into oligomers.



KEYWORDS: *Lignocellulosic biomass, pretreatment, fractionation, mass balance, glycerol*

INTRODUCTION

Natural materials have supramolecular structures that originate from the self-assembly processes that are under cellular control. For small assemblies like enzymes, these proteins fold into specific architectures that impart functionality to the material. It is well-known that natural architectures of the biopolymers are transformed by heat, chaotropic agents, or other solvents into non-native structures. Changes in pH or heat overwhelm secondary interactions between amino acids, carbohydrates, or other building blocks. This denaturing of structure is utilized industrially to create materials like gelatin derived from the chemical treatment of animal collagen or processing natural materials like starch on extrusion equipment to create thermoformable bioplastics.[1] For these systems, thermal energy is applied with a plasticizer that lowers the glass transition temperature of the material, allowing polymer chains to undergo flow without degradation.[2] However, some biopolymers have specific chemical linkages, such as disulfide linkages found in keratin, which must be chemically broken before they can be denatured and melt processed into moldable bioplastics. In this case, specific reducing agents are applied to transform proteinaceous biomass such as feather during melt processing, keeping most of the polymer backbone chemically unmodified.[3] For very complex materials like woody biomass, this concept of denaturing, at first glance, seems unusual to apply. The wood cell wall involves multiple structural biopolymers such as cellulose, lignin, and heteropolysaccharides like glucoronoxylan and glucomannan that have varying sensitivities to thermal treatments. Typically, a number of side-products are formed during thermal processing that self-catalyze the degradation. An example of this type of pretreatment is steam-explosion processing where hydrothermal treatment plasticizes the cell wall, causes chemical changes, and explosive decompression shears apart fibers.[4] The native structure changes significantly and there is severe disruption of the polymeric backbones arising from the acid catalyzed reactions associated with the saponification of the hemicelluloses. This result in turn significantly reduces the molecular weight of the polysaccharide backbone, forms furfural byproducts, and modifies the lignin to varying extents from depolymerization and repolymerization reactions.[5−7] While the native cell wall architecture is denatured,[8] the biopolymers are significantly chemically altered.[9]

Because wood and natural biomass is increasingly valued for its inherent stored energy in glucose, there is burgeoning research in "clean fractionation" to separate the polymeric components of biomass into cellulose-rich, hemicellulose-rich, and lignin-rich fractions that are not significantly altered.[6,10−17]

Received: August 28, 2014
Revised: December 9, 2014
Published: February 4, 2015

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413−420

The polymeric components serve as valuable coproducts, offsetting the cost associated with bioconversion. For instance, dry distiller grains are the byproduct of corn ethanol production and are usually sold as animal feed. This valuable coproduct can make the difference between operating with a profit when corn prices are high because of seasonal price fluctuations. Analogous to the grains, xylan and lignin have been assessed to have significant added value to the cellulosic biorefinery.[18]

Glycols are known to protect biopolymers from high temperature dehydration reactions[19] and lower the glass transition temperature[20] with the benefit of limited acid formation occurring in anhydrous conditions. Hence, the glycerol softens the cell wall and protects it from the usual acid reactions found in autohydrolysis, additionally serving as a heat transfer agent. The literature describes aqueous glycerol treatments used as an organosolv pulping process where hydronium and hydroxide ions can have significant impact in catalyzing reactions.[21−24] Demirbas reported up to 94% delignification accompanied by extensive hemicellulose loss from aqueous glycerol treatment for 9 h in the presence of alkaline catalysts.[21] Moreover, biomass was liquefied with aqueous glycerol and alkali, which resulted in lignin degradation into small fragments.[25] Sun et al. and Novo et al. studied delignification using noncatalytic aqueous glycerol to produce biomass substrates for enzymatic saccharification from wheat straw and bagasse. Both studies indicated 65−80% delignification after processing 2 to 4 h at temperatures of 200 to 240 °C.[22,23,25] At these conditions in aqueous systems, autohydrolysis of the hemicelluloses occurred to catalyze the degradation of the biomass.

Here we report on the idea of denaturing wood to recover the biopolymers through thermally processing in the presence of the plasticizer glycerol. This present method differs from the organosolv pulping technologies that hydrolyze hemicellulose backbones and actively attack covalent linkages and create side-reactions. A series of nine different processing conditions are investigated here to track the severity of the processing for controlled denaturation and degradation with the intent to separate the biopolymers from thermally processed, glycerol-plasticized wood. The study is optimized to allow the isolation of the biopolymers to understand the chemical changes among the biopolymers in a sequential fractionation treatment. The work provides a simple path to generate coproducts from biomass using a benign processing solvent and short heating times.

MATERIALS AND METHODS

Chemicals and reagents used in this research were purchased from Sigma-Aldrich, Alfa Aesar, and MP Biomedicals, and used as received. Deionized water (DI-water) was produced by Millipore Direct Q3UV with a resistivity of 18.2 mΩ.

Lignocellulosic Biomass. A mature sweet gum (*Liquidambar styraciflua*) hardwood tree from Blacksburg, VA was debarked, machined to cubes, and stored in a freezer before use. Prior to pretreatment, the biomass was knife milled using a Wiley mill and sorted to a particle size between 40 to 60 mesh on a metal screen (250−420 μm). Then, the sweet gum particles were Soxhlet extracted using toluene/ethanol (427 mL/1000 mL) followed by ethanol (ASTM D1105-96)[26] to produce extractive-free wood. The resulting extractive-free sweet gum particles (SG) were air-dried at ambient temperature for 48 h.

