The Effect of Ethanol on Hydroxyl and Carbonyl Groups in Biopolyol Produced by Hydrothermal Liquefaction of Loblolly Pine: ^{31}P -NMR and ^{19}F -NMR Analysis

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Abstract

The goal of this study was to investigate the role of ethanol and temperature on the hydroxyl and carbonyl groups in biopolyol produced from hydrothermal liquefaction of loblolly pine (*Pinus spp*) carried out at 250, 300, 350 and 390 °C for 30 min. Water and water/ethanol mixture (1/1, wt/wt) were used as liquefying solvent in the HTL experiments. HTL in water and water/ethanol is denoted as W-HTL and W/E-HTL, respectively. It was found that 300 °C and water/ethanol solvent was the optimum liquefaction temperature and solvent, yielding up to 68.1 wt.% bio-oil and 2.4 wt.% solid residue. ³¹P-NMR analysis showed that biopolyol produced by W-HTL was rich in phenolic OH while W/E-HTL produced more aliphatic OH rich biopolyols. Moreover, biopolyols with higher hydroxyl concentration were produced by W/E-HTL. Carbonyl groups were analyzed by ¹⁹F-NMR, which showed that ethanol reduced the concentration of carbonyl groups.

Keywords: Biopolyol, Bio-oil, Hydrothermal liquefaction, ³¹P-NMR, ¹⁹F-NMR.

1. Introduction

Lignocellulosic biomass is being used as an alternative feedstock for the production of bio-based polyols (biopolyols) in order to reduce our dependency on petroleum. Polyol is a compound that has multiple hydroxyl groups in its structure and is essential for the synthesis of a variety of polymers such as epoxy, polyurethane, polyester, polycarbonate, and phenolic resin. However, today the major source for polyols is petroleum. Researchers have thus focused on biopolyol in the face of depleting petroleum reserves, increased greenhouse effect, as well as an increasing demand by the consumers for bio-based products. Moreover, the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA) have prioritized the development of bioenergy and bioproducts with the goal to produce 18% of the current U.S. chemical commodities from lignocellulosic biomass by 2020, and 25% by 2030 (Perlack et al. 2005).

Bio-oil, the black liquid produced by degradation of lignocellulosic biomass through thermomechanical processes, has attracted interest to be used as a biopolyol because of its high hydroxyl concentration. Gas chromatography–mass spectrometry (GC-MS) analysis of bio-oil showed that bio-oil is a highly complex mixture, mainly composed of carbohydrates, furans, phenols, guaiacols, syringols type of compounds (Thangalazhy-Gopakumar et al. 2010). Hydrothermal liquefaction (HTL), also known as direct liquefaction, is a thermomechanical conversion technique to produce bio-oil where lignocellulosic biomass is converted into liquid (bio-oil), gas and solid products using subcritical or supercritical water at elevated temperature (250 – 370 °C) and pressure (2 – 24 MPa). Bio-oil produced by the HTL process has a significant potential for commercialization in terms of price and life cycle assessment (Elliott et al. 2015). The effect of process parameters such as temperature, pressure, reaction time and feedstock type

on the bio-oil yield in HTL process has been intensively studied and valuable literature reviews can be found elsewhere (Akhtar and Amin 2011, Toor et al. 2011). Approximately 30 – 40 wt.% bio-oil (based on dry mass of biomass) is obtained from the hydrothermal liquefaction of lignocellulosic biomass (Akhtar and Amin 2011). Recently, ethanol has received attention to be used as a co-solvent in the HTL process due to its high hydrogen solvent capability. Yuan et al. (2007) proposed that the highly reactive free radicals generated from the HTL of biomass were stabilized by the ethanol which acts as a hydrogen-donor solvent, and Cheng et al. (2010) reported that bio-oil yield could be increased from 40 to 65 wt.% when ethanol was used along with water in a sub-critical condition. In another study, alkaline lignin was liquefied in hot compressed water/ethanol medium, and found that addition of ethanol increased the degradation of lignin which resulted in a lower amount of solid residue (Yuan et al. 2010). This improvement may be due to the low dielectric constant of ethanol which facilitates dissolving of high molecular weight lignin at supercritical temperatures (Krammer and Vogel 2000). The synergistic effect of water/ethanol mixture in the liquefaction of rice husk for bio-oil production via the HTL process was also observed by Liu et al. (2013). Besides the high bio-oil yield, the addition of ethanol to water also affects the distribution of phenolics such as phenol, ethylphenol and guaiacols, ethylguaiacol and syringol in the bio-oil as well (Ye et al. 2012, Ouyang et al. 2015). Recently, Kosinkova et al. (2015) reported that aqueous ethanol improved the higher heating value (HHV) of bio-oil to be used in the field of biodiesel applications. Moreover, the addition of medical stone as a catalyst into aqueous ethanol could further increase the yield of bio-oil produced by HTL of cotton seed (Yan et al. 2015).

