The Addition of Water to Extract Maximum Levoglucosan from the Bio-oil Produced via Fast Pyrolysis of Pretreated Loblolly Pinewood

Qi Li,[a] Philip H. Steele,[a,*] Brian Mitchell,[a] Leonard L. Ingram,[a] and Fei Yu [b]

Levoglucosan is one of the major polar compounds that can be initially extracted into an aqueous fraction with water as the solvent. Levoglucosan can be hydrolyzed by an acid catalysis into monomeric sugars (primarily glucose), which can be further converted biochemically into alcohols or lipids, or converted catalytically into hydrogen. It has been demonstrated that the levoglucosan yield can be greatly increased if the proper pretreatment is applied to demineralize the feedstock prior to pyrolysis. In this study, bio-oil with a high levoglucosan concentration was produced via the fast pyrolysis of a dilute acid pretreated loblolly pine wood in an auger reactor. The water-to-bio-oil ratio, temperature, and time were selected as the three parameters to investigate the optimal condition for extracting the maximum amount of levoglucosan from the bio-oil. A response surface design (Box Behnken Design) was utilized to determine the direct and interactive effects of the three parameters on the extraction yield of the levoglucosan from the bio-oil. The optimal condition for the levoglucosan extraction was found to be 1.3:1 (water-to-bio-oil ratio), 25 ^{o}C, and 20 min, with a levoglucosan yield of 12.7 wt%.

Keywords: Levoglucosan; Anhydrosugars; Bio-oil; Biomass pretreatment; Pyroligneous fraction; Aqueous fraction

Contact information: a: Department of Forest Products, College of Forest Resources, Mississippi State University, Box 9820, Mississippi State, MS 39762 USA; b: Department of Agricultural and Biological Engineering, Mississippi State University, Box 9632, Mississippi State, MS 39762 USA.
** Corresponding author: psteele@cfr.msstate.edu*

INTRODUCTION

Levoglucosan (1, 6-anhydro-β-D-glucopyranose) is a major degradation product of cellulose during fast pyrolysis (Scott *et al.* 1995). Previous research has shown that the levoglucosan yield can be greatly increased if a mild acid pretreatment is applied to demineralize the feedstock prior to the pyrolysis (Dobele *et al.* 2003; Mourant *et al.* 2011; Patwardhan *et al.* 2010; Scott *et al.* 2001). The interest in levoglucosan production stems from the fact that it provides a route to the production of monomeric sugars, primarily glucose, which can be utilized to produce biochemically derived fuels (ethanol, butanol, lipids, *etc.*) (Bennett *et al.* 2009; Lian *et al.* 2010; Yu and Zhang 2003), or the sugars can be converted into hydrogen (Fermoso *et al.* 2012; Quéméneur *et al.* 2012; Sigurbjornsdottir and Orlygsson 2012).

So and Brown (1999) performed an economic analysis of three lignocellulose-to-ethanol conversion technologies. They were fast pyrolysis integrated with a fermentation step, simultaneous saccharification and fermentation (SSF), and dilute sulfuric acid hydrolysis and fermentation. An estimation of $1.57, $1.28, and $1.35 per gallon was

reported for the ethanol production cost for fast pyrolysis, SSF, and acid hydrolysis technologies, respectively. An estimated ethanol production yield of 360 liters per ton of feedstock was also reported for the fast pyrolysis route. It was proposed that the fast pyrolysis integrated with a fermentation step was economically comparable and had the potential for further development.

Bio-oils are dark brown, free-flowing liquids with water-emulsive suspensions of more than 100 organic compounds (Bridgwater *et al.* 1999; Czernik and Bridgwater 2004; Mohan *et al.* 2006; Peacocke and Bridgwater 1994; Peacocke *et al.* 1994). Bio-oils can be easily separated into two phases (Chen *et al.* 2011; Song *et al.* 2009). Water extraction is the first important step in order to isolate polar water-soluble organic compounds from the bio-oil into the aqueous fraction (Vitasari *et al.* 2011). Levoglucosan is one of the major polar compounds that can be initially extracted into the aqueous fraction with water as the solvent (Chan and Duff 2010). Vitasari *et al.* (2011) concluded that the optimal condition for extracting forest residue-derived bio-oil was at a water-to-bio-oil ratio of 0.65 to 0.7, while the optimal condition for extracting pine-derived bio-oil was at a water-to-bio-oil ratio of 0.5. However, the levoglucosan concentrations in both of the bio-oils analyzed in that study were very low, with 1.7 wt% for forest residue-derived bio-oil and 1.6 wt% for the pine-derived pyrolysis oil.

Previous research showed that the levoglucosan yield can be greatly elevated by applying the proper pretreatment to lignocellulosic materials prior to fast pyrolysis, *e.g.*, hot water or a mild acid pretreatment of the cellulose or lignocellulosic biomass (Dobele *et al.* 2003). Demineralization by washing the biomass with distilled water has also been demonstrated as effective for increasing the levoglucosan yield (Dobele *et al.* 2003; Johnson *et al.* 2009). Scott *et al.* (2001) performed a preliminary study on the rate of cation removal in poplar wood by ion exchange using a very dilute acid treatment. It was found that the majority of the alkaline cations were removed from the poplar wood via an ion exchange process using 0.1 wt% of nitric acid solution. The yield of the levoglucosan during fast pyrolysis increased from 3 wt% for untreated poplar wood to 17.1 wt% for demineralized poplar wood.

Bennett *et al.* (2009) examined the potential to produce levoglucosan from bio-oil without biomass demineralization or hydrolysis to remove hemicellulose. Rather, the water-to-raw-bio-oil ratio was varied to determine an optimum level for the water fractionation method to produce the highest concentration of levoglucosan in the bio-oil aqueous fraction. For the optimum water addition treatment of 41 wt%, at 34 ^{o}C, with an extraction time of 22 minutes, the yield of the levoglucosan was 7.8% of the total raw bio-oil weight, and the levoglucosan concentration was 87 g L^{-1} in the resultant aqueous fraction. This aqueous fraction was then hydrolyzed to glucose at various acid concentrations, hydrolysis temperatures, and reaction times. The maximum glucose yield was obtained for a reaction time of 44 minutes at 124 ^{o}C, where a 0.5 M concentration of sulfuric acid was used for hydrolysis. Based on the original levoglucosan weight, the yield of the glucose was 216% following the hydrolysis step. A concentration of 16 g L^{-1} of glucose was obtained in that study. While this previous study focused on the optimum water addition for bio-oil fractionation to produce highest concentration of levoglucosan, the results did not provide information on the optimum water addition to obtain maximum levoglucosan yields as opposed to higher concentrations that may represent lower yields. Further, the study was based on the analysis of bio-oil produced from a feedstock not subjected to a method that would increase the levoglucosan yield such as a mild acid pretreatment.

Currently, there is no information available on the optimal method to extract the highest yields of levoglucosan into the aqueous fraction following the application of a levoglucosan maximization treatment. We hypothesized that a higher water-to-bio-oil ratio may be required to extract bio-oils with higher levoglucosan concentrations because a higher water-to-bio-oil ratio may promote the mass transfer of levoglucosan from the bio-oils into the aqueous fractions. The objective of this research was to investigate the optimal conditions to extract the maximum amount of the levoglucosan from the bio-oil, produced from fast pyrolysis of pretreated loblolly pine wood, into the aqueous phase via the water addition method.

EXPERIMENTAL

Materials

The pyrolysis feedstock utilized in this study was 10-year-old loblolly pinewood randomly selected from a plantation at the Starr Memorial Forest of the Forest and Wildlife Research Center, Mississippi State University, Starkville, MS. The tree stems were first debarked, and the clear wood was then chipped in a wood chipper (Model 39, Carthage Machine Inc.) into paper chip size (20 to 35 mm). The wood chips were then ground in a Bauer mill (Model 5K4324A21, Bauer Brothers Co.) and sieved in a screener (Model Type S #1354, Universal Vibrating Screen) to a particle size of 0.6 to 3 mm. The ground pinewood particles were air-dried for one to two weeks to 8 to 10 wt% moisture content. The air-dried wood samples were stored in sealed plastic containers for the subsequent pretreatment and pyrolysis experiments.

Methods

Biomass pretreatment

Pinewood particles were pretreated with a 0.1 wt% phosphoric acid solution. The same pretreatment method has been described previously (Li *et al.* 2013). The pinewood samples were immersed and heated in an aqueous solution of 0.1 wt% phosphoric acid (sample/solution weight ratio = 1: 10) to 100 ^{o}C for 1 h in a water bath (Model 2385, Thermo Fisher Scientific Inc., Hanover Park, IL). Following the phosphoric acid pretreatment, the pinewood samples were washed with distilled water in a 0.43 m (length) × 0.25 m (width) × 0.20 m (height) rectangular stainless steel basket with 0.53 mm mesh openings (30 mesh × 0.3 mm woven) until reaching a neutral pH; then they were dried to a moisture content below 3 wt%. After drying, the pretreated feedstock was stored in sealed plastic containers for the pyrolysis experiment. The chemical and elemental composition of the untreated and pretreated pinewood was reported by Li *et al.* (2013).

Fast pyrolysis in an auger reactor

A 7 kg h^{-1} auger-fed proprietary pyrolysis reactor, described previously (Li *et al.* 2013), produced the required bio-oil. The pyrolysis of the 3 wt% moisture content pretreated pinewood occurred in a reactor tube at 450 ^{o}C, and the pyrolysis vapor moved through a condenser train where it was condensed to bio-oil. Nitrogen was supplied continuously, at a flow rate of 0.2 standard cubic feet per min (scfm), to the reactor heated zone as an inert gas to exclude oxygen from the pyrolysis reactor. The temperature of the initially formed vapor was about 30 ^{o}C below the set pyrolysis temperature (450 ^{o}C). The pyrolysis vapor exited the heated reactor tube into a water-cooled first

condenser, where its temperature dropped to about 75 to 80 °C. A second condenser lowered the temperature to the range 25 to 35 °C, and aerosol (fog-like) liquid/vapor continued into the third and the fourth condensers, which both maintained the temperature within the range 25 to 35 °C.

The non-condensable gases formed during the pyrolysis were collected at the exit of the last condenser by using a gas sampler (GAV-200 MK 2 gas sampler kit, SGE Analytical Science) and were analyzed by utilizing a Varian CP-4900 Micro Gas Chromatograph coupled with a Thermal Conductivity Detector (TCD). The detailed gas analysis procedures applied are available in a recent publication (Li *et al.* 2013). Liquid condensate was collected from all four of the condensers and was combined to be analyzed as the bio-oil sample. Char was collected into a sealed vessel at the exit of the reactor tube and weighed. The non-condensable gases passed through a Ritter gas flow meter to measure the volume and flow rate. All of these measurements enabled the calculation of the pyrolysis yield and a relatively good mass balance closure of 91.8 % as shown in Table 1. A total run time of 30 min was employed to ensure a steady status of the reactor and, therefore, an accurate mass balance calculation, and also to obtain sufficient bio-oil for the various study analyses.

Table 1. Mass Balance Closure for Fast Pyrolysis of Pretreated Pinewood

Parameters	Pretreated Pinewood
Pyrolysis Temperature (°C)	450
Feedstock Moisture Content (mf wt%)	3.0
Yields (mf wt%)	
Bio-oil	58.6
Char	21.1
Gas	12.1
Closure (%)	91.8

Bio-oil characterization

The bio-oil water content was measured by Karl Fisher titration according to the ASTM standard E203 with an HI 903 Karl Fischer Volumetric Titrator (Hanna Instruments). The bio-oil kinematic viscosity at 40 °C was measured according to the ASTM standard D445. The bio-oil pH value was measured with a pH bench-top meter (Orion 4 STAR, Thermo Fisher Scientific Inc.). The total acid number (TAN) was determined by titrating 1 g of the bio-oil sample (dissolved in 35/65 vol/vol isopropanol and water mixture) with 0.05 N NaOH to a pH of 8.5 as the end point. The higher heating value (HHV) was determined using a Parr 6200 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Carbon, hydrogen, nitrogen content, and oxygen (by subtraction) were measured with a CE-440 Elemental Analyzer (Exeter Analytical, North Chelmsford, MA) (Li *et al.* 2013). The bio-oil characteristics resulting from the various analyses are given in Table 2.

Effects of water-to-bio-oil ratio on the bio-oil fractionation components

The interest of this study was to investigate the optimal water addition to extract a maximum amount of levoglucosan from the bio-oil into the aqueous phase. Preliminary experiments were performed by adding different ratios of water to the bio-oil to

accomplish fractionation of the bio-oil into pyroligneous and aqueous fractions. The water-to-bio-oil weight ratios investigated are listed in Table 3, with the water-addition ratios ranging from 0.25 to 4.50.

Table 2. Characteristics of the Bio-oil Produced from Fast Pyrolysis of Dilute Acid Pretreated Loblolly Pine Wood

Bio-oil Characteristics	Value
Water Content, wt%	24.16
Viscosity @ 40 °C, cSt	16.93
Density, g mL^{-1}	1.22
pH	2.32
Total Acid Number (TAN), mg KOH g^{-1}	85.26
Higher Heating Value (HHV), MJ kg^{-1}	16.89
Carbon, wt%	41.10
Hydrogen, wt%	7.56
Nitrogen, wt%	0.10
Oxygen*, wt%	51.24
*Oxygen content was calculated as remaining by subtraction.	

Bio-oil samples of 10 grams were weighed into 11 labeled 125 mL Erlenmeyer flasks. The weights were as given in Table 3 with some variance of 10 grams due to measurement difficulties caused by the bio-oil viscosity in which one drop, more or less, resulted in a departure from the 10 gram target weight. Different amounts of distilled water were added into the Erlenmeyer flasks to create the water-to-bio-oil ratios as listed in Table 3. The Erlenmeyer flasks were placed in a water bath shaker (Reciprocal Shaking Bath Model 50, Precision) to mix the water and bio-oil for 20 min. The temperature was set at 25 ^{o}C, and the shaking speed was 120 rounds per minute. Upon extraction, the mixture was immediately transferred into 50 mL disposable centrifuge tubes and centrifuged for 4 hours at 4000 g to separate the aqueous and pyroligneous fractions (AccuSpin 3R, Fisher Scientific). After the separation, the aqueous fraction was decanted, and the weight and volume were measured and recorded.

Table 3. Water to Bio-oil Weight Ratios Applied for Investigation

Sample No.	Bio-oil (g)	Added Water (g)	Ratio (water to bio-oil)	Total Water (expressed as wt% of the original bio-oil)*
1	10.00	2.52	0.25	49.36
2	10.00	4.99	0.50	74.05
3	10.00	7.53	0.75	99.43
4	10.02	9.99	1.00	123.82
5	9.97	12.57	1.25	150.21
6	10.01	15.01	1.50	174.20
7	10.00	17.89	1.75	203.11
8	10.00	20.87	2.00	232.88
9	10.01	25.07	2.50	274.50
10	10.11	35.54	3.50	375.56
11	10.03	45.00	4.50	473.04
* The original water content of the study bio-oil was 24.16 wt%.				

The concentration of the levoglucosan in the aqueous fraction was analyzed with an Agilent 1200 Series High Performance Liquid Chromatography (HPLC, Agilent) with a Bioradd Aminex HPX-89P column equipped with a guard column and a refractive index detector (RID). The column and detector were operated at 80 ^{o}C and 40 ^{o}C, respectively. The flow rate of the mobile phase (HPLC grade water, 0.2 µm filtered and degassed) was held constant at 0.6 mL per minute and the injection volume was 10 µL. Calibration curves were developed based on a levoglucosan standard with concentrations ranging from 0.2 mg mL^{-1} to 10.0 mg mL^{-1}. The original water content of the study bio-oil was 24.16 wt%. Therefore, in Table 3 total water was calculated and expressed as a wt% of the original bio-oil.

Effects of temperature, time, and water-to-bio-oil ratio on the extraction yield of levoglucosan into the aqueous fraction

Based on the preliminary results in the previous section, two more factors (temperature and time) were included in the extraction experiments in an attempt to improve the extraction yield of the levoglucosan from the bio-oil. The Box Behnken Design (NIST/SEMATECH 2012) is a response surface design that was utilized to determine the direct and interactive effects of the three parameters (water-to-bio-oil ratio, temperature, and time) on the extraction yield of levoglucosan from the bio-oil. The parameter levels are listed in Table 4.

Table 4. Parameter Levels for the Box Behnken Design Experiments

Level (coded)	Ratio (water to bio-oil)	Temperature (°C)	Time (min)
Low (-1)	0.5	25	20
Middle (0)	1	45	40
High (1)	1.5	65	60

The experimental design was based on the following Box Behnken Design model, from which the relationship between the independent variables and the dependent response was calculated,

$$Y=\beta_0+\sum\beta_i X_i+\sum\beta_{ii}X_i^2+\sum\beta_{ij}X_iX_j \quad (1)$$

where Y is the predicted response (levoglucosan yield in wt% of bio-oil), β_0 is the intercept term, β_i is the linear effect, X_i and X_j are the independent variables (water-to-bio-oil ratio X_1, temperature X_2, and time X_3), β_{ii} is the squared effect, and β_{ij} is the interaction effect.

A total of 3^3=27 experiments were required if every combination of the parameters was used. The Box Behnken Design was applied to reduce the experimental size to 15 experiments, without losing the power of developing a valid predictive model by covering enough data points. The combination of parameters for the extraction experiments is listed in Table 5.

All of the experiments were performed in the same water bath shaker (Reciprocal Shaking Bath Model 50, Precision) as described earlier with a shaking speed of 120 rounds per minute. After the extraction, the mixture was centrifuged, and the aqueous fraction was decanted as described before. The yield of the levoglucosan in the aqueous fraction were determined via Agilent 1200 Series High Performance Liquid Chromatography (HPLC, Agilent) with the same method as described above.

Statistical Analysis

JMP IN (version 10.0) software (SAS Institute Inc., Cary, NC, USA) was applied for the analysis of the response-surface factorial designed experiment. Response surface design is a type of experimental design that allows finding the optimal condition within a specified range of parameters (Amouzgar *et al.* 2010; Wu *et al.* 2010). The Box Behnken Design is one type of the most widely used response surface designs (NIST/SEMATECH 2012). JMP IN software has built-in experimental design and data analysis functions for the Box Behnken Design.

RESULTS AND DISCUSSION

Effects of Water-to-Bio-oil Ratio on the Bio-oil Fractionation

As mentioned above, the interest of this study was to capture a maximum amount of levoglucosan present in the study of bio-oil into an aqueous fraction. Theoretically, a larger water-addition ratio is beneficial for the levoglucosan to transfer from the bio-oil to the aqueous fraction. The levoglucosan molecules are hydrophilic and tend to dissolve in water because of the hydroxyl groups in their molecular structure. However, this phenomenon has not been scientifically proven yet. This study will contribute to an understanding of the effects of the water-to-bio-oil ratio on the bio-oil fractionation components. The influence of the water-to-bio-oil ratio on the yield of aqueous and pyroligneous fractions is illustrated in Fig. 1.

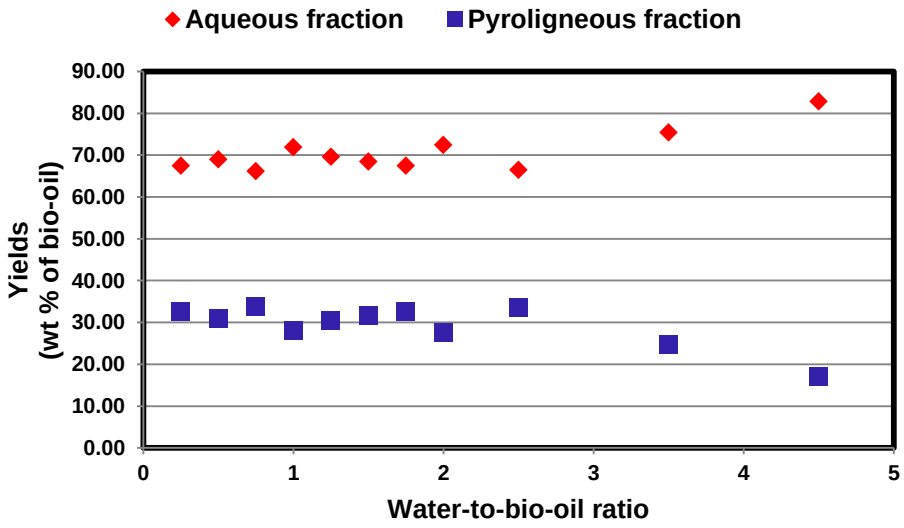

Fig. 1. Effect of the water-to-bio-oil ratio on the yields of aqueous and pyroligneous fractions

In this study, bio-oil phase separation occurred in all of the treatments tested, even at the lowest level of the water-addition ratio of 0.25 for a total water content of 49.4% (Table 3). This matches the reported range for bio-oil phase separation in previous work (Bennett *et al.* 2009; Chan and Duff 2010; Peacocke *et al.* 1994; Oasmaa and Peacocke 2001). Figure 1 shows that the total mass percentage transferred into the aqueous fraction based on the total bio-oil weight (the yield of aqueous fraction) increased at an increasing rate with an increase in the water-addition ratio after reaching a water-to-bio-oil ratio above 2.5. Correspondingly, Fig. 1 also shows that the total mass percentage left in the

pyroligneous fraction based on the total bio-oil weight (the yield of pyroligneous fraction) decreased at an increasing rate with increasing the water-addition ratio after reaching a water-to-bio-oil ratio of above 2.5. These phenomena agreed with the mass transfer equilibrium theory, in which more solvent is beneficial for reaching the maximum mass transfer. It is clear that a critical mass of water must be added to the bio-oil in order to capture more of the polar compounds in the aqueous fraction.

The bio-oil aqueous fraction mainly contains anhydrosugars, particularly levoglucosan, which is formed from the pyrolysis of cellulose. A certain amount of acetic acid, furfural, and some other organic compounds are also present in the bio-oil aqueous fraction (Bhattacharya *et al.* 2010; Chan and Duff 2010; Song *et al.* 2009; Vitasari *et al.* 2011). The bio-oil pyroligneous fraction is primarily composed of pyrolytic lignin, containing between 75% and 85% of phenolic compounds (Dobele *et al.* 2011; Song *et al.* 2009; Sukhbaatar *et al.* 2009). The original content of levoglucosan in the raw bio-oil is about 13 to 15 wt%. Previous literature reported a range of 10 to 20 wt% of levoglucosan in the bio-oils produced from various demineralized feedstocks.

Figure 2 shows the liquid chromatogram of a typical bio-oil aqueous fraction sample. Levoglucosan was eluted at around 31.8 min under the conditions provided in the Experimental section. Levoglucosan was detected and quantified via an HPLC device equipped with an FID detector.

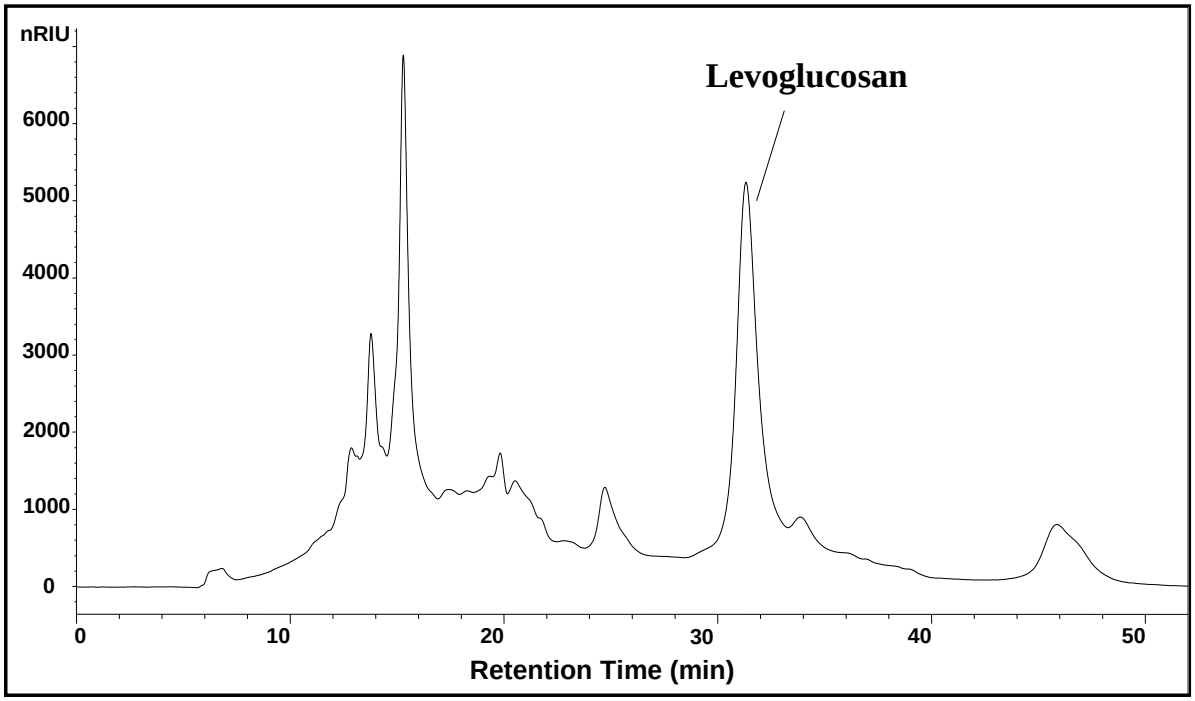

Fig. 2. Liquid chromatogram of a typical bio-oil aqueous fraction sample

Figure 3 shows the influence of the water-to-bio-oil ratio on the levoglucosan yield expressed in a wt% of the bio-oil. It is shown that the maximum levoglucosan yield was reached in the approximate range of 0.5 to 1.5 of the water-to-bio-oil ratio, a range in agreement with a previous finding (Chan and Duff 2010). At a water-to-bio-oil ratio higher than 2.5, the levoglucosan yield remained almost constant, although the total mass transferred into the aqueous fraction increased as shown in Fig. 1. This might be because,

instead of levoglucosan, other polar compounds are present in the study bio-oil such as organic acids, furans, and aldehydes, which started to transfer into the aqueous fraction with the increasing water-addition ratio. Therefore, the range of the water-to-bio-oil ratio of 0.5 to 1.5 was selected for the subsequent Box Behnken Design experiments to determine the optimal condition for the maximum levoglucosan yield in the aqueous fraction.

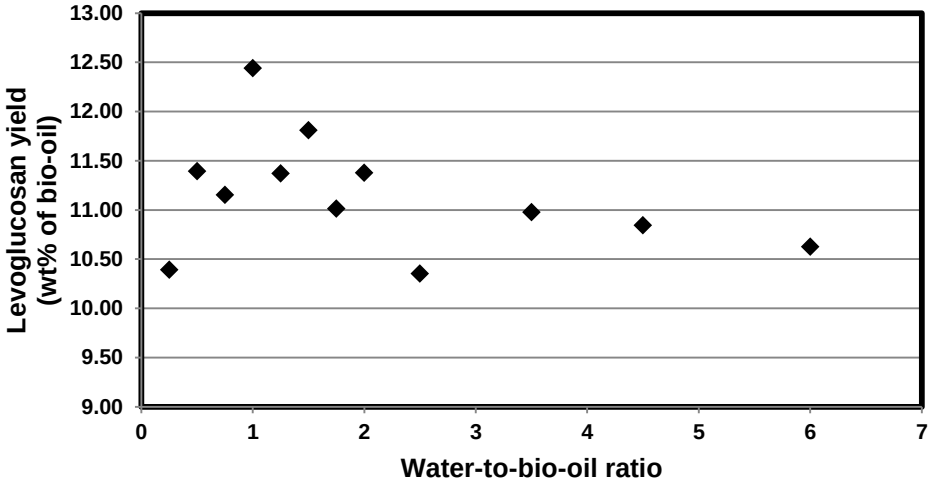

Fig. 3. Effect of the water-to-bio-oil ratio on the yield of levoglucosan

The Optimal Conditions for the Maximum Extraction Yield of Levoglucosan into the Aqueous Fraction

Results are shown in Table 5:

Table 5. Parameter Combinations and Actual Levoglucosan (LG) Yields (wt%) for the Box Behnken Design Extraction Experiments

Treatment No.	Temperature (^{o}C)	Time (min)	Ratio (water to bio-oil)	LG Yield (wt%)
1	45	20	1.5	9.86
2	25	60	1	10.62
3	45	40	1	11.43
4	65	40	0.5	10.19
5	45	40	1	11.94
6	25	20	1	12.44
7	45	20	0.5	9.86
8	25	40	0.5	9.29
9	65	20	1	11.86
10	45	60	0.5	9.88
11	45	60	1.5	10.68
12	65	60	1	11.33
13	65	40	1.5	10.08
14	45	40	1	11.25
15	25	40	1.5	11.09

Table 5 represents an attempt to further improve the extraction yield of the levoglucosan from the bio-oil based on the preliminary results. Two more factors (temperature and time) were included in the Box Behnken Design extraction experiments. The parameter combinations and the levoglucosan yields are listed in the table.

These 15 data points were input into the JMP IN software for a statistical analysis. The summary of the analysis of variance (ANOVA) for the model of the levoglucosan yield is shown in Table 6. The ANOVA resulted in a P value of 0.0318 (<0.05), showing that the model was significant at the 0.05 level. Moreover, the lack of fit F-value was 0.4313, which means that the lack of fit is not significant in relation to the pure error. Regression analysis showed that the coefficient of determination (R^2=0.95) was satisfactory to confirm the significance of the model as shown in Fig. 4.

Table 6. ANOVA of the Model for Levoglucosan Yield as a wt% in the Bio-oil

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Prob>F
Model	9	10.39	1.155	7.7748	0.0318
Error	4	0.594	0.1485	-	-
Total	13	10.98	-	-	-
Lack of Fit	2	0.3378	0.1689	1.3186	0.4313

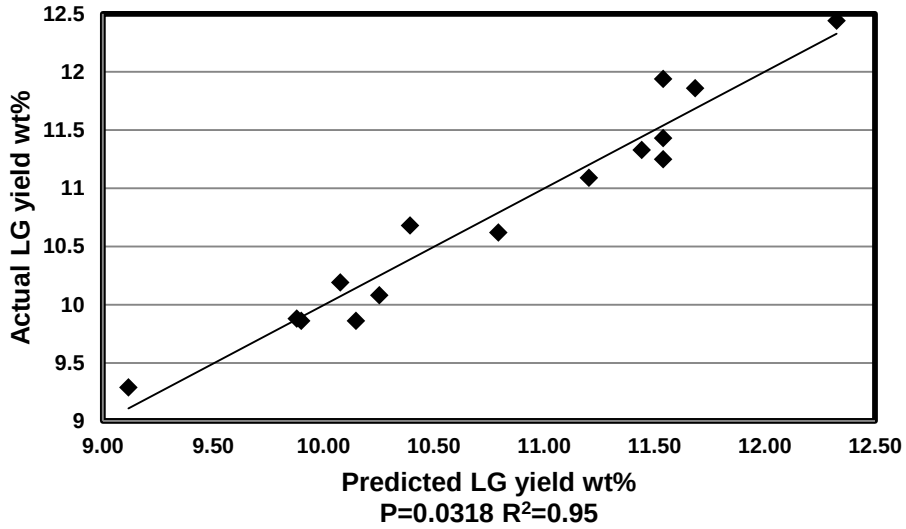

Fig. 4. The relationship between the actual and predicted levoglucosan yield extracted into the aqueous fraction as predicted by regression analysis

The mathematical regression model determined by the JMP IN software is shown as Eq. 2. Only terms determined as statistically significant (at p = 0.05) were included in the prediction equation for the levoglucosan yield,

$$Y=11.54 + 0.57X_1–1.18X_1^2 \quad (2)$$

where Y is the levoglucosan yield (wt% of bio-oil) and $X_1$is the water-to-bio-oil ratio.

Over the ranges examined for temperature (25 to 65 ^oC) and time (20 to 60 min), both temperature and time had no significant effects and no interaction effects on the levoglucosan yield. Therefore, these two parameters were not included in the prediction

equation. The equation estimated by the JMP IN software contains a negative X_1^2 term, indicating a curvilinear relationship that increases at a decreasing rate. Over the ranges tested in this study, the optimal condition for the levoglucosan extraction (solved by the JMP IN software) was 1.3:1 (water-to-bio-oil ratio), 25 ^oC, and 20 min, with a predicted levoglucosan yield of 12.7 wt%. The previous study by Bennett *et al.* (2009) directed to the determination of the water addition to maximize the levoglucosan concentration found that the optimal extraction condition was 41 wt% of water at 34 ^oC over a reaction time of 22 minutes. The results for the maximization levoglucosan yield determined in our current study differ considerably. The water addition level for the levoglucosan yield maximization was found to be 154.2 wt% compared to 41 wt% for the concentration maximization. Also, the extraction temperature was found to be insignificant, with 25 ^oC for the current yield maximization study compared to 34 ^oC for the concentration maximization. Finally, 20 minutes was found to be adequate for the levoglucosan yield maximization compared to 22 minutes for the levoglucosan concentration maximization. A recent publication (Wang *et al.* 2012) demonstrated the successful detoxification and fermentation of the sugar source from this current study to produce ethanol.

CONCLUSIONS

1. It was found that the yield of the aqueous fraction increased at an increasing rate with an increasing of the water-addition ratio. Correspondingly, the yield of the pyroligneous fraction decreased at an increasing rate with increasing the water-addition ratio. These phenomena agreed with the mass transfer equilibrium theory in which more solvent is beneficial for reaching the maximum mass transfer.
2. It was also found that the density of the aqueous fraction decreased at a decreasing rate with an increasing water-addition ratio. When the water-to-bio-oil ratio was higher than 2.5, the density of the obtained aqueous fraction was almost equal to the density of water (1.0 g mL^{-1}).
3. In an attempt to improve the extraction yield of the levoglucosan from the bio-oil, two more factors (temperature and time) were included in addition to the water-addition ratio in the Box Behnken Design extraction experiments. Over the ranges examined for temperature (25 to 65 ^oC) and time (20 to 60 min), both temperature and time had no significant effect on the levoglucosan yield.
4. A regression equation was estimated, and the optimal condition for the levoglucosan extraction (solved mathematically by JMP IN software) was 1.3:1 (water-to-bio-oil ratio), 25 ^oC, and 20 min, giving a predicted levoglucosan yield of 12.7 wt%.
5. The water addition to maximize the levoglucosan yield (154.2 wt%) is more than three times higher than the 41 wt% determined in previous research to maximize the levoglucosan concentration.

ACKNOWLEDGMENTS

This research is based upon work performed through the Sustainable Energy Research Center at Mississippi State University and is supported by the Department of Energy under Award Number DE-FG3606GO86025.

REFERENCES CITED

Amouzgar, P., Abdul Khalil, H. P. S., Salamatinia, B., Zuhairi Abdullah, A., and Issam, A. M. (2010). "Optimization of bioresource material from oil palm trunk core drying using microwave radiation; A response surface methodology application," *Bioresource Technology* 101(21), 8396-8401.

Bennett, N. M., Helle, S. S., and Duff, S. J. B. (2009). "Extraction and hydrolysis of levoglucosan from pyrolysis oil," *Bioresource Technology* 100(23), 6059-6063.

Bhattacharya, P., Hassan, E. B., Steele, P., Cooper, J., and Ingram, L. (2010). "Effect of acid catalysts and accelerated aging on the reaction of methanol with hydroxyacetaldehyde in bio-oil," *BioResources* 5(2), 908-919.

Bridgwater, A. V., Czernik, S., and Diebold, J. (1999). *Fast Pyrolysis of Biomass: A Handbook*. Newbury, UK: CPL Scientific Publishing Services, Ltd.

Chan, J. K. S., and Duff, S. J. B. (2010). "Methods for mitigation of bio-oil extract toxicity," *Bioresource Technology* 101(10), 3755-3759.

Chen, H. W., Song, Q. H., Liao, B., and Guo, Q. X. (2011). "Further separation, characterization, and upgrading for upper and bottom layers from phase separation of biomass pyrolysis oils," *Energy and Fuels* 25(10), 4655-4661.

Czernik, S., and Bridgwater, A. V. (2004). "Overview of applications of biomass fast pyrolysis oil," *Energy and Fuels* 18(2), 590-598.

Dobele, G., Dizhbite, T., Ponomarenko, J., Urbanovich, I., Kreicberga, J., and Kampars, V. (2011). "Isolation and characterization of the phenolic fractions of wood pyrolytic oil," *Holzforschung* 65(4), 503-510.

Dobele, G., Dizhbite, T., Rossinskaja, G., Telysheva, G., Meier, D., Radtke, S., and Faix, O. (2003). "Pre-treatment of biomass with phosphoric acid prior to fast pyrolysis: A promising method for obtaining 1,6-anhydrosaccharides in high yields," *Journal of Analytical and Applied Pyrolysis* 68–69, 197-211.

Fermoso, J., He, L., and Chen, D. (2012). "Sorption enhanced steam reforming (SESR): A direct route towards efficient hydrogen production from biomass-derived compounds," *Journal of Chemical Technology and Biotechnology* 87, 1367-1374.

Johnson, R. L., Liaw, S. S., Garcia-Perez, M., Ha, S., Lin, S. S. Y., McDonald, A. G., and Chen, S. (2009). "Pyrolysis gas chromatography mass spectrometry studies to evaluate high-temperature aqueous pretreatment as a way to modify the composition of bio-oil from fast pyrolysis of wheat straw," *Energy and Fuels* 23(12), 6242-6252.

Li, Q., Steele, P. H., Yu, F., Mitchell, B., and Hassan, E.-B. M. (2013). "Pyrolytic spray increases levoglucosan production during fast pyrolysis," *Journal of Analytical and Applied Pyrolysis* 100, 33-40.

Lian, J., Chen, S., Zhou, S., Wang, Z., O'Fallon, J., Li, C. Z., and Garcia-Perez, M. (2010). "Separation, hydrolysis and fermentation of pyrolytic sugars to produce ethanol and lipids," *Bioresource Technology* 101(24), 9688-9699.

Mohan, D., Pittman Jr, C. U., and Steele, P. H. (2006). "Pyrolysis of wood/biomass for

bio-oil: A critical review," *Energy and Fuels* 20(3), 848-889.
Mourant, D., Wang, Z., He, M., Wang, X. S., Garcia-Perez, M., Ling, K., and Li, C. Z. (2011). "Mallee wood fast pyrolysis: Effects of alkali and alkaline earth metallic species on the yield and composition of bio-oil," *Fuel* 90(9), 2915-2922.
NIST/SEMATECH. (2012). NIST/SEMATECH e-Handbook of Statistical Methods, http://www.itl.nist.gov/div898/handbook/.
Oasmaa, A., and Peacocke, C. (2001). "A guide to physical property characterisation of biomass-derived fast pyrolysis liquids," Espoo 2001. Technical Research Centre of Finland, VTT Publications 450. 65 p. + app. 34 p.
Patwardhan, P. R., Satrio, J. A., Brown, R. C., and Shanks, B. H. (2010). "Influence of inorganic salts on the primary pyrolysis products of cellulose," *Bioresource Technology* 101(12), 4646-4655.
Peacocke, G. V. C., and Bridgwater, A. V. (1994). "Ablative plate pyrolysis of biomass for liquids," *Biomass and Bioenergy* 7, 147-154.
Peacocke, G. V. C., Russell, P. A., Jenkins, J. D., and Bridgwater, A. V. (1994). "Physical properties of flash pyrolysis liquids," *Biomass and Bioenergy 7*, 169-177.
Quéméneur, M., Bittel, M., Trably, E., Dumas, C., Fourage, L., Ravot, G., Steyer, J. P., and Carrère, H. (2012). "Effect of enzyme addition on fermentative hydrogen production from wheat straw," *Intl. J. Hydrogen Energy* 37(14), 10639-10647.
Scott, D. S., Paterson, L., Piskorz, J., and Radlein, D. (2001). "Pretreatment of poplar wood for fast pyrolysis: Rate of cation removal," *Journal of Analytical and Applied Pyrolysis* 57(2), 169-176.
Scott, D. S., Piskorz, J., Radlein, D., and Majerski, P. (1995). 5395455. U. S. Patent.
Sigurbjornsdottir, M. A., and Orlygsson, J. (2012). "Combined hydrogen and ethanol production from sugars and lignocellulosic biomass by Thermoanaerobacterium AK 54, isolated from hot spring," *Applied Energy* 97, 785-791.
So, K., and Brown, R. (1999). "Economic analysis of selected lignocellulose-to-ethanol conversion technologies," *Applied Biochemistry and Biotechnology* 79(1), 633-640.
Song, Q. H., Nie, J. Q., Ren, M. G., and Guo, Q. X. (2009). "Effective phase separation of biomass pyrolysis oils by adding aqueous salt solutions," *Energy and Fuels* 23(6), 3307-3312.
Sukhbaatar, B., Steele, P. H., and Kim, M. G. (2009). "Use of lignin separated from bio-oil in oriented strand board binder phenol-formaldehyde resins," *BioResources* 4(2), 789-804.
Vitasari, C. R., Meindersma, G. W., and de Haan, A. B. (2011). "Water extraction of pyrolysis oil: The first step for the recovery of renewable chemicals," *Bioresource Technology* 102(14), 7204-7210.
Wang, H., Livingston, D., Srinivasan, R., Li, Q., Steele, P., and Yu, F. (2012). "Detoxification and fermentation of pyrolytic sugar for ethanol production," *Applied Biochemistry and Biotechnology* 168(6), 1568-1583.
Wu, J., Wang, J. L., Li, M. H., Lin, J. P., and Wei, D. Z. (2010). "Optimization of immobilization for selective oxidation of benzyl alcohol by *Gluconobacter oxydans* using response surface methodology," *Bioresource Technology* 101(23), 8936-8941.
Yu, Z., and Zhang, H. (2003). "Ethanol fermentation of acid-hydrolyzed cellulosic pyrolysate with *Saccharomyces cerevisiae*," *Bioresource Technology* 90(1), 95-100.

Article submitted: Dec. 19, 2012; Peer review completed: Jan. 25, 2013; Revised version received and accepted: Feb. 15, 2013; Published: February 21, 2013.