Glycerol Thermal Processing (GTP) Pretreatment. The extractive-free biomass was pretreated with glycerol using a benchtop internal mixing head with a high intensity shear roller blade driven by a C.W. Brabender Prep-Center drive. The rotation speed of internal blades was 100 rpm. The biomass (∼6% MC)-to-glycerol weight ratio was 1:3. The processing time was chosen as 4, 8, and 12 min at 200, 220, and 240 °C, respectively. To simplify the GTP conditions, a single severity parameter (R_0) calculated from time and temperature was adopted according to the equation defined by Overend and Chornet[27]

$$R_0 = t \times e^{(T-100)/14.75}$$

where t is the pretreatment time (min) at temperature T (degrees Celsius).

The GTP pretreatment conditions and corresponding logarithm of R_0 used in this research are shown in Table 1.

Table 1. Glycerol Thermal Processing Conditions and Corresponding Log Severity Parameters

sample label	T (°C)	t (min)	log(R_0)
SG#1	200	4	3.55
SG#2	200	8	3.85
SG#3	200	12	4.02
SG#4	220	4	4.14
SG#5	220	8	4.44
SG#6	220	12	4.61
SG#7	240	4	4.72
SG#8	240	8	5.03
SG#9	240	12	5.20

After pretreatment, the GTP pretreated sweet gum (GTPSG) was collected from the mixing head and stored at 4 °C until further analysis. Samples for each severity condition were run in triplicates.

Water Extraction. The GTPSG was first DI-water extracted for 2 h to remove the glycerol residue and any degraded components in a 40 °C water bath. The solid-to-liquid ratio was 1/10 (m/v). The water extracted biomass was collected through centrifugation and rinsed by deionized water until colorless supernatant before freeze-drying. For the accurate mass balance calculation, the water extracted GTPSG was stored in a vacuum oven (0.9 mmHg, 40 °C) for an additional 48 h over phosphorus pentoxide (P_2O_5) after freeze-drying. Unless otherwise noted, this vacuum drying procedure was used throughout the study.

Solvent Extraction. The water extracted GTPSG was subjected to 96% (v/v) 1,4-dioxane[28] (azeotrope) extraction at a solid to liquid ratio of 1/25 (m/v) with continuous stirring for lignin fractionation. This extraction occurred at ambient temperature for 24 h without additional catalysts. The residual biomass was collected through filtration, and subsequently washed by 96% dioxane, deionized water, and acetone until the wash was colorless. Then, the solvent extracted GTPSG was vacuum-dried thoroughly. At the same time, the dioxane extract was subjected for lignin recovery.

Alkaline Extraction. The solvent extracted GTPSG was further extracted by 1.0 N sodium hydroxide solution at a solid to liquid ratio of 1/40 (m/v) for xylan fractionation.[29] The alkaline extraction was performed at ambient temperature with continuous stirring in nitrogen environment. After 24 h of extraction, the mixture was centrifuged to separate biomass solid residue and alkaline extract. Sequentially, 100 mL of 1 M sodium hydroxide solution and 100 mL of deionized water were used to wash the solid residue, and the washing liquids were collected and combined with the alkaline extract. Continually, the solid residue was thoroughly washed by deionized water until pH was neutral and freeze-dried followed by the vacuum drying procedure. The combined alkaline extract and wash liquid was used to recover xylan.

Compositional Analysis of Extracted GTPSG. Lignin and carbohydrate contents of nonpretreated SG, water, solvent, and alkaline extracted GTPSG were analyzed according to the NREL laboratory analytical procedure (LAP): Determination of structural carbohydrates and lignin in biomass.[30] Each analysis was run in

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413−420

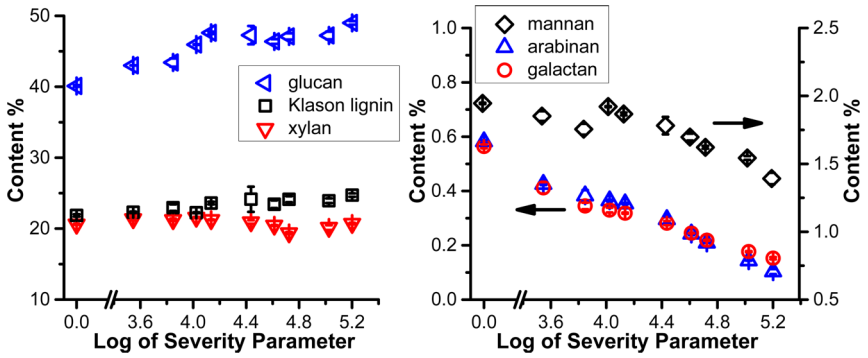


Figure 1. Compositional analysis (wt %) of the relative amounts of structural biopolymer components within water-extracted GTP sweet gum based on severity level. Severity 0 is unprocessed sweet gum, used as a control.

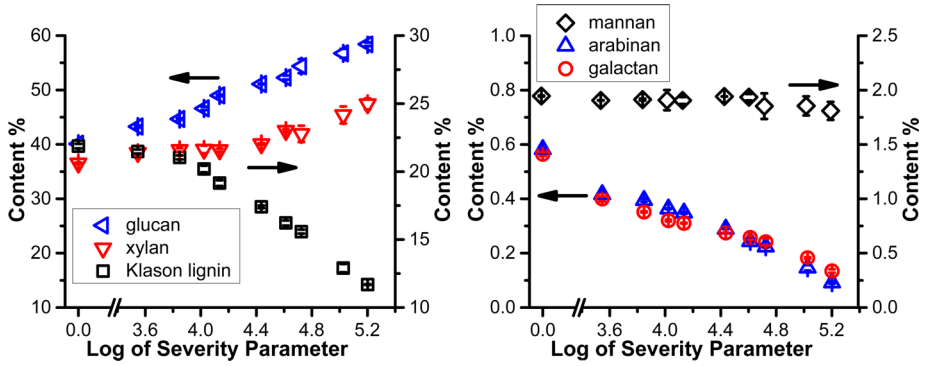


Figure 2. Compositional analysis (wt %) of the relative amounts of structural biopolymer components within solvent-extracted GTP sweet gum based on severity level. Severity 0 is unprocessed sweet gum fiber, used as a control.

duplicates. The acid insoluble lignin (Klason lignin) was analyzed gravimetrically through the mass difference before and after heating the acid-hydrolyzed residue at 575 °C. The carbohydrates in the filtrate were analyzed in duplicates using Metrohm Ion Chromatography (IC) installed with a pulsed amperometric detector (PAD), Metrohm Inc., USA. Monosaccharides in the filtrate were separated by a Hamilton RCX-30 (250 × 4.6 mm) column with DI-water as the eluent. The eluent flow rate was 1 mL/min, and the column temperature was 32 °C. 350 mmol/L NaOH with a flow rate of 0.43 mL/min was introduced after column separation to aid the signal generation in PAD at 35 °C. Five sugars including L-(+)-arabinose, D-(+)-galactose, D-(+)-glucose, D-(+)-xylose, and D-(+)-mannose were quantified in the Mag IC. Net software. Cellobiose was not detected in all of the samples and was excluded from the analysis. Linear calibration curves were run prior to every batch test with $R^2 > 0.9999$ and relative standard error < 5%. The monosaccharide concentration was converted to the relative percentage of its anhydro-form in the biomass according to the NREL standard.

Specific Surface Area of Water Extracted GTPSG. Nitrogen adsorption specific surface area of nonpretreated SG and water extracted GTPSG was determined using Autosorb-1C (model: AX1C-MP-LP, Quantachrome Instruments, USA). Prior to analysis, samples of SG and GTPSG were dried completely under vacuum.

About 0.8 g of SG or GTPSG was filled in a Quantachrome brand 6 mm large bulb cell for surface area analysis. The samples were degassed at 45 °C until outgas pressure rise was less than 50 μm Hg per minute. The multipoint analysis was performed at a temperature of 77 K provided by liquid nitrogen. The Autosorb-1C measured the weight of the adsorbed nitrogen gas on the solid surface at different relative pressure during the test. Sample specific surface area was calculated using Brunauer–Emmett–Teller (BET) theory[26,31,32] for the pressure range P/P_0 of 0.05–0.3 with the linear coefficient $R^2 > 0.998$.

RESULTS

Glycerol thermal processing (GTP) pretreatment was conducted on extractive-free sweet gum at nine pretreatment severities related to a range of processing times and temperatures (Table 1). Compositional analysis of pretreated and water extracted biomass, shown as a function of pretreatment severity, revealed GTP processing at higher severity caused several changes to the biomass (Figure 1). After GTP pretreatment and water washing, the relative contents of the three predominant hardwood constituents (cellulose, xylan and lignin) remained constant or slightly increased regardless of the pretreatment severity. Only the contents of minor carbohydrates of the heteropolysaccharides (arabinose and galactose) were observed to decrease during processing and water washing, while mannan decreased at a lesser extent. Although GTP pretreatment occurred at comparable pretreatment severities as autohydrolysis or steam-explosion treatment, this treatment did not result in severe degradation of the xylan into water-soluble monomers and oligomers.[33] This finding suggested the glycerol thermal processing was different from the aqueous treatments where acidity arising from degradation of the wood autocatalyzes the decomposition of the backbone linkages of the polysaccharides. In fact, the water-soluble extract consisting of glycerol and the extracted wood components had pH values that were near neutral independent of pretreatment severity (pH = 5.8 to 6.1), similar to the pH of glycerol solution at the same concentration.

After freeze-drying the GTP pretreated biomass, a mild solvent extraction using 96% 1,4-dioxane at ambient temperature without any acid or alkali catalysts was adopted to isolate

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413–420

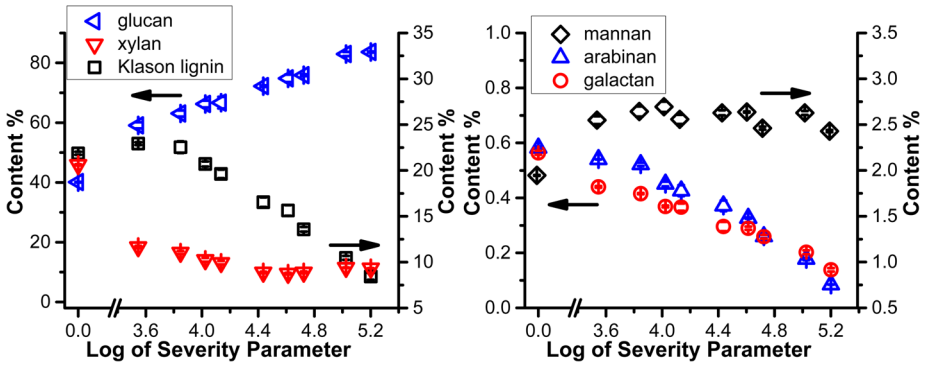


Figure 3. Compositional analysis (wt %) of the relative amounts of structural biopolymer components within alkaline-extracted GTP sweet gum based on severity level. Severity 0 is unprocessed sweet gum fiber, used as a control.

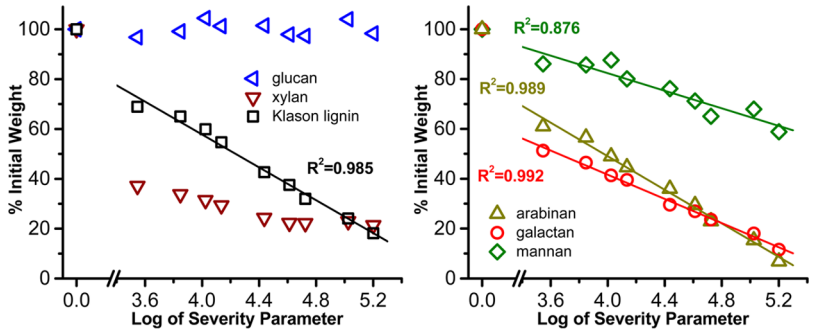


Figure 4. Mass balance of wood components after GTP pretreatment and extraction (calculated based on the average of duplicates with COV < 5%) relative to the initial weight of components in untreated sweet gum.

lignin from pretreated sweet gum. This extraction solvent was used for comparative studies in the literature and not meant as an optimized fractionation scheme for industrial development. Compositional analysis of solvent extracted biomass residue revealed a strong correlation of lignin extraction with increasing GTP pretreatment severity (Figure 2). As a comparison, no recoverable lignin was isolated from nonpretreated sweet gum powder by this extraction procedure. The relative content of residual lignin in solvent extracted biomass decreased at increased GTP severity. Correspondingly, the relative glucan and xylan concentrations were observed to increase, considering this content was expressed as weight percentage of the extracted biomass. For the most severe GTP pretreatment, the residual Klason lignin accounted for 11% (w/w) in the solvent-extracted GTPSG, compared to 22% in native biomass and 25% right before solvent extraction. Up to 56% of the Klason lignin was removed after GTP pretreatment following organic solvent extraction. Without a catalyst, this result was unusual and must be tied to the breakage of linkages in the cell wall because of heat. Although, lignin has been noted to have the highest overall thermal stability of the biopolymers found in wood, close examination of the thermal treatment of lignin revealed significant bond breakage occurred in the aryl ether structures at temperatures below 200 °C.[34] This thermal sensitivity of interunit linkages was below the processing range of the GTP biomass, providing the possibility of bond scission occurring at GTP treatment temperatures.

After organic solvent extraction, the GTP biomass was dried and extracted with aqueous alkali. According to Figure 3, the relative glucan content of the extracted fiber related to the cellulose increased to nearly 85%, while there was significant reduction of the xylan content across most of the severity treatments. The mannan content was not impacted by the alkali extraction staying relatively constant at 2.5% of the extracted fiber weight. This data showed that xylan extraction was not as sensitive to processing conditions relative to the other components, as there was significant removal at low severity conditions.

To better understand the impact of the processing conditions on the fractionation, the biopolymer concentration of the fully extracted fiber was reported as a percent of the mass in the starting material (Figure 4). As indicated in Figure 4, cellulose content remained constant across the severity levels, when we disregard fluctuations related to experimental error. At the highest severity level, 98% of the cellulose remained showing that GTP pretreatment has little impact on the cellulose content. Lignin extraction was dictated by the processing condition severity, as there was a linear−log relationship with an R^2 factor of 0.985. The data revealed that extraction is dependent upon GTP severity removing up to 80% of the lignin without the aid of any catalysts as found in chemical pulping cooks. The main hemicellulose component in the wood, xylan, was the most extractable across the majority of the processing severities and the extraction reached a maximum at the four highest severity conditions. Figure 4 also revealed that mannan content was reduced with processing severity and was resistant to extraction as more than 50% of the initial mannan remained in the pretreated and extracted biomass residue at the highest severity levels. However, on a total percentage basis this concentration accounted for only 2% of the dry mass. Additionally, processing conditions were closely linked to the extraction of the side-chain hemicellulose groups, galactan, and arabinan, with R^2 correlations near 0.99 for both components.

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413−420

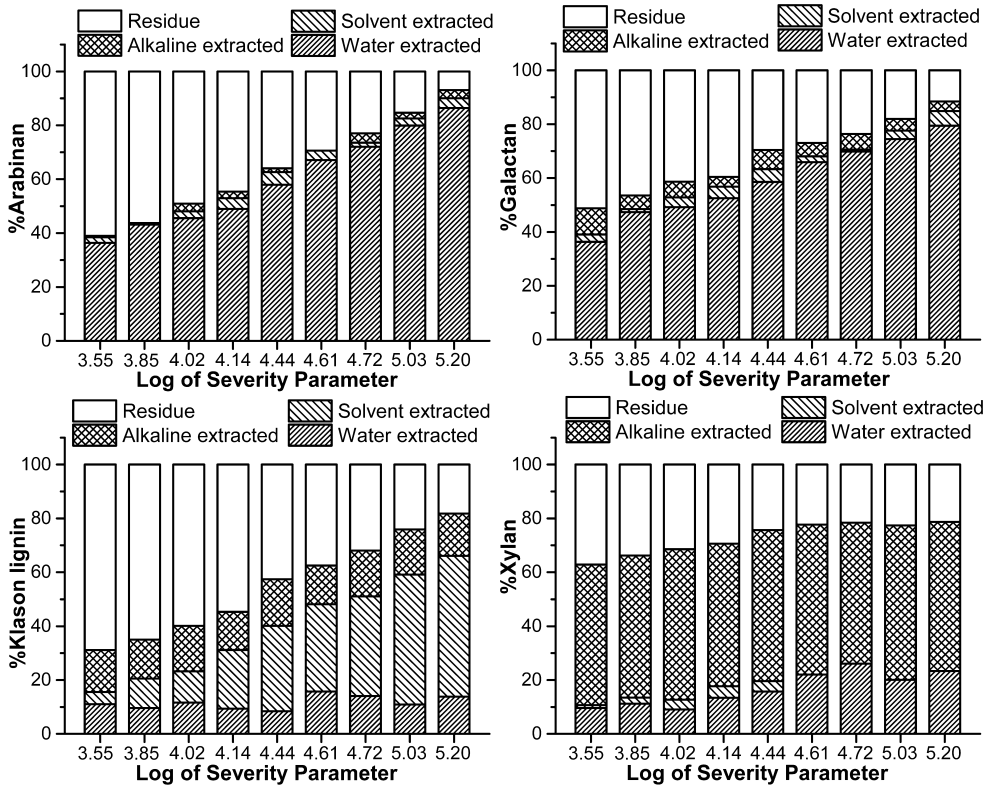


Figure 5. Biopolymer mass distribution (wt %) in relation to log(R_0) after GTP pretreatment and sequential extractions of water, aqueous dioxane, and 1.0 N NaOH solution (calculated based on the average of duplicates).

The extraction of components in each step is plotted in Figure 5 to show the amount of biopolymer removed in each extraction stage. The majority of the arabinan and galactan residues were removed during the water extraction stage with minor losses in the other extraction stages accounting for less than 5% of the arabinan and less than 10% of the galactan. The loss of lignin and xylan during the water extraction stage ranged from 10 to 20% dependent upon the severity and biopolymer type. Although lignin itself is not known to be water-soluble, the removal of lignin in this solvent did not correlate with severity. This observation suggested that lignin extracted in the water was related to a more surface active lignin−carbohydrate complex that would show natural solubility in water. Similar results have been reported in previous research for steam-assisted fractionation, where 10% of the lignin was associated with the water-soluble fraction.[9] Moreover, there was a preferential extraction of the lignin with the organic solvent and with minimal xylan removed during this extraction stage, yielding a high purity lignin based on simple mass balances. In contrast, the subsequent alkali extraction revealed a near constant removal of lignin and xylan, irrespective of the processing conditions with a 1:3.5 of lignin to xylan ratio.

DISCUSSION

Glycerol thermal processed biomass disrupted the cell wall allowing the extraction of the biopolymer constitutive components to various degrees. Processing the biomass in this manner opened the cell wall, as revealed by a change in specific surface area of the fiber (Table 2). For mild and high severity conditions, the surface area of the fiber doubled and tripled, respectively. This data suggested the access of the solvent into the cell wall structure of the biomass is enhanced

Table 2. BET Specific Surface Area (SSA) of GTP Pretreated Biomass before Solvent Extraction

log(R_0)	SSA (m^2/g)
SG control	0.807 (0.019)
4.44	1.785 (0.064)
5.03	2.428 (0.237)

after disruption of the native cell wall network. The disruption is evident as the hemicellulose side chains that have been proposed to serve as connections to lignin through ether bonds[35,36] are cleaved as a function of temperature and removed through water extraction. In one sense, this bond rupture is analogous to the breakage of disulfide linkages when processing materials like keratin with reducing agents[3] to allow polymer flow. In this case, there is a strong correlation between the delignification and the loss of side-chain hemicellulose components (Figure 6), which suggests either common activation energy for these changes to occur or key linkages that need to be broken prior to delignification.

The thermal decomposition of hemicelluloses are reported in the literature beginning at temperatures of 180 to 220 °C.[37,38] However, for model studies, carbohydrate structure, linkage type, degree of polymerization, and the degree of substitution impact the degradation temperatures[39,40] and this would suggest the ability to selectively cleave carbohydrate linkages. For example, α-1,6 linkages are reported to be less stable than β-1,4 linkages, providing some insight into the selective removal of certain units. For the current work, galactose residues in hardwoods are linked along the main chain of glucomannan in the α-1,6 position.[41] Additionally, arabinose units are α-linked residues at the 2 or 3 position along the xylan backbone.[42] Both of these cases, based on the model study using pyrolysis,

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413−420

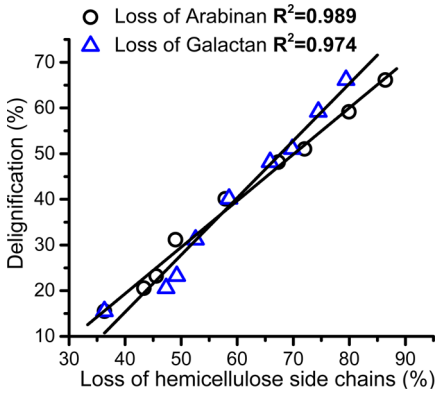
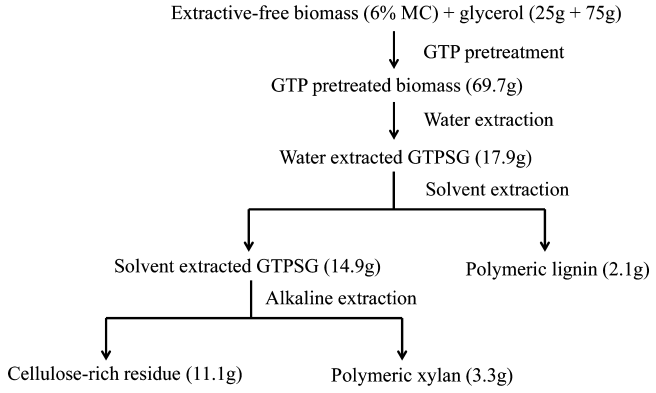


Figure 6. Delignification (after solvent extraction) compared to the loss of hemicellulose side-chain groups (after water extraction) (calculated based on the average of duplicates with COV < 5%).