Compounds containing hydroxyl and carbonyl groups in bio-oil play a major role in its utilization as a biopolyol. There are a variety of polymers synthesized using bio-oil including

phenolic resin (Choi et al. 2015), polyurethane (Hu and Li 2014), epoxy (Kuo et al. 2014) and polyester (Yu et al. 2006), and the hydroxyl groups in bio-oil are utilized to synthesize these polymers. Wei et al. (2014) used bio-oil to modify an epoxy resin, and found that hydroxyl number (OHN) of the bio-oil had a significant effect on the glass transition temperature (T_g) of the modified epoxy resin. In another study, bio-oil was used to synthesize a new adhesive system for application in particle boards. Here, the hydroxyl groups in bio-oil contributed to the condensation reactions with melamine-formaldehyde and melamine-urea-formaldehyde resin precursors which ultimately affected the properties of the resulting resin system (Kunaver et al. 2010). Zou et al. (2012) synthesized a polyurethane foam using bio-oil, and reported that a high number of primary hydroxyl groups from the bio-oil was necessary to increase the mechanical and thermal properties of polyurethane foam. Thus, it could be hypothesized that the reaction behavior and the mechanical/thermal properties of the resulting polymer depend on the interaction between hydroxyl groups in bio-oil and the formaldehyde for phenol formaldehyde resin, isocyanate for the polyurethane, epoxide groups for epoxy resin, and ester groups for polyester production.

Compounds containing carbonyl groups in the bio-oil such as aldehydes, ketones and quinones are found to play a major role in the aging of bio-oil during storage (Czernik et al. 1994). Reactions between compounds containing carbonyl and hydroxyl groups in the bio-oil have been reported to be a reason for bio-oil aging which results in the polymerization and consequently a change in viscosity (Czernik et al. 1994). The production of carboxylic acid as a result of oxidation of carbonyl groups is another problem which may cause equipment corrosion. Most recently, our group has studied the curing reaction of a commercial epoxy resin (EPON828) with a bio-oil, and found that carbonyl groups in the bio-oil contributed the curing of

epoxy resin along with the hydroxyl groups as well (Celikbag et al. 2015). Therefore, the characterization and quantification of hydroxyl and carbonyl groups in the bio-oil is crucial to determine its quality as well as optimization for bio-oil based polymer synthesis.

The literature on liquefaction of lignocellulosic biomass in water/ethanol mixture via HTL process lacks specific information regarding the source and variation of hydroxyl and carbonyl groups in the bio-oil. Characterization of these functional groups plays an important role in the improvement of bio-oil for its effective utilization as a biopolyol. Therefore, a comprehensive hydroxyl and carbonyl group analysis is necessary to understand the source and variation of bio-oil hydroxyl and carbonyl group content which will make it possible to engineer the properties of bio-based polymers and their synthesis in future studies. To the best of our knowledge, this is the first study that has used the ^{31}P -NMR and ^{19}F -NMR analytical tools to partition out the hydroxyl and carbonyl groups to understand the role of ethanol in the HTL process of lignocellulosic biomass. Thus, the objectives of this study were to (i) investigate the effect of ethanol and liquefaction temperature and on hydroxyl and carbonyl groups, and (ii) analyze the variation and source of these functional groups in the bio-oil using ^{31}P -NMR and ^{19}F -NMR.

