



Changes in composition and sugar release across the annual rings of *Populus* wood and implications on recalcitrance

Jaclyn D. DeMartini, Charles E. Wyman*

Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, 1084 Columbia Avenue, Riverside, CA 92507, United States

ARTICLE INFO

Article history:

Received 9 July 2010

Received in revised form 30 August 2010

Accepted 31 August 2010

Available online 6 September 2010

Keywords:

Pretreatment

Enzymatic hydrolysis

Biomass recalcitrance

Age effects

Populus wood

ABSTRACT

Understanding structural characteristics that are responsible for biomass recalcitrance by identifying why it is more difficult for some plants, or portions of plants, to release their sugars would be extremely valuable in overcoming this barrier. With this in mind, this study investigated the recalcitrance of wood by considering the effects of aging in two *Populus tremuloides* cross sections. By applying our novel small scale systems, including a multi-well pretreatment and enzymatic hydrolysis system and a downscaled compositional analysis procedure, we were able to follow ring-by-ring compositions and sugar release patterns. Observed variations were then related to structural changes that occur across the radial direction of trees, providing an important step toward understanding the influence of these changes on recalcitrance.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

There is an urgent need for utilization of renewable resources to reduce our dependence on petroleum and associated greenhouse gas emissions. However, options are limited, and currently the only promising resource for large-scale sustainable production of organic chemicals and liquid fuels is lignocellulosic biomass (Lynd et al., 1991; Perlack et al., 2005; Farrell et al., 2006). The cellulose and hemicellulose fractions that comprise about two thirds to three quarters of such materials can be broken down to release sugars that in turn can be converted biologically or chemically into a wide range of fuels, chemicals, and materials (Wyman, 2007). Woody biomass represents one potentially important source of lignocellulosic materials, but lignocellulosic biomass, and particularly woody material, is recalcitrant to sugar release. As a result, severe pretreatments are needed to achieve reasonable sugar yields, which result in some losses through degradation while still requiring high enzyme loadings in the subsequent enzymatic hydrolysis operation. The resulting high energy inputs and high enzyme protein doses translate into high costs for conversion that have stymied commercialization. Unfortunately, the understanding of biomass recalcitrance is limited, making it difficult to optimize harvest age and conversion technologies, and also limiting rational genetic engineering efforts to produce less recalcitrant species.

Although there is little consensus on what specific structural characteristics cause biomass, or wood in particular, to be recalci-

trant, some of the most commonly proposed features include the degree of lignification (Mansfield et al., 1999; Chang and Holtzap- ple, 2000; Himmel et al., 2007; Chen and Dixon, 2007), the distribution of microfibrils and matrix polymers (Himmel et al., 2007), the level of cellulose crystallinity and degree of polymerization (Mansfield et al., 1999; Chang and Holtzap- ple, 2000), the acetyl content (Chang and Holtzap- ple, 2000), and the available surface area (Mansfield et al., 1999; Chang and Holtzap- ple, 2000). To expand on current knowledge, we sought to examine the effect of age on composition and sugar release of *Populus* wood and thereby develop a better understanding of recalcitrance in trees. In particular, we sought to evaluate the influence of structural changes resulting from juvenile-to-mature aging processes on recalcitrance and sugar yields by addressing the following questions:

- (1) Are significant differences present in the composition of, and sugar release from juvenile versus mature wood?
- (2) Do these differences translate into digestibility and recalcitrance being defined by the maturity of wood?
- (3) Can the recalcitrance of wood be correlated to structural changes that are caused by juvenile-to-mature aging? If not, can it be related to other structural, compositional, or environmental factors?

In pursuit of answers, we investigated the radial variation in composition and performance in pretreatment and enzymatic hydrolysis for two different-aged *Populus tremuloides* trees to determine whether significant radial variation existed, and if so, whether it followed any observable trends. Results were then re-

* Corresponding author. Tel.: +1 951 781 5703; fax: +1 951 781 5790.

E-mail address: Charles.wyman@ucr.edu (C.E. Wyman).

lated to structural changes that are known to occur across the radial direction of trees so that the influence of these changes could be evaluated for their influence on sugar yields.

Previous studies have examined the variation in composition across individual annual rings of a tree's cross section (Shupe et al., 1997; Bertaud and Holmbom, 2004; Sykes et al., 2008), however, the evaluation of ring-by-ring sugar release from pretreatment and enzymatic hydrolysis has been limited by the significant amounts of material that are typically required. As a result, conversion data is usually developed from large amounts of materials that include the entire cross section of the tree. To overcome this limitation, downscaled processes can be employed to study the variation in both composition and sugar release of individual annual rings, of which there is often very small amounts of material (less than 1 g). Such a study is valuable in understanding how wood age affects sugar release because the use of samples from a single tree eliminates the influence of genetic factors associated with comparison of multiple trees and also reduces the impact of environmental factors. The result is that observed differences can be more confidently attributed to wood age and juvenile-to-mature transitions within a tree.

To facilitate the analysis of radial variation within trees, cross sections from 8 and 26 year old *P. tremuloides* trees were fractionated into their individual annual rings and analyzed for composition using a novel downscaled wet chemistry method (DeMartini et al., 2010). Each ring was then subjected to a hot water pretreatment followed by enzymatic hydrolysis in a similar high throughput, scaled-down system as used for the compositional analysis (Studer et al., 2010). Composition, sugar release, and resulting sugar yields were then determined for each ring and analyzed for variation.

2. Methods

2.1. Biomass samples

Trembling Aspen (*P. tremuloides*) samples were obtained by Benchmark International within a 2 km radius in High Level, Alberta, Canada. Variable aged stands were located, and trees were destructively sampled to collect approximately 20–80 mm thick disks, or cross sections, from individual trees at 0.3 meters from the point of germination. The sections were grouped into age class in 10 year increments, labeled, and sent to the University of California Riverside, USA.

2.2. Cross section fractionation

Cross sections classified as 1–10 and 20–30 years in age were chosen for this study. They were sanded with decreasingly course sand paper (grit #50 through #150) to produce clean and smooth surfaces. Cross sections were then cut into different pieces, including two bulk sections that were prepared to serve as control materials representative of the entire cross section. For both cross sections, Bulk 1 included the bark from its section, while for Bulk 2, the bark was removed.

The remaining strip of wood was fractionated into its individual annual rings. Annual rings were usually identified by general observation. However, to aid in distinguishing between rings in the 20–30 year section, in which ring width was generally more compressed, a thin strip of the ring section was cut and examined under a microscope (Nikon Optiphot-66 equipped with Imaging Source IS-2CU USB CCD Camera, Tokyo, Japan). In this way, individual rings were identified and then fractionated using a wood chisel and hammer. The 20–30 year section was fractionated into 26 rings plus the bark, and the 1–10 year section was fractionated into

8 rings plus the bark. Following fractionation, all samples were milled (Wiley Laboratory Mill Model 4, Arthur H. Thomas Company, Philadelphia, PA, USA) until they passed through a 20-mesh screen (<0.85 mm).

2.3. Compositional analysis

Glucan, xylan, and lignin contents were determined by performing a novel downscaled compositional analysis that is nearly identical to conventional wet chemistry procedures (Sluiter et al., 2008) but uses 100 times less biomass. The entire process, which is described in detail elsewhere (DeMartini et al., 2010), was performed in 1.5 mL high recovery glass high performance liquid chromatography (HPLC) vials (Agilent, Santa Clara, CA, USA) using only 3 mg dry biomass per test. It should be noted that the lignin contents determined by this method are approximate values because the analysis method measures the total acid insoluble residue, which also includes acid insoluble ash. However, since the whole ash contents of the Bulk 1 and 2 materials are quite small, $2.8\% \pm 0.5\%$ and $1.2\% \pm 0.3\%$, respectively, the acid insoluble residue should provide a good estimate of the Klason lignin content for the ring samples.

2.4. Pretreatment and enzymatic hydrolysis

All samples were subjected to combined pretreatment and enzymatic hydrolysis to determine resulting sugar release using a novel high throughput pretreatment and enzymatic hydrolysis (HTPH) system described elsewhere (Studer et al., 2010). In this process, 2.5 mg dry, milled biomass was loaded into individual wells of a custom-built metal well plate in which both pretreatment and enzymatic hydrolysis were performed. Next, 247.5 μL deionized (DI) H_2O was pipetted into all wells (8 channel pipetter, 30–300 μL , Eppendorf, Hamburg, Germany) to achieve a solids loading of 1%w/w. The well plate was then clamped between two stainless steel plates with a flat silicone gasket (thickness 1.5875 mm, durometer hardness A40) in between. The sealed plate assembly was placed in a custom-built steam chamber for pretreatment with condensing steam (Studer et al., 2010).

After pretreatment, the reaction was quenched with cold water and the plate assembly was removed from the chamber and opened. 20 μL of a mixture of 1 M citric acid buffer (pH 4.95), sodium azide solution (1 g/L), and enzyme mixture was pipetted into each well (8 channel pipetter, 10–100 μL , Eppendorf, Hamburg, Germany). The mixture contained 5 mL of buffer, 1 mL of sodium azide solution, and 2.0 mL of a dilute cellulase and xylanase solution prepared at a protein mass ratio of 3:1, respectively, to which DI water was added at a volume ratio of 3:1. The resulting enzyme loading corresponded to 75 + 25 mg of cellulase and xylanase protein, respectively, per g of glucan + xylan in raw biomass for the 8 year old Bulk 1 material, which had a composition of 40.7% glucan, 17.0% xylan, 27.8% lignin + ash. After enzyme addition, the plate was re-sealed and placed on its side in an incubation shaker (Multitron Infors-HT, ATR Biotech, Laurel, MD, USA) at 50 °C for 72 h at 150 rpm.

Following enzymatic hydrolysis, the slurry was transferred to, and centrifuged in 1.5 mL polypropylene (PP) centrifuge tubes (Safe-Lock 2.0 mL test tubes, Eppendorf, Hamburg, Germany). 300 μL of the contents were transferred to a PP 96-well plate (Agilent, Santa Clara, CA, USA) for HPLC analysis.

Prior to testing all individual ring samples in the HTPH system, the bulk materials were used to establish a pretreatment optimization curve. Based on the resulting sugar release data, a slightly suboptimal pretreatment condition was selected for testing all ring samples to reduce sugar degradation. The selected pretreatment condition was a 70 min reaction at 160 °C.

2.5. Sugar analysis and results

Sugar concentrations were measured on an Aminex HPX-87H column (BioRad, Hercules, CA, USA) heated to 65 °C used in a separation module (Agilent 1200, Agilent, Santa Clara, CA, USA) equipped with a refractive index detector (1200 Agilent) using 0.005 M sulfuric acid as the eluent.

Sugar release results (g/g) were defined as the amount of glucose (or xylose) monomer released into solution per the amount of dry biomass used, while sugar yields (%) were defined as the amount of glucose (or xylose) monomer released into solution divided by the maximum amount of glucose (or xylose) that could be released, based on the given glucan (or xylan) content, times 100.

2.6. Statistical analysis

An unpaired two-tailed student's-*t* test was used to evaluate whether differences observed between juvenile and mature wood were significant. Unless otherwise stated, parameters were considered to be significantly different for $p < 0.05$. Analysis was performed using Igor Pro (Wavemetrics Inc., Lake Oswego, OR, USA).

3. Results and discussion

3.1. Composition results

Fig. 1 demonstrates that the composition of individual annual rings from the 8 and 26 year old trees were highly variable within the cross sections. In particular, rings from the young tree (excluding the bark) ranged from 27.5% to 42.1% in glucan content, from 16.2% to 21.2% in xylan content, and 26.9% to 38.2% in lignin content. For rings from the 26 year old section (again excluding bark), the glucan content ranged from 29.0% to 48.9%, while the xylan and lignin contents ranged from 13.1% to 18.8% and 21.5% to 33.5%, respectively. Compositional variation across both sections were also found to follow the same trend, namely that glucan content in-

creased in the direction of pith to bark, lignin content decreased in the same direction, while the variation in xylan content across the sections showed no clear trend.

3.2. Sugar release results

Significant variation was also discovered in sugar release from individual annual rings resulting from pretreatment and enzymatic hydrolysis, as displayed in Fig. 1. For the younger tree, the xylose release varied slightly from 0.15 to 0.18 g/g, while the glucose and glucose + xylose releases varied from 0.26 to 0.41 g/g and 0.41 to 0.57 g/g, respectively. Similar results were found for rings from the 26 year section, in which the xylose release ranged from 0.15 to 0.20 g/g, and the glucose and glucose + xylose releases varied from 0.27 to 0.47 g/g and 0.42 to 0.66 g/g, respectively.

3.3. Sugar yield results

Fig. 2 shows that sugar yields calculated from the composition and sugar release data varied much less than the composition and sugar release (g/g). The xylose and glucose yields of the 8 year's individual rings ranged between 72.7% and 88.6%, and 85.4% and 91.8%, respectively, corresponding to a range of glucose + xylose yields of 81.5%–88.7%. For the 26 year section, both the yields themselves and the extent of variation were somewhat higher than that of the 8 year old tree. In particular, the xylose and glucose yields varied between 90.9% and 104.7%, and 70.2% and 96.0%, respectively, while the glucose + xylose yields ranged between 76.3% and 98.4%. However, closer examination shows that although the overall variation was larger for the older tree, this was due primarily to rings aged 7 and 8, which performed significantly worse than the remaining rings within that same cross section. If these two rings were not included in the analysis, the glucose and glucose + xylose yields only varied by between 82.3% and 96.0%, and 87.8% and 98.4%, respectively, which is similar to the variation observed for the younger tree. The performance of rings 7 and 8 will be discussed in more detail in Section 3.5.

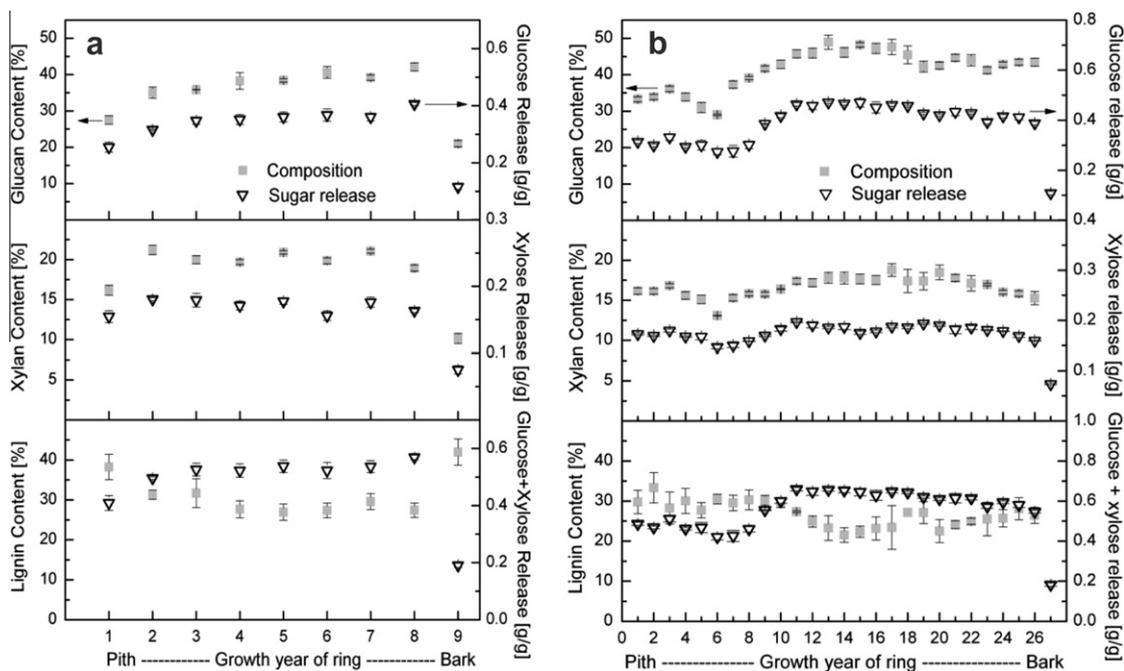


Fig. 1. Ring-by-ring composition including glucan, xylan, and lignin contents (in mass percent), as well as ring-by-ring sugar release (in g sugar released/g biomass) for glucose, xylose, and glucose + xylose. Results are shown for the 8 year old (a) and 26 year old (b) section, with the horizontal axis plotting the age of each ring from pith (left) to bark (right), where the final data point represents the bark material. Error bars represent the standard deviation of triplicate experiments.

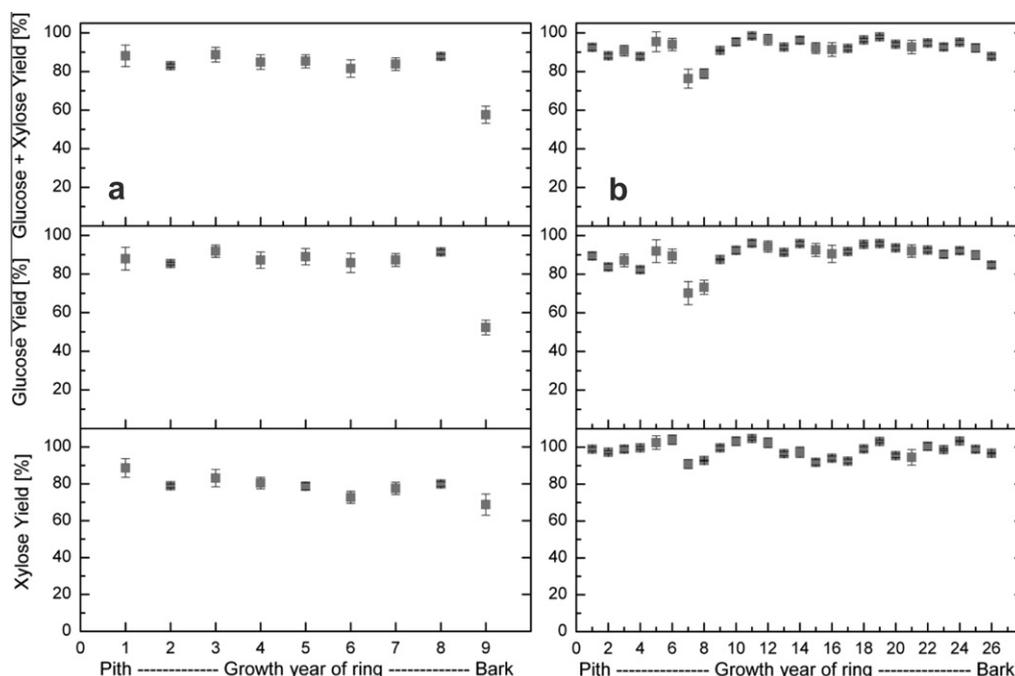


Fig. 2. Xylose, glucose, and glucose + xylose yields (%) for the 8 year old (a) and 26 year old (b) cross section. The horizontal axis plots the age of each ring from pith (left) to bark (right), where the final data point represents the bark material. Error bars represent the standard deviation of triplicates.

3.4. Evaluating variability

Analysis of the composition and sugar release patterns of individual annual rings from two *P. tremuloides* cross sections revealed substantial within tree radial variation. Fig. 1 demonstrates that compositional variation was more pronounced for the older tree, in which the glucan, xylan, and lignin contents varied among the 26 annual rings by up to 41%, 30%, and 36% of their respective values. However, the 8 year old tree also covered a large range, with glucan, xylan, and lignin contents varying by up to 35%, 24%, and 36% of their respective values. While the variability and observed trends of increasing glucan and decreasing lignin contents from pith to bark agreed with results from past work (Shupe et al., 1997; Bertaud and Holmbom, 2004; Sykes et al., 2008; Fengel and Wegener, 1984), the extent of variation was unexpected.

Fig. 1 further demonstrates that these trends were not gradual changes within the 26 year tree, but instead two major regions were discernible in the cross section. The first region encompassed mostly, if not all, of the juvenile wood (rings grown in approximately years 1–6) and was characterized by low glucan and slightly higher lignin contents. Rings aged 7 and 8 years appeared to transition to the second region that included more mature wood (rings grown in approximately years 9–26) and was characterized by higher glucan and somewhat lower lignin contents. The tested parameters of glucan and lignin content were found to be significantly different between the two groups. This trend was not observed in the 8 year cross section, likely due to the younger age and the resulting reduced, or non-existent presence of mature wood.

By subjecting rings to a high throughput and downscaled pretreatment and enzymatic hydrolysis screen, the sugar release across each cross section was found to vary significantly, and in a remarkably similar manner to the observed composition trends. Overall, glucose release (g sugar/g biomass) increased from pith to bark, and xylose release fluctuated slightly. Glucose release was also found to be significantly different between the juvenile and mature regions described above for compositional characteris-

tics. Comparison of these two distinct regions suggested that they could be distinguished by darker colored wood, which may reflect the presence of heartwood caused by the deposition of extractives into the dead xylem long after cells have become metabolically inactive (Sjostrom, 1993; Taylor et al., 2002). Once the cell is dead, wood chemistry and structure do not change, suggesting that the observed sugar release patterns were not a direct reflection of the presence of heartwood or sapwood but instead may be due to juvenile-to-mature transitions over the life of a tree. Although these transitions vary from genotype to genotype and with environment (Bendtsen and Senft, 1986; Peszlen, 1994), heartwood formation is related to age, which may explain the correlation seen between sugar release and darker wood color.

3.5. Investigating glucan digestibility

After observing significant variations in sugar release, it was surprising to discover that there was very little variation in glucose yield within each cross section, and that the ring-by-ring composition explained almost all of the observed trends and differences in sugar release performance. In fact, with the exception of bark, which has been previously shown to be inhibitory to enzymatic and microbial actions (Walch et al., 1992; Robinson et al., 2002), only rings 7 and 8 from the 26 year section deviated significantly from the other rings in terms of glucose yield. The glucose yields of rings 7 and 8 were 70.2% and 73.2%, respectively, while the average glucose yield of all other rings in that set was 90.9%. Because Fig. 3 shows a general trend of decreasing glucose yield with increasing lignin content, we first tried to explain the performance of these two rings by checking whether they exhibited higher lignin contents. Rings 7 and 8 had lignin contents of 29.3% and 30.3%, which were slightly higher than the average of all rings (26.8%). However, this alone could not explain the low yields because other rings with virtually the same lignin content performed substantially better and exhibited glucose yields of between 89.4% and 92.3%, a 20% improvement. Thus, factors other than lignin and carbohydrate contents apparently affected sugar yields, and as a re-

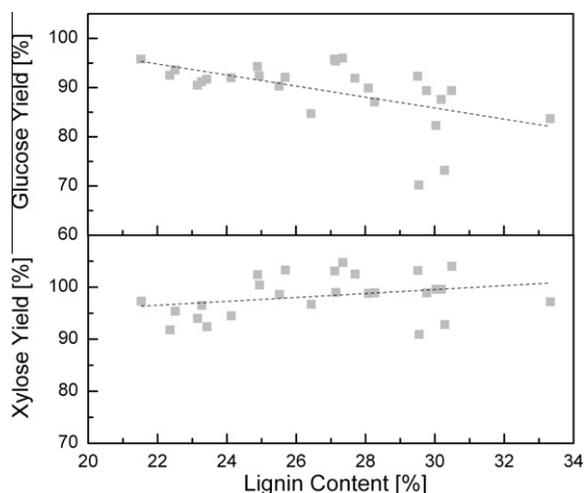


Fig. 3. Glucose and xylose yields (%) plotted versus lignin content for the 26 year old section. Dotted lines represent the linear fit of yield data in each subplot to emphasize the observed trends in sugar yields.

sult, we sought to investigate other possible causes for the low yields observed in these two rings.

Monthly and annual weather histories were examined for High Level, Alberta, with particular attention paid to the growing season, but no correlation could be found between the low glucose yields of rings 7 and 8 and average or extreme temperatures or precipitation (http://www.climate.weatheroffice.gc.ca/climateData/canada_e.html). Forest fire history was also investigated, but no correlation was found between sugar yields and fires in the growth area and the time of interest (<http://fire.cfs.nrcan.gc.ca/home-accueil-eng.php>). It is possible that the low yields observed for rings 7 and 8 may correspond to the transition between heartwood and sapwood, which is characterized by a high accumulation of extractives (Bertaud and Holmbom, 2004; Sykes et al., 2006). However, this zone is also reported to have high cellulose and low lignin contents, in contrast to what we found for these two rings. Thus, other unmeasured biochemical or anatomical changes that occurred initially during transition from juvenile-to-mature wood and that stabilized after the transition was complete appeared to be responsible for the low glucose yields observed in these rings.

3.6. Mechanistic implications for recalcitrance

With the exception of rings 7 and 8, results from this study demonstrated that the digestibility of the *Populus* trees examined did not change with age, and in particular, that the recalcitrance of wood was not defined by juvenility, maturity, or changes caused by this transition. Although the exact age of demarcation between juvenile and mature wood varies, it is almost certain that the transition will occur within the 26 year life span of the older tree, making results from this cross section particularly applicable in the context of studying wood maturity and recalcitrance (Bendtsen and Senft, 1986). As did Bendtsen and Senft (1986), we assumed that rings aged 6 years or less were representative of juvenile wood and that rings aged 20 years and older were representative of mature wood. In this way, we could be confident that our defined juvenile and mature woods were safely distant from the age of demarcation. Analysis of these two regions demonstrated that there was no statistically significant variation between them in terms of glucose yields, confirming that wood maturity did not define conversion potential.

Evaluating structural characteristics of juvenile and mature wood could provide clues for understanding factors that control the recalcitrance of *P. tremuloides* wood and help explain these findings. A review of the literature on wood quality as related to mechanical and anatomical properties demonstrated that the radius, length, and cell wall thickness of fiber increases from juvenile-to-mature wood, resulting in enhanced strength and stiffness of mature wood (Yanchuk et al., 1984; Bendtsen and Senft, 1986; Peszlen, 1994; Lei et al., 1996; Bao et al., 2001), while microfibril angle (MFA) has consistently been demonstrated to be higher in juvenile wood than in mature wood (Bendtsen and Senft, 1986; Lichtenegger et al., 1999; Evans et al., 2000; Barnett and Bonham, 2004). While little work has been reported that systematically studied the effect of these or other structural features in wood on recalcitrance, a multitude of studies on the digestibility of grasses as ruminant feedstocks have demonstrated similar structural characteristics to be important. For example, the distribution and relative amounts of various tissue types have been observed to affect digestibility in grasses because they define cell shape, size, wall thickness, and corresponding surface area to cell wall volume, all of which have been suggested to impact digestibility (Grabber et al., 1992; Grabber and Allinson, 1992; Wilson and Mertens, 1995; Buxton and Redfearn, 1997). It has also been proposed that the structural heterogeneity and complexity of cell wall components such as microfibrils may play a role in biomass recalcitrance (Himmel et al., 2007). Investigating whether these structural characteristics affect conversion of *P. tremuloides* to sugars may help advance our understanding of recalcitrance in wood. However, the lack of significant variation in glucose yields between the defined juvenile and mature wood suggests that structural differences in cell radius, length, and wall thickness, as well as MFA, could be ruled out of impacting the digestibility of *P. tremuloides* wood.

3.7. Influence of lignin content

The plot in Fig. 3 of xylose and glucose yields versus lignin content was prepared to determine the influence of lignin content on sugar conversion for the individual rings of the 26 year old cross section. A dotted line showing the linear fit of each subplot is included to represent the observed trends in sugar yields. While the glucose yield decreased somewhat with increasing lignin content, the xylose yield appears to have increased slightly for rings with higher lignin content.

In addition to evaluating the impact of structural features on recalcitrance, it has been reported that digestibility can be affected by cell wall composition, particularly lignin composition and content. Increased lignin content has been shown to adversely effect glucan digestibility, possibly due to restriction of enzyme access to cellulose and increased non-productive binding of enzyme (Chang and Holtzaple, 2000; Berlin et al., 2005; Chen and Dixon, 2007). The negative influence of lignin was observed in the older cross section of this study, in which the glucose yield dropped with increasing lignin content. However, much less clear is the effect of lignin on hemicellulose convertibility, which was shown to generally increase with higher lignin contents. It is also important to note that the lignin and xylan contents were inversely related, and that samples with higher lignin contents exhibited lower xylan contents. These observations were surprising because lignin is known to impart a high level of mechanical strength to cell walls by forming numerous crosslinks with polysaccharides (Carpita and Gibeau, 1993; Iiyama et al., 1994). As a result, increased lignin content should produce hemicellulose that is more tightly bound within the cell wall matrix which in turn would be expected to have negative consequences on xylose release and conversion.

Further investigation will be required to determine the cause of this trend.

3.8. Implications for harvest age

Results from this study have implications on the optimal harvest age of trees to be converted to sugars for fermentation to ethanol and other products. Because it was observed that sugar yields were fairly consistent across the rings and that juvenile-to-mature changes occurring in the wood did not affect digestibility, sugar yields should be similar for trees harvested at a later age as those harvested earlier in their life. In fact, comparison of sugar yields of the 8 and 26 year sections showed that on average, the glucose and xylose yields were higher for the older tree. However, despite the proximity of the two trees used in this study, it is unknown if they were suckers off of the same parent and thus directly related to one another. As a result, the difference in yields cannot be attributed exclusively to age, but may in fact be an effect of natural variation.

This study confirmed that the older tree had a larger proportion of mature wood which contained high levels of glucan, which was further demonstrated to be converted to glucose with the same efficiency as the glucan in juvenile wood. Thus, there will be an optimum harvest age when glucan content and tree productivity maximize the mass of sugar produced per mass of feedstock per unit time. Although the growth of almost all trees follows an S-shaped sigmoidal curve, the rates of growth and final tree height will vary by genotype and by site location (Chen et al., 2002), and thus broad generalizations about optimum harvest times are difficult to make. However, the results of this study strongly suggest that this optimum is not likely to occur during the juvenile growth phase, which could have implications on the 6–10 year rotations that are typically proposed for short rotation woody crops. Additional studies are needed to examine cross sections of varying ages for trees of interest to determine whether this observation can be generalized to all *P. tremuloides* trees as well as to other hardwoods.

4. Conclusions

Our novel small-scale high throughput multi-well pretreatment and hydrolysis system and downscaled compositional analysis procedure were able to determine ring-by-ring compositions and sugar release patterns of two *P. tremuloides* trees of different ages. Although significant within tree radial variation in composition and sugar release was found, digestibility remained almost constant. These results suggest that wood maturity does not influence the recalcitrance of *P. tremuloides* wood and further allowed us to speculate that a number of structural features that have previously been proposed to impact digestibility, did not effect the recalcitrance for the wood used in this study.

Acknowledgements

We gratefully acknowledge support for this research by the Office of Biological and Environmental Research in the DOE Office of Science through the BioEnergy Science Center (BESC). The authors would also like to extend their appreciation to Pat Guidera, Dave Patterson and Dan Wilkinson of Alberta Sustainable Resource Development, Len Bykowski of VRM Management Solutions, and Benchmark Environmental for providing biomass materials. We also wish to thank Gerald Tuskan of Oak Ridge National Laboratory for his valuable discussions and insights, as well as the Ford Motor Company for their support of the Chair in Environmental Engineering at UCR that augments our ability to perform such research.

References

- Bao, F.C. et al., 2001. Differences in wood properties between juvenile wood and mature wood in 10 species grown in China. *Wood Sci. Technol.* 35, 363–375.
- Barnett, J.R., Bonham, V.A., 2004. Cellulose microfibril angle in the cell wall of wood fibres. *Biol. Rev.* 79, 461–472.
- Bendtsen, B.A., Senft, J., 1986. Mechanical and anatomical properties in individual growth rings of plantation-grown eastern cottonwood and loblolly pine. *Wood Fiber Sci.* 18 (1), 23–38.
- Berlin, A., Neil, G., Arwa, K., Renata, B., Tu, M., 2005. Weak lignin-binding enzymes. *Appl. Biochem. Biotechnol.* 121 (124), 163–170.
- Bertaud, F., Holmbom, B., 2004. Chemical composition of earlywood and latewood in Norway spruce heartwood, sapwood and transition zone wood. *Wood Sci. Technol.* 38, 245–256.
- Buxton, D.R., Redfearn, D.D., 1997. Plant limitations to fiber digestion and utilization. *J. Nutr.* 127, 814S–818S.
- Carpita, N.C., Gibeaut, D.M., 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 3 (1), 1–30.
- Chang, V.S., Holtzapfel, M.T., 2000. Fundamental factors affecting biomass enzymatic reactivity. *Appl. Biochem. Biotechnol.* 84 (86), 5–37.
- Chen Han, Y.H., Krestov, P.V., Klinka, K., 2002. Trembling aspen site index in relation to environmental measures of site quality at two spatial scales. *Can. J. For. Res.* 32, 112–119.
- Chen, F., Dixon, R., 2007. Lignin modification improves fermentable sugar yields for biofuel production. *Nat. Biotechnol.* 25 (7), 759–761.
- DeMartini, J.D., Studer, M.H., Wyman, C.E., 2010. Small scale and automatable high-throughput compositional analysis of biomass. *Biotechnol. Bioeng.*, doi:10.1002/bit.22937.
- Evans, R., Stringer, S., Kibblewhite, R.P., 2000. Variation of microfibril angle, density and fibre orientation in twenty-nine *Eucalyptus nitens* trees. *Appita J.* 53, 450–457.
- Farrell, A.E. et al., 2006. Ethanol can contribute to energy and environmental goals. *Science* 311, 506–508.
- Fengel, D., Wegener, G., 1984. *Wood-Chemistry, Ultrastructure and Reactions*. Walter de Gruyter New York, NY, USA.
- Grabber, J.H., Allinson, D.W., 1992. Anatomical structure and digestibility of reed canarygrass cultivars and hybrid ryegrass. *Grass Forage Sci.* 47, 400–404.
- Grabber, J.H., Jung, G.A., Abrams, S.M., Howard, D.B., 1992. Digestion kinetics of parenchyma and sclerenchyma cell walls isolated from orchardgrass and switchgrass. *Crop Sci.* 32, 806–810.
- Himmel, M. et al., 2007. Biomass recalcitrance: engineering plants n enzymes for biofuels production. *Science* 315 (804), 804–807.
- Iiyama, K., Bach-Tuyet Lam, T., Stone, B.A., 1994. Covalent cross-links in the cell wall. *Plant Physiol.* 104, 315–320.
- Lei, H., Milota, M.R., Gartner, B.L., 1996. Between- and within-tree variation in the anatomy and specific gravity of wood in Oregon White Oak (*Quercus Garryana* Dougl.). *IAWA J.* 17 (4), 445–461.
- Lichtenegger, H., Reiterer, A., Stanzl-Tschegg, S.E., Fratzl, P., 1999. Variation of cellulose microfibril angles in softwoods and hardwoods – a possible strategy of mechanical optimization. *J. Struct. Biol.* 128, 257–269.
- Lynd, L.R., Cushman, J.H., Nichols, R.J., Wyman, E.E., 1991. Fuel ethanol from cellulosic biomass. *Science* 251, 1318–1323.
- Mansfield, S.D., Mooney, C., Saddler, J.N., 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnol. Prog.* 15, 804–816.
- Perlack R.D. et al., 2005. Biomass as a feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-bon annual supply. (ORNL/ TM-2005/66Oak Ridge National Laboratory, Oak Ridge, TN). Also available at http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf.
- Peszlen, I., 1994. Influence of age on selected anatomical properties of *Populus* clones. *IAWA J.* 15 (3), 311–321.
- Robinson, J., Keating, J.D., Boussaid, A., Mansfield, S.D., Saddler, J.N., 2002. The Influence of bark on the fermentation of Douglas-fir whitewood pre-hydrolysates. *Appl. Microbiol. Biotechnol.* 59, 443–448.
- Shupe, T.F., Hse, C.Y., Choong, E.T., Groom, L.H., 1997. Differences in some chemical properties of innerwood and outerwood from five silviculturally different Loblolly Pine stands. *Wood Fiber Sci.* 29 (1), 92–97.
- Sjostrom, E., 1993. *Wood Chemistry: Fundamentals and Applications*. Academic Press, CA, San Diego, USA.
- Sluiter, A. et al., 2008. Determination of Structural Carbohydrates and Lignin in Biomass Laboratory Analytical Procedure. National Renewable Energy Laboratory, Golden, Colorado.
- Studer, M.H., DeMartini, J.D., Brethauer, S., McKenzie, H.L., Wyman, C.E., 2010. Engineering of a high-throughput screening system to identify cellulosic biomass, pretreatments, and enzyme formulations that enhance sugar release. *Biotechnol. Bioeng.* 105, 231–238.
- Sykes, R., Li, B., Isik, F., Kadla, J., Chang, H.-M., 2006. Genetic variation and genotype by environment interreaction of juvenile wood chemical properties in *Pinus taeda*. *L. Ann. For. Sci.* 63, 897–904.
- Sykes, R., Kodrzycki, B., Tuskan, G., Foutz, K., Davis, M., 2008. Within tree variability of lignin composition in *Populus*. *Wood Sci. Technol.* 42 (8), 649–661.
- Taylor, A.M., Gartner, B.L., Morrell, J.J., 2002. Heartwood formation and natural durability – a review. *Wood Fiber Sci.* 34 (4), 587–611.

- Walch, E., Zemann, A., Schinner, F., Bonn, G., Bobleter, O., 1992. Enzymatic saccharification of hemicellulose obtained from hydrothermally pretreated sugar cane bagasse and beech bark. *Bioresour. Technol.* 39, 173–177.
- Yanchuk, A.D., Dancik, B.P., Micko, M.M., 1984. Variation and heritability of wood density and fibre length of trembling aspen in alberta, Canada. *Silvae Genetica*. 33 (1), 11–16.
- Wilson, J.R., Mertens, D.R., 1995. Cell wall accessibility and cell structure limitations to microbial digestion of forage. *Crop Sci.* 35, 251–259.
- Wyman, C.E., 2007. What is (and is not) vital to advancing cellulosic ethanol. *Trends Biotechnol.* 25, 153–157.

Further reading

- Environment Canada. National Climate Data and Information Archive. http://www.climate.weatheroffice.gc.ca/climateData/canada_e.html. Date accessed: April 27, 2010.
- Natural Resources Canada. Forest Fire in Canada. <http://www.fire.cfs.nrcan.gc.ca/home-accueil-eng.php>. Date accessed: April 27, 2010.