Scheme 1. Biopolymer Fractionation for Cellulose, Lignin, and Xylan Stream from GTP Pretreated Biomass



suggest a preferential mechanism in cleavage for these side groups. Currently, there is no mechanism proposed in the retention of xylans at these high temperatures when xylan degradation occurs above 220 °C in an inert atmosphere.[37] Xylan in hardwood has regular backbone chemistry and is known to be acetylated at a molar ratio near 1:0.5 Xyl:Acetyl.[37] It has been reported that acetylated xylans have lower thermal stability than there deactylated counterparts;[43] however derivatized xylan acetates are more thermally stable providing competing evidence to the impact of substitution on thermal stability.[44] Furthermore, it is reported in the literature that the predominant aryl ether linkages of lignin are sensitive to temperatures above 180 °C[45] and the potential breakage of these linkages at the processing temperatures would contribute to the delignification of the pretreated biomass. Hence, the processing conditions that approach the thermal degradation temperatures of hemicelluloses and fragmentation of lignin, give rise to reactions for fractionation with minimal degradation byproducts of the main polymers in the presence of glycerol. Glycols are capable of preventing polysaccharide chain degradation at high temperature and pressure via hydrogen bond formation.[19,46] In other words, glycerol offers protection to prevent dehydration, impacting glycosidic bond cleavage. Additionally, glycerol may serve as a source for hydrogen abstraction to prevent further radical degradation pathways. These findings suggest that thermal processing in glycerol can be used to tailor the heteropolysaccharides, dependent upon the temperature, developing more uniform biopolymer streams from biomass.

Developing a clean fractionation concept requires the recovery of components in nonmodified form and high yield. Currently, pulp and paper processes isolate a highly purified fiber of 80 to 95% cellulose, dependent upon pulping technologies and bleaching sequences. In the current research, glycerol thermal processing was applied as a novel pretreatment to denature the native cell wall structure facilitating biopolymer fractionation (Scheme 1). Lignin from the highest severities could be recovered at 41% dry weight from precipitation of the organic phase. This amount would be equivalent to 9% of the starting mass providing opportunity to develop added coproducts from lignin. Additionally, 68% of the xylan is recovered after precipitation or 14% of the initial biomass weight. In contrast, pulping processes do not recover hemicelluloses and the main industrial source of isolated hemicellulose is processed agriculture grains. Most isolation procedures for fractionation of biomass involve acid catalysts, as used in organosolv pulping, or use dilute acid and these technologies cleave the xylan backbones to various extents. Similarly, autohydrolysis, prehydrolysis kraft pulp, and steam-explosion technologies significantly break the hemicellulose backbone to various degrees leaving an oligosaccharide-rich solution. An example of this is seen in the clean fractionation concept using steam-explosion[47] that achieves similar levels of lignin extraction and cellulose purity to the present study, but significant loss of the xylan into oligosaccharides occurs.[9] Altogether, glycerol thermal processing of biomass produces three potential sources of polymer components that equate to nearly 70% of the starting mass, which have potential values ranging from ca. $0.70/kg for glucose[48] to ca. $1.40/kg in equivalent polymer precursors, such as ethylene.[49] Although this research provided a conceptual framework to treat biomass with glycerol and heat, the study is of a lab-scale nature with a "clean" starting material for analytical purposes. Additional work must be performed to narrow the solvent recovery and biopolymer isolation steps, such as coextracting lignin and xylan with alkali and selectively precipitating out a component with a nonsolvent to offset processing costs. Additional analysis of the biopolymer molecular structure and the cellulose digestibility has been performed and will be reported in upcoming studies.

CONCLUSIONS

The present study investigated the efficient removal of the majority of the noncellulosic biopolymers simply through a series of extractions after treatment of the biomass on polymer processing equipment in the presence of glycerol. To evaluate this process, the time and temperature variables were combined in a single severity parameter to control the degree of cell wall disruption. Delignification and removal of side-chain hemicellulose components were directly correlated to the severity of the processing condition. At the highest severity, 240 °C and 12 min, a total of over 80% of initial lignin became extractable with more than 40% of the initial lignin recovered in a precipitated solid powder from the solvent extraction. Furthermore, greater than 65% of xylan was recovered, leaving a cellulose-fiber-rich substrate that was 84% in purity. Thermal processing in the presence of glycerol without catalysis caused controlled denaturing and deconstruction of the woody biomass useful for a clean fractionation process where xylan, lignin, and cellulose are recovered in good yield, providing a source to develop value-added coproducts in a biorefinery concept.

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413−420

AUTHOR INFORMATION

Corresponding Author

*S. Renneckar. E-mail: scott.renneckar@UBC.ca. Phone: +1(604)-827-0637.

Notes

The authors declare the following competing financial interest(s): Drs. Barone and Renneckar are listed as inventors for a patent filed by the Virginia Tech Foundation.

ACKNOWLEDGMENTS

The authors greatly acknowledge financial support from USDA NIFA 2010-65504-20429 for the work along with support from the Institute for Critical Technology and Science of Virginia Tech and the Virginia Tech Graduate School.

ABBREVIATIONS

BET, Brunauer–Emmett–Teller theory
DI-water, deionized water
GTP, glycerol thermal processing
GTPSG, glycerol thermal pretreated sweet gum
IC, ion chromatography
LAP, laboratory analytical procedure
PAD, pulsed amperometric detector
SG, extractive-free sweet gum

REFERENCES

(1) Fakirov, S.; Bhattacharyya, D. *Handbook of Engineering Biopolymers: Homopolymers, Blends and Composites*; Hanser Verlag: Cincinnati, OH, 2007.

(2) Barone, J. R.; Schmidt, W. F.; Liebner, C. F. Thermally processed keratin films. *J. Appl. Polym. Sci.* **2005**, *97* (4), 1644–1651.

(3) Barone, J. R.; Schmidt, W. F.; Gregoire, N. Extrusion of feather keratin. *J. Appl. Polym. Sci.* **2006**, *100* (2), 1432–1442.

(4) Brownell, H. H.; Saddler, J. N. Steam pretreatment of lignocellulosic material for enhanced enzymatic hydrolysis. *Biotechnol. Bioeng.* **1987**, *29* (2), 228–235.

(5) Li, J.; Henriksson, G.; Gellerstedt, G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour. Technol.* **2007**, *98* (16), 3061–3068.

(6) Chua, M. G. S.; Wayman, M. Characterization of autohydrolysis aspen (*P. tremuloides*) lignins. Part 1. Composition and molecular weight distribution of extracted autohydrolysis lignin. *Can. J. Chem.* **1979**, *57*, 1141–1149.

(7) Chua, M. G. S.; Wayman, M. Characterization of autohydrolysis aspen (*Populus tremuloides*) lignins. 3. Infrared and ultraviolet studies of extracted autohydrolysis lignin. *Can. J. Chem.* **1979**, *57* (19), 2603–2611.

(8) Donaldson, L.; Wong, K.; Mackie, K. Ultrastructure of steam-exploded wood. *Wood Sci. Technol.* **1988**, *22* (2), 103–114.

(9) Glasser, W. G.; Wright, R. S. Steam-assisted biomass fractionation. I. Fractionation behavior of various biomass resources. *Biomass Bioenergy* **1998**, *14* (3), 219–235.

(10) Bozell, J. J.; Black, S. K.; Myers, M.; Cahill, D.; Miller, W. P.; Park, S. Solvent fractionation of renewable woody feedstocks: Organosolv generation of biorefinery process streams for the production of biobased chemicals. *Biomass Bioenergy* **2011**, *35* (10), 4197–4208.

(11) Brudecki, G.; Cybulska, I.; Rosentrater, K. Optimization of clean fractionation process applied to switchgrass to produce pulp for enzymatic hydrolysis. *Bioresour. Technol.* **2013**, *131*, 101–112.

(12) Brudecki, G.; Cybulska, I.; Rosentrater, K. Integration of extrusion and clean fractionation processes as a pre-treatment technology for prairie cordgrass. *Bioresour. Technol.* **2013**, *135*, 672–682.

(13) Cybulska, I.; Brudecki, G. P.; Hankerson, B. R.; Julson, J. L.; Lei, H. Catalyzed modified clean fractionation of switchgrass. *Bioresour. Technol.* **2013**, *127*, 92–99.

(14) BlackS. K.HamesB. R.MyersM. D.Method of separating lignocellulosic material into lignin, cellulose and dissolved sugars. U.S. Patent 5,730,837, March 24, 1998.

(15) Brudecki, G.; Cybulska, I.; Rosentrater, K.; Julson, J. Optimization of clean fractionation processing as a pre-treatment technology for prairie cordgrass. *Bioresour. Technol.* **2012**, *107*, 494–504.

(16) Wayman, M.; Lora, J. H. Simulated autohydrolysis of aspen milled wood lignin in the presence of aromatic additives: Structural modifications. *J. Appl. Polym. Sci.* **1980**, *25* (10), 2187–2194.

(17) Wayman, M.; Lora, J. Aspen autohydrolysis: The effects of 2 naphthol and other aromatic compounds. *Technol. Assoc. Pulp Pap. Ind.* **1978**, 55–57.

(18) Zhang, Y. H. P.; Ding, S. Y.; Mielenz, J. R.; Cui, J. B.; Elander, R. T.; Laser, M.; Himmel, M. E.; McMillan, J. R.; Lynd, L. R. Fractionating recalcitrant lignocellulose at modest reaction conditions. *Biotechnol. Bioeng.* **2007**, *97* (2), 214–223.

(19) Marchessault, R.; Coulombe, S.; Morikawa, H.; Robert, D. Characterization of aspen exploded wood lignin. *Can. J. Chem.* **1982**, *60* (18), 2372–2382.

(20) Chowdhury, S.; Frazier, C. E. Compressive-torsion DMA of yellow-poplar wood in organic media. *Holzforschung* **2013**, *67* (2), 161–168.

(21) Demirbas, A. Aqueous glycerol deligninfication of wood chips and ground wood. *Bioresour. Technol.* **1998**, *63*, 179–185.

(22) Sun, F.; Chen, H. Evaluation of enzymatic hydrolysis of wheat straw pretreated by atmospheric glycerol autocatalysis. *J. Appl. Chem. Biotechnol.* **2007**, *82* (11), 1039–1044.