2. Materials and Methods

2.1. Materials

Loblolly pine wood chips that are free from bark and leaf were obtained from a local chipping plant in Opelika, AL, USA. Loblolly pine composition (48.5% cellulose, 31.3% hemicellulose, 25.8% lignin and 4.5% extractives) was determined using standard wet chemistry analysis protocol. All chemicals were purchased from VWR; except the phosphorylating agent

for ^{31}P -NMR analysis, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP), purchased from Sigma Aldrich. All chemicals were reagent grade and used as received.

2.2. Hydrothermal Liquefaction (HTL) of Loblolly Pine

Loblolly pine was ground by a hammer mill (New Holland grinder model 358, New Holland, PA.) using 40 mesh sieve size for particle size reduction, and the pine passed through 40 mesh were used. HTL was carried out in 1 L high pressure/high temperature Parr® Reactor (Parr Instrument Company, Model 4577 HP/HT pressure reactor, Moline, IL., USA) equipped with mechanical stirrer and reactor controller (Parr Instrument Company, Model 4848, Moline, IL., USA). The reactor was charged with 25 g of 40 mesh loblolly pine and 250 g of solvent (biomass/solvent=1/10, wt/wt) which consisted of either DI water or DI water/ethanol mixture (1/1, wt/wt). The HTL process with water and water/ethanol is denoted as W-HTL and W/E-HTL, respectively. Before heating up the reactor, high purity nitrogen was purged into the reactor to remove the air, and then reactor was pressurized to 2 MPa with nitrogen to force all the reactive materials into the liquid phase. Hydrothermal liquefaction was carried out at 300 rpm for 30 min. at four different conditions: 250 °C (far-critical), 300 °C (sub-critical), 350 °C (near-critical), and 390 °C (super-critical). HTL time was started once the set temperature was reached. For the 390 °C super-critical condition, the reactor was not initially pressurized to 2 MPa with nitrogen due to the safety limit of the reactor. The final pressure in the reactor at each temperature was read from the reactor controller and is shown in Table 1. A typical temperature-pressure profile during HTL experiment is illustrated in Fig. S1 (see supporting information). After 30 min. reaction, the reactor is immersed in iced water to quench the reaction.

2.3. Product Separation and Calculation of the Product Yields

The procedure of product separation is illustrated in Fig. 1. Once the reactor was cooled down to 24 °C, the gas fraction was released, and the reactor was opened. The composition of the gas fraction was not analyzed in this study. The amount of gas produced was calculated by the weight difference of the reactor after venting the gas (Eq. 1), where W1 is the weight of the reactor before HTL containing biomass and solvent; and W2 is the weight of the reactor (containing liquefied biomass) after HTL and venting the gas. The slurry (liquefied wood) was poured into a flask, and the reactor was rinsed with dichloromethane (DCM) to collect all the contents in the reactor. The slurry was then diluted with 400 mL DCM. The solid and the liquid part in the slurry was vacuum filtered using preweighed Whatmann#5 filter paper. The solid part, accumulated on the filter paper, was oven dried at 105 °C for overnight, and assigned as “residue”. The yield of residue was calculated using Eq. 2. The liquid part was poured into a 1 L separatory funnel to isolate the organic and the aqueous phases. Since water is not miscible in DCM, organic and aqueous products separated naturally in the separatory funnel. The organic products were carefully transferred to a rotary evaporator. The DCM and the solvent (water and/or ethanol) retained in the organic phase was removed by rotary evaporation at 65 °C. The DCM soluble compounds, a viscous-black liquid, were collected in the flask, which was donated as “bio-oil”, and the yield of bio-oil was calculated using Eq. 3. The aqueous products (water and water soluble products) accumulated in the top layer, and assigned as “light oil”. Since the major interest of this study was the bio-oil, the light oil was not further analyzed in this study. Quantification of light-oil is one challenge in HTL studies due to the high boiling point of water (Cheng et al. 2010). Therefore, the yield of the light oil was calculated by difference using Eq. 4 for simplicity. However, it should be noted that calculating the yield of light-oil by difference results experimental errors. All experiments were carried out in duplicates and ANOVA was

performed at 95% confidence interval to find out whether the results were statistically significant.

$$\text{Gas yield (wt. \%)} = \frac{W1-W2}{\text{wt.of the loblolly pine (dry basis)}} \times 100 \quad (\text{Eq. 1})$$

$$\text{Residue yield (wt. \%)} = \frac{\text{wt.of the solid products}}{\text{wt.of the loblolly pine (dry basis)}} \times 100 \quad (\text{Eq. 2})$$

$$\text{Bio – oil yield (wt. \%)} = \frac{\text{wt.of the bio–oil after rotary evaporator}}{\text{wt.of the loblolly pine (dry basis)}} \times 100 \quad (\text{Eq. 3})$$

$$\text{Light oil yield (wt. \%)} = 100 - \text{Yield}_{\text{Gas+residue+bio–oil}} \quad (\text{Eq. 4})$$

2.4. Hydroxyl (OH) Group Analysis of Bio-oil: ³¹P-NMR

³¹P-NMR provides great deal of information to determine the content of OH groups present in the bio-oil. In this technique, OH groups belonging to aliphatic, phenolic, carboxylic units were phosphitylated by TMDP followed by quantitative ³¹P-NMR. In the phosphitylation reaction, TMDP reacted with the free OH groups in the bio-oil, and yielded the derivatized compound and hydrochloric acid (HCl). Phosphitylation of bio-oil was performed according to Celikbag et al. (2015), and ³¹P-NMR spectra were acquired with a Bruker Avance II 250 MHz spectrometer using inverse gated decoupling pulse sequence, 90° pulse angle, 25 s pulse delay and 128 scans following the methods of Ben and Ragauskas (2011). Detailed method is presented in the Supporting Information. All experiments were performed in duplicates with ANOVA at 95% confidence interval.

2.5. Carbonyl Groups Analysis of Bio-oil: ¹⁹F-NMR

The carbonyl groups in bio-oil was quantitatively determined by ¹⁹F-NMR according to the method reported by Huang et al. (2014). In this technique, the carbonyl groups (aldehydes,

ketones and quinones) in the bio-oil were derivatized with 4-(tri-fluoromethyl)phenylhydrazine. For quantitative analysis, 3-trifluoromethoxybenzoic was used as the internal standard. Detailed method is presented in the Supporting Information. ^{19}F -NMR spectra were acquired with a Bruker Avance II 250 MHz spectrometer using 90° pulse angle without proton decoupling, 10 s pulse delay and 400 scans. All experiments were performed in triplicates and ANOVA was performed at 95% confidence interval.

3. Results and Discussion

3.1. Product Yields

3.1.1. Effect of Temperature on Product Yields

Product yields obtained under different HTL conditions are illustrated in Fig. 2. It was found that temperature had a significant effect on bio-oil yield (p -value=0.0007 and <0.0001 for W-HTL and W/E-HTL, respectively), and residue yield (p -value=0.0001 and <0.0001 for W-HTL and W/E-HTL, respectively). The maximum bio-oil yield of 68 wt.% and minimum residue yield of 2 wt.% was obtained at W/E-HTL at 300 °C. The bio-oil yield first increased when the temperature increased from 250 °C to 300 °C, and then decreased as the temperature further increased to 390 °C. The first increase in the bio-oil yield could be attributable to enhanced degradation of lignocellulosic biomass into bio-oil due to the elevated temperature. The minimum temperature for hemicellulose decomposition in water was reported to be 180 °C (Changi et al. 2015), and higher temperatures (>260 °C) are required to overcome the activation energy for lignin degradation (Yuan et al. 2010); therefore, it is reasonable that HTL at 300 °C resulted in higher degradation of biomass than at 250 °C, and consequently led to increased bio-oil yield. However, bio-oil yield decreased while residue and gas yield increased as the HTL

temperature increased to 390 °C as can be seen in Fig. 2. Many of the HTL studies reported similar trends regarding a critical temperature for HTL of lignocellulosic biomass at which bio-oil yield begins to drop and more solid residue and gaseous products are produced at above the critical temperature (Akhtar and Amin 2011). In this study, 300 °C was found to be the best temperature in terms of bio-oil and residue yield for both W-HTL and W/E-HTL. A kinetic study also showed that gaseous products and solid residue are formed by the secondary reactions of bio-oil at higher temperatures as a result of cracking and recondensation of bio-oil (Valdez and Savage 2013). Thus, it is possible that the decrease in bio-oil yield at higher temperatures is attributable to the enhanced cracking and condensation reactions. The decrease in the dielectric constant (ϵ) of water at higher temperature and pressure could be another reason for the decrease in bio-oil yield. Since the dielectric constant of water measures the polarity, it could be defined as the rough measurement of water's ability to dissolve polar compounds, and it decreases from 27.1 at subcritical conditions ($150\text{ }^{\circ}\text{C} < T < 350\text{ }^{\circ}\text{C}$, $0.4\text{ MPa} < p < 20\text{ MPa}$) to 5.9 at supercritical condition ($T > 370\text{ }^{\circ}\text{C}$, $p = 25\text{ MPa}$) (Krammer and Vogel 2000). Moreover, water at subcritical conditions acts like an acid/base catalyst precursor which is desirable for biomass liquefaction while water at supercritical conditions exhibits more like a non-polar solvent properties (Kruse and Dinjus 2007). Therefore, it could also be conjectured that the lower dielectric constant of water at higher temperatures could have contributed to lower bio-oil yield observed in this study.

3.1.2. Effect of Ethanol on Product Yields

ANOVA analysis with 95% confidence interval showed that addition of ethanol significantly increased the bio-oil yield obtained at each HTL temperature (p -value=0.0016, 0.0017, 0.0076 and 0.0014 for 250, 300, 350 and 390 °C, respectively), and decreased the amount of residue generated during the HTL (p -value=0.0063, <0.0001, 0.0027 and 0.0003 for

250, 300, 350 and 390 °C, respectively). All the W/E-HTL experiments exhibited higher bio-oil yield and lower residue yield than the W-HTL experiments; in fact, the lowest bio-oil yield (30 ± 0.5 wt.%) obtained from W/E- HTL was even higher than the maximum bio-oil yield (26 ± 1.6 wt.%) obtained from the W-HTL suggesting that water/ethanol shows great synergy effect for HTL of the lignocellulosic biomass. Moreover, much lower solid residue (2 wt.%) was produced by W/E- HTL at 300 °C while W-HTL produced 39 wt.% solid residue at 300 °C which indicates that addition of ethanol effectively inhibited the re-condensation of degradation products of lignocellulosic biomass. During the HTL, biomass is degraded to fragments and free radicals are generated. When there is no hydrogen donor solvent, these reactive free radicals recombines and forms high molecular weight products called solid residue or char. In case a sufficiently high amount of a hydrogen donor solvent such as ethanol is introduced to the HTL, free radicals can be stabilized and the formation of solid residue is decreased (Vasilakos and Austgen 1985). Thus, lower residue content at W/E-HTL is attributable to the hydrogen donor capability of ethanol. As Liu et al. (2013) summarized the synergetic effect of water/ethanol, the high bio-oil and low residue yield from W/E-HTL experiments could be attributed to (i) enhanced hydrogen donor capability of ethanol at subcritical and supercritical conditions and acting as a reaction substrate, (ii) high ability of ethanol to dissolve oily products, (iii) ability of ethanol to stabilize the free radicals resulting in lower residue content, and (iv) the increased solubility of high molecular weight products in water/ethanol mixture at subcritical conditions. In addition to the synergistic effects mentioned above, conversion of highly reactive carbonyl groups in the bio-oil to more stable acetal groups by ethanol could be another reason for the low residue content in W/E- HTL. Compounds containing carbonyl groups such as aldehydes and ketones are primarily responsible for the repolymerization of bio-oil due to their high reactivity and consequently

result in the generation of solid residue as well as an increase in the viscosity of bio-oil (Czernik et al. 1994). In this study, it was found that bio-oil produced from W/E- HTL had a lower carbonyl concentration (see below, section 3.3. ^{19}F -NMR analysis of bio-oil) suggesting that ethanol stabilized the carbonyl groups, and as a result, a lower amount of solid residue was generated.

3.2. Hydroxyