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Hot water and dilute acid pretreatment of high and low specific gravity *Populus deltoides* clones

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Abstract *Populus* sp. are hardwood feedstocks that grow in forest management areas that are logged for softwoods; however, they are also being considered as an energy-destined feedstock. The objective of this work was to determine the effect of xylose yield from dilute acid and hot water pretreatments performed in unstirred batch stainless steel reactors at temperatures ranging from 140 to 200°C. *Populus deltoides* clones S13C20 and S7C15 used in this study originated from Eastern Texas and were cultivated for 14 years in Pine Tree, AR. *P. deltoides* clones S13C20 and S7C15 had specific gravities of 0.48 and 0.40, respectively. Bark and wood were examined separately. As expected, hot water pretreatments, in the tested temperature range, resulted in very little direct xylose recovery. However, the 140°C dilute acid pretreatment of the lower specific gravity clone, S7C15, wood yielded the highest average xylose recovery of 56%. This condition also yielded the highest concentration of furfural, 9 mg/g sample, which can be inhibitory to the fermentation step. The highest xylose recovery from bark samples, 31%, was obtained with clone S7C15, using the 160°C dilute acid pretreatment for 60 min.

Keywords Hybrid poplar · Populus · Hemicellulose · Pretreatment · Xylose

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Introduction

The development of petroleum-free fuels is of utmost importance for economic and ecological reasons. Corn-based ethanol has been the primary player in the alternative energy field. However, corn is not only used for ethanol production, but also for food, creating a large and potentially competing demand for this commodity. Cellulosic biomass sources, especially those not linked to food, are being considered. These include herbaceous and woody energy crops as well as agricultural and forestry product residues [9]. Hybrid poplars, *Populus deltoides,* are hardwoods that often grow in forest areas that are logged for softwood. Additionally, *P. deltoides* is being increasingly planted and managed in the United States as short-rotation plantations for timber and pulp, and as a potential source of biomass energy [5]. Woody biomass, including *P. deltoides*, contains cellulose, hemicellulose and lignin. When biomass is pretreated and hydrolyzed, it releases various compounds, including monomeric sugars that can be fermented to biofuels.

Recently, the Consortium for Applied Fundamentals and Innovation (CAFI) reported on the effect of five pretreatments on monomeric xylose recovery from poplar feedstock [11, 12]. Of the tested pretreatment conditions, SO_2

steam explosion gave the highest xylose recovery yield, 74%, in the poplar exudates. The second best pretreatment was that of dilute acid with a xylose recovery yield of 63%, indicating that this pretreatment could be a viable option for monomeric sugar production.

This study examines the effect of pretreatment, 140–200°C dilute acid and hot water, on the xylose recovery from bark and wood of two *P. deltoides* clones. One clone was cultivated in a situation where the water supply was maintained, while the other clone was grown under dry land conditions. These differences in clones and cultivation practices led to material with differences in their specific gravity.

Materials and methods

Poplar feedstock

P. deltoides samples were collected from the University of Arkansas Pine Tree Branch Station (Pine Tree, AR), which has study plots of several representative *P. deltoides* clones. The nine clones grown on this site were from Mississippi and East Texas, and represented 14 years of growth. The particular clones used in this study were S13C20 and S7C15, and were selected because they had the highest and lowest oven-dried specific gravity (personal communication from Umesh Chaudhari, University of Arkansas, Monticello). Dykes were arranged around clone S13C20, such that a constant water supply was provided; therefore, this clone was never in a drought situation. On the other hand, *P. deltoides* clone S7C15 was cultivated in an open flat field, and the source of water was solely natural rainfall. In early January 2010, *P. deltoides* clones S13C20 and S7C15 were harvested and chipped into 2.5-cm pieces using a BX62 3 Point Hitch tractor mounted wood chipper (EMB Manufacturing Inc., St Clements, ON, Canada). Twenty-kilogram samples of S13C20 and S7C15 wood and bark chips were delivered to Fayetteville, AR, in mid-January 2010 and stored at 4°C until needed.

Specific gravity determination

The oven-dried specific gravity was calculated as the ratio of the oven-dried density (oven-dried mass of the sample divided by its oven-dried volume displaced in water) to the density of water. Ten individual cross-sectional samples of *P. deltoides* clones S13C20 and S7C15 were secured from the harvest site, debarked, and placed into pre-weighed plastic bags for transport. The wood samples were subsequently oven-dried at 103°C until the mass of the wood sample remained constant for 24 h. Once dried, the wood was dipped in very hot paraffin wax, such that it was now sealed with an extremely thin coating; the wax-coated sample was then dipped into a pan of water that had been placed on a digital scale and tared to zero. The new mass was recorded, and from this, the mass of the displaced water was calculated. Using the value for the density of water, the mass of displaced water was converted to the volume of displaced water, which was equivalent to the volume of the wood. The specific gravity of the wood sample, which is dimensionless, was calculated as the mass of the oven-dried sample divided by its volume, all of which was divided by the density of water.

Moisture content determination

One gram of ground sample was analyzed for moisture content using a MB45 Moisture Analyzer (Ohaus Corporation, Pine Brook, NJ). Bark and wood from both clones were analyzed. This was repeated three times for each sample.

Pretreatment

To prepare the material for pretreatment, wood and bark chips from *P. deltoides* clones S13C20 and S7C15 were ground to a 10-mesh size, using a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ). The samples were pretreated in either water or dilute acid at temperatures ranging from 140 to 200°C. Essentially, 1 g of the ground sample and 20 ml of either Millipore-filtered water or 1% sulfuric acid (EMD, Gibbstown, NJ) were placed into a thick-walled stainless steel tube (interior diameter 14.22 mm, wall thickness 5.59 mm, length 200 mm, for a total chamber volume capacity of 32 ml); the sample was characterized as a 5% slurry. Before the assigned pretreatment, the Techne Industrial fluidized sand bath (Techne Incorporated, Burlington, NJ) was allowed to reach the desired pretreatment temperature. When the reaction temperature was attained, the stainless tubes were placed in the sand bath for the specific reaction time; this was determined as the start of the reaction. The reaction times and temperatures were the following for the wood and bark pretreatments: 140°C for 100 min, 160°C for 60 min, 180°C for 40 min, and 200°C for either 20 or 40 min. After each pretreatment run, the tubes were removed and placed in cold running water for 1 min and then cooled to 4°C for 30 min. When opened, the slurry was poured into 50-ml beakers, where the pH and the recovered volume were measured. Calcium carbonate (Fisher Scientific, Fairlawn, NJ) was added to the solution and stirred overnight, such that a neutral pH was obtained. This is critical for maintaining the longevity of the high pressure liquid chromatography (HPLC) pre-column and column. The neutralized sample solution was subsequently placed into 15-ml screw-cap centrifuge tubes (Corning Inc., Corning, NY) and centrifuged for

2 min at 500*g*. The supernatant was placed into other centrifuge tubes and filtered through a 0.2-μm filter (National Scientific, Rockwood, TN) for HPLC analysis. Pretreatments were performed at least three times for each time/temperature combination.

Monomeric and oligomeric determination

D-(−) arabinose and D-(+) glucose were purchased from Sigma–Aldrich (St. Louis, MO) and Alfa Aesar (Heysham, Lancashire). D-(+) mannose and D-(+) xylose were purchased from Fluka Analytical (Buchs, Switzerland). Xylotriose, xylopentaose and xylohexose were purchased from Megazyme (Wicklow, Ireland). All carbohydrate standards were prepared in sterile deionized water at concentrations of 1 mg/ml.

The HPLC and protocol used in this work for quantifying the carbohydrate monomers were as previously described by Martin et al. [8]. Analysis of each sample was performed at least in duplicate. Samples chosen for xylose oligomer content were analyzed and quantified using a Waters Alliance HPLC system (Model 2695, Waters Corporation, Milford, MA) employing a Micro-Guard De-Ashing precolumn and an Aminex HP-87A column (300 × 7.8 mm) (Bio-Rad, Hercules, CA). The column temperature was 85°C. Millipore water, filtered to 0.2 μm, was used as the eluent, with a flow rate of 0.2 ml/min. Then 10-μl samples were injected, and the oligomers were monitored by a Waters 2414 Refractive Index detector.

Furfural determination

Samples chosen for furfural content were analyzed and quantified using a Waters model 2695 Separations Module equipped with a Biorad Aminex HPX-87H column (300 × 7.8 mm), hydrogen form, 9-μm particle size, 8% cross linkage, and pH range 1–3. A pre-column, Micro-Guard Cation H Refill Cartridges (30 × 4.6 mm) guard column, hydrogen form, pH range 1–3, was placed in line prior to the column. The column temperature was set at 35°C. The mobile phase was 0.005 M sulfuric acid. The flow rate was set at 0.6 ml/min for 70 min, with an injection volume of 20 μl. Detection was measured at 280 nm, using a Waters 2996 Detector.

Data analysis

Percent extraction yields of carbohydrates from bark and wood were analyzed using Microsoft Excel. Analysis of variance (ANOVA) was run using the Fit Least Squares Model and LSMeans Differences Tukey, HSD JMP 8.02 (SAS Institute, Cary, NC). Significance was determined at $P < 0.05$.

Results and discussion

Eastern cottonwood (*Populus deltoides),* a common minor species in the southeast US, is a source of biomass that has the potential to become an economically valuable feedstock in the production of cellulosic biofuels [4, 5]. The feedstocks used in this study were *P. deltoides* clones, both harvested at 14 years. The *P. deltoides* clone S13C20 was cultivated under a constant water supply, while the S7C15 clone was cultivated under dry land conditions. The clone of origin and/or the water management practices had an effect on the specific gravity values; the specific gravity of clones S13C20 and S7C15 were 0.48 and 0.40, respectively. At the time of the study, the moisture content of clones S13C20- and S7C15-derived wood chips were 43 and 48%, respectively. Interestingly, the forester responsible for the Pine Tree site noted that clone S13C20 did not survive in dry land conditions, while clone S7C15 perished when cultivated under constant water supply, indicating a possibly important genetic predisposition between the two clones.

Hot water pretreatment

In the biochemical conversion platform of biomass to biofuels, pretreatment is a key step prior to enzymatic hydrolysis. Of the pretreatments, high temperature water pretreatment presents an interesting option because this methodology does not require additional chemicals, such as dilute acid, for hydrolysis of the hemicellulose. Results presented in Table 1 show the recovered xylose values of wood and bark of *P. deltoides* clones S13C20 and S7C15, which were pretreated with water at 160°C for 60 min and 200°C for 20 min. It is important to note that the baseline composition of *P. deltoides* clones S13C20 and S7C15 was not determined, and, for the sake of recovery calculations, the *P. deltoides* composition reported by Kim et al. [4] was used as the baseline composition. The percent recoveries reported in this paper are based on the percent of the content of that sugar in the original wood and bark as reported by Kim et al. [4]. As shown in Table 1, no xylose was recovered in the water exudates, which is in accordance with the report by Kim et al. [4]. There are variations of percent xylose content in hybrid poplar, varying from 13.1 to 18.7%, with the average being 15.8%, as reported by Sannigrahi et al. [9]. There is not a significant difference in using 14.9 compared to 15.8%; therefore, for comparison with Kim [4], 14.9% xylose content was chosen for this study. Water pretreatments induce the release of xylans in the form of oligomeric sugars, as opposed to monomeric sugars such as xylose. Figure 1a presents a chromatogram of the oligomer standards, while Fig. 1b shows an oligomer analysis chromatogram that was obtained from clone

Table 1 Recovery of monomeric sugars, oligomeric sugar (DP3), and furfural from the filtrate after 160 and 200°C hot water pretreatments

	SC13C20 wood	S7C15 wood	SC13C20 bark	S7C15 bark
160°C				
Xylose (%)	n.d.	n.d.	n.d.	n.d.
Glucose (%)	0.78 ± 0.48	0.22 ± 0.10	2.62 ± 1.23	0.58 ± 0.24
DP3 (mg/g)	0.07 ± 0.02	0.04 ± 0.07	0.23 ± 0.01	0.13 ± 0.02
Furfural mg/ml	n.d.	n.d.	n.d.	n.d.
200°C				
Xylose (%)	0	0	0	0
Glucose (%)	0.40 ± 0.17	0.79 ± 0.03	1.77 ± 1.29	0.61 ± 0.41
DP3 (mg/g)	0.05 ± 0.07	0.00 ± 0.00	0.26 ± 0.14	0.10 ± 0.03
Furfural mg/ml	n.d.	n.d.	n.d.	n.d.

n.d.: Area of sample was below the detection limit

± indicates the standard deviation of the sample

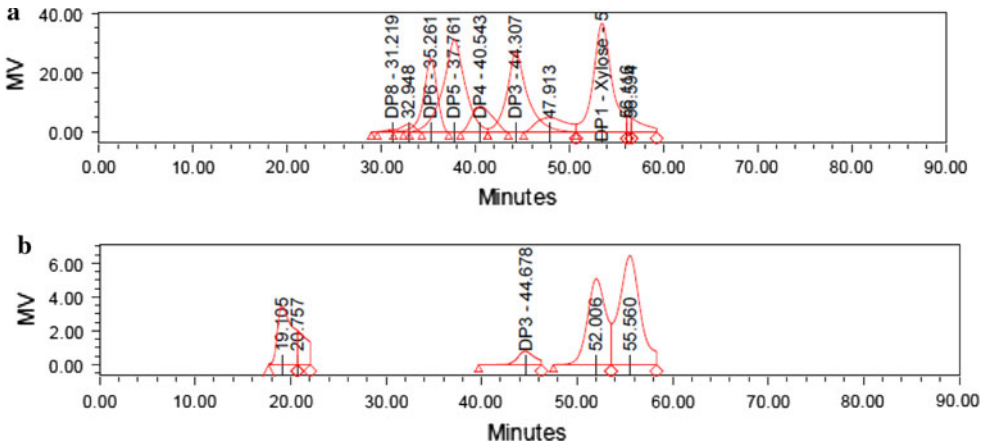

Fig. 1 **a** Chromatogram of oligomer standards, prepared at 1 mg/ml. **b** Chromatogram of HPLC run for oligomer detection of *Populus deltoides* higher specific gravity wood (clone S13C20) pretreated with hot water at 200°C for 20 min in a fluidized sand bath. Presence of DP3 was noted

S13C20 pretreated in water at 200°C for 40 min. Xylotriose (DP3) was detected in the water-pretreated chromatogram at the retention time of 44 min. The HPLC system used for this oligomer analysis was not set up to detect oligomers with degrees of polymerization higher than ten units, so it is possible that the 200°C pretreatment yielded oligomers that were not detected during the analysis.

Figure 2a presents a chromatogram of the furfural standard, while Fig. 2b shows a furfural analysis chromatogram that was obtained from clone S13C20 pretreated in water at 200°C for 40 min. Only negligible amounts of furfural were detected (below 0.002 AU) as shown in Fig. 2b, which were below the integration levels. Table 1 presents results pertaining to the glucose concentrations of water-pretreated clones S13C20 and S7C15; between 0.22 and 0.79% of the glucose present in higher and lower specific gravity wood was recovered with 160°C for 60 min and 200°C for 20 min pretreatments, respectively. Higher glucose recovery was also calculated for the bark of S13C20 clone.

Dilute acid pretreatment

In addition to water pretreatments, 1% dilute acid pretreatments at temperatures ranging from 140°C for 100 min to 200°C for 20 min were performed for bark. Because bark accounts for a significant amount of tree material [10], its ensuing saccharification potential must be determined. Figure 3 presents the xylose recovery from the bark of clones S13C20 and S7C15 during acid pretreatment. The highest xylose recovery, 31% (with an average recovery of 29%), was obtained with the lower specific gravity clone, S7C15, pretreated at 160°C for 60 min, while the other pretreatment conditions enabled recoveries ranging between 10 and 28%. Using a stirred Parr reactor, Torget et al. [10] also reported that dilute acid pretreatments at 160°C gave the highest xylose recovery of 76% for poplar hybrid NE 388. The difference in reactor configuration between the latter work and this work likely accounts for differences in calculated xylose recoveries, but, nonetheless, shows that bark is a material that can yield a significant fraction of its xylose content.

For dilute acid pretreatment of wood, Fig. 4 presents the xylose recovery from the wood of *P. deltoides* clones S13C20 and S7C15 At the conditions tested, the 140°C pretreatment of wood from the lower specific gravity clone S7C15 yielded the highest average xylose recovery of 56%, and increasing the pretreatment temperature reduced the xylose recovery. These results are somewhat different than

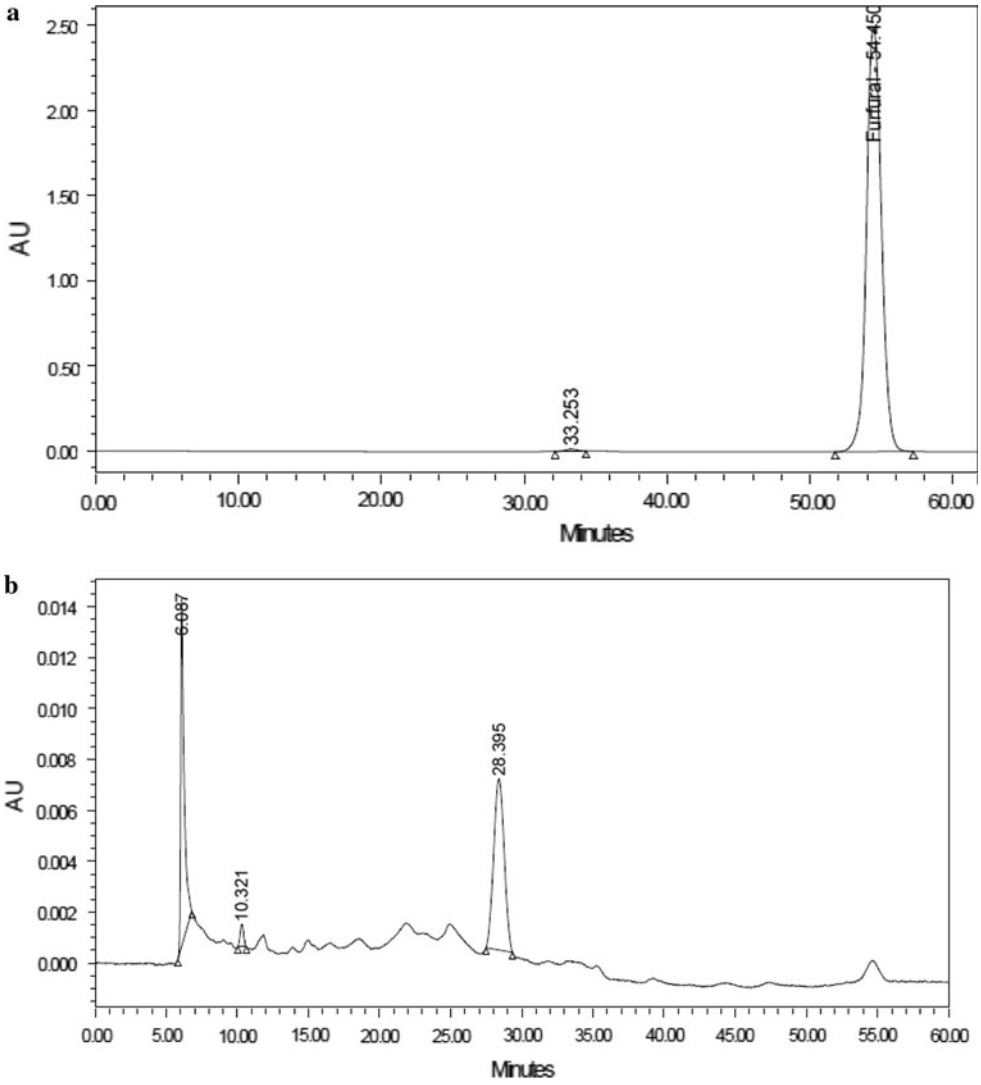

Fig. 2 **a** Furfural standard at 1 mg/ml analyzed by UV with a retention time of 54.45 min. **b** Chromatogram of HPLC run of *Populus deltoides* higher specific gravity wood (clone S13C20) pretreated with hot water at 200°C for 40 min in a fluidized sand bath. Furfural peak was detected at 54.45 min for this sample, but was too small to be integrated

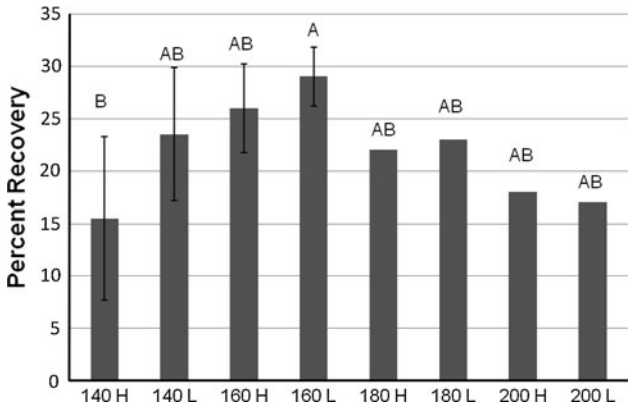

Fig. 3 Percent xylose recovery from bark of *P. deltoides* clones S13C20 (higher specific gravity) and S7C15 (lower specific gravity) pretreated with 1% sulfuric acid. X-axis refers to temperatures (°C) of experiments and density of woods (*H* higher specific gravity bark; *L* lower specific gravity bark). Analysis from JMP 8.02 program, LSMeans Differences Tukey HSD, where $\alpha = 0.050$, $Q = 3.48153$. Levels not connected by the *same letter* are significantly different

what was previously reported for poplar pretreated in a stirred reactor [1], where xylose recovery increased with increasing acid concentration and pretreatment temperature. These differences may be due to the differences in reactor configuration and length of reaction. The severity parameters for the 140 to 200°C wood pretreatments, calculated as described [6], were: 2.18, 2.54, 2.96, and 3.55, respectively, for the 140°C for 100 min, 160°C for 60 min, 180°C for 40 min, and 200°C for 40 min pretreatments, respectively. The calculated severity parameters in this work are higher than what was reported for corn stover [6]. With respect to the severity parameter/xylose recovery relationship, Kabel et al. [3] showed that the xylose recovery from pretreated wheat straw exudates leveled off as the severity of the pretreatment increased. Interestingly, results presented by Jung et al. [2] show that hemicellulose liberation from poplar cell wall when pretreated with dilute acid is a complicated process, where the resulting xylan in the cell wall is inversely proportional to the pretreatment severity.

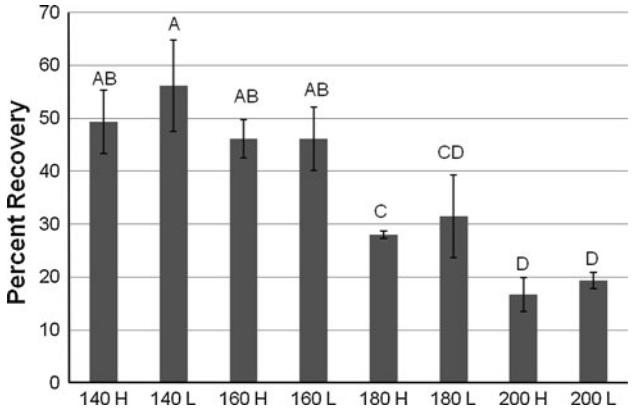

Fig. 4 Percent xylose recovery from wood of *P. deltoides* clones S13C20 (higher specific gravity) and S7C15 (lower specific gravity) pretreated with 1% sulfuric acid. X-axis refers to temperatures (°C) of experiments and density of woods (*H* higher specific gravity wood; *L* lower specific gravity wood). Analysis from JMP 8.02 program, LSMeans Differences Tukey HSD, where $\alpha = 0.050$, $Q = 3.20547$. Levels not connected by the *same letter* are significantly different

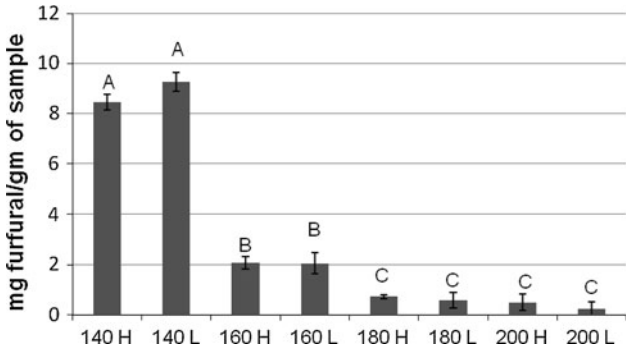

Fig. 5 Recovery of furfural (mg/g of sample) from *P. deltoides* clones S13C20 (higher specific gravity) and S7C15 (lower specific gravity) pretreated with 1% sulfuric acid. X-axis refers to temperatures (°C) of experiments and density of woods (*H* higher specific gravity bark; *L* lower specific gravity bark). Analysis from JMP 8.02 program, LSMeans Differences Tukey HSD, where $\alpha = 0.050$, $Q = 3.57019$. Levels not connected by the *same letter* are significantly different

The results presented in this work show that pretreatment temperatures of 180°C and higher for 40 min are too severe for the poplar feedstock. On the other hand, the results presented in Fig. 4 show that the 140°C pretreatment of the S7C15 clone yielded a higher xylose recovery but was not significantly different from clone S13C20 at 140°C; the higher xylose recovery was most likely due to the less dense and lignified cell walls being more amandable to releasing xylose. Analysis of the 140 and 200°C dilute acid pretreatment exudates for the presence of oligomers showed, as expected, that only xylose was present in the liquor.

Figure 5 presents furfural production from clones S13C20 and S7C15 wood as a function of pretreatment. Of the conditions tested, the 140°C acid pretreatment resulted in the highest furfural yields for both higher and lower specific gravity clones. Kabel et al. [3] reported furfural concentrations as a function of increased severity and showed that furfural formation was proportional to pretreatment severity. The results presented in this work are reported in terms of mg furfural per g of sample, but when combined with those of Lu et al. [7], where furfural concentrations greater than 10 g/l inhibited the fermentation, processing conditions that minimize furfural formation from poplar pretreatment could be sought.

In conclusion, results from this study showed that the best pretreatment condition yielding the highest monomeric xylose recoveries from the *P. deltoides* wood was 140°C for 100 min, using a 1% sulfuric acid pretreatment; a higher xylose recovery was obtained with the lower specific gravity clone wood, S7C15. The lower specific gravity clone was cultivated under dry land conditions, which did not have a continuously maintained water supply. This positive attribute of periodically dry land grown wood could become important if *P. deltoides* becomes a key contributor to the feedstock supply chain. The results presented in this study also indicate that the bark component is amendable to pretreatment and can be a source of carbohydrates. Thus, most likely, bark does not need to be removed before processing. It is important to note that this study focused solely on the pretreatment step. It is possible that the pretreatment conditions developed in this work, once integrated with the hydrolysis step, will not be conducive to maximum monomer xylose and glucose recoveries. If such is the case, pretreatment process parameters will need to be revised. This is currently under investigation and will be reported in future work.

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