(23) Sun, F.; Chen, H. Enhanced enzymatic hydrolysis of wheat straw by aqueous glycerol pretreatment. *Bioresour. Technol.* **2008**, *99* (14), 6156–6161.

(24) Novo, L. P.; Gurgel, L. V. A.; Marabezi, K.; Curvelo, A. A. D. Delignification of sugarcane bagasse using glycerol-water mixtures to produce pulps for saccharification. *Bioresour. Technol.* **2011**, *102* (21), 10040–10046.

(25) Demirbas, A. Liquefaction of biomass using glycerol. *Energy Sources, Part A* **2008**, *30* (12), 1120–1126.

(26) Bose, S.; Francis, R. The role of β-O-4 cleavage in acidic organosolv pulping of softwoods. *J. Pulp Pap. Sci.* **1999**, *25* (12), 425–430.

(27) Overend, R. P.; Chornet, E. Fractionation of lignocellulosics by steam-aqueous pretreatments. *Philos. Trans. R. Soc., A* **1987**, *321* (1561), 523–536.

(28) Ikeda, T.; Holtman, K.; Kadla, J. F.; Chang, H.-m.; Jameel, H. Studies on the effect of ball milling on lignin structure using a modified DFRC method. *J. Agric. Food Chem.* **2002**, *50* (1), 129–135.

(29) Glasser, W. G.; Kaar, W. E.; Jain, R. K.; Sealey, J. E. Isolation options for non-cellulosic heteropolysaccharides (hetps). *Cellulose* **2000**, *7* (3), 299–317.

(30) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure*; Technical Report No. NREL/TP-510-42618; NREL: Golden, CO, 2008; pp 1–15.

(31) Evtuguin, D. V.; Neto, C. P.; Silvestre, A. J. Condensation reactions of lignin during oxygen delignification under acidic conditions. *J. Wood Chem. Technol.* **1997**, *17* (1–2), 41–55.

(32) Butt, H.-J.; Graf, K.; Kappl, M. *Physics and Chemistry of Interfaces*; John Wiley & Sons: Hoboken, NJ, 2006.

(33) Li, J.; Henriksson, G.; Gellerstedt, G. Carbohydrate reactions during high-temperature steam treatment of aspen wood. *Appl. Biochem. Biotechnol.* **2005**, *125*, 175–188.

(34) Kishimoto, T.; Sano, Y. Delignification mechanism during high-boiling solvent pulping. *Holzforschung* **2001**, *55* (6), 611–616.

(35) Sjöström, E. *Wood Chemistry: Fundamentals and Applications*; Academic Press: San Diego, CA, 1993.

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413–420

(36) Ebringerova, A.; Hromadkova, Z.; Heinze, T. Hemicellulose. In *Polysaccharides I*; Heinze, T., Ed.; Springer: Berlin, Heidelberg, New York, 2005.
(37) Fengel, D.; Wegener, G. *Wood: Chemistry, Ultrastructure, Reactions*; Walter de Gruyter: New York, 1983.
(38) Hill, C. A. *Wood Modification: Chemical, Thermal and Other Processes*; John Wiley & Sons: Hoboken, NJ, 2007.
(39) Ramos-Sanchez, M. C.; Rey, F.; Rodriguez, M. L.; Martin-Gil, F.; Martin-Gil, J. DTG and DTA studies on typical sugars. *Thermochim. Acta* **1988**, *134*, 55−60.
(40) Rey, F.; Ramos-Sanchez, M.; Rodriguez-Mendez, M.; Martin-Gil, J.; Martin-Gil, F. Dtg and dta studies on sugar derivatives. *Thermochim. Acta* **1988**, *134*, 67−72.
(41) Lora, J.; Wayman, M. Delignification of hardwoods by autohydrolysis and extraction. *Tappi* **1978**, *61*, 47−50.
(42) Ebringerova, A.; Heinze, T. Xylan and xylan derivatives − Biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties. *Macromol. Rapid Commun.* **2000**, *21* (9), 542−556.
(43) Beall, F. Thermogravimetric analysis of wood lignin and hemicelluloses. *Wood Fiber Sci.* **1969**, *1* (3), 215−226.
(44) Fundador, N. G. V.; Enomoto-Rogers, Y.; Takemura, A.; Iwata, T. Acetylation and characterization of xylan from hardwood kraft pulp. *Carbohydr. Polym.* **2012**, *87* (1), 170−176.
(45) Windeisen, E.; Strobel, C.; Wegener, G. Chemical changes during the production of thermo-treated beech wood. *Wood Sci. Technol.* **2007**, *41* (6), 523−536.
(46) Thring, R. W.; Chornet, E.; Overend, R. P. Recovery of a solvolytic lignin: Effects of spent liquor/acid volume ratio, acid concentration and temperature. *Biomass* **1990**, *23* (4), 289−305.
(47) Ibrahim, M.; Glasser, W. G. Steam-assisted biomass fractionation. Part iii: A quantitative evaluation of the "clean fractionation" concept. *Bioresour. Technol.* **1999**, *70* (2), 181−192.
(48) Gierer, J. Chemistry of delignification. *Wood Sci. Technol.* **1985**, *19* (4), 289−312.
(49) Vanasse, C.; Chornet, E.; Overend, R. Liquefaction of lignocellulosics in model solvents: Creosote oil and ethylene glycol. *Can. J. Chem. Eng.* **1988**, *66* (1), 112−120.

DOI: 10.1021/sc500564g
ACS Sustainable Chem. Eng. 2015, 3, 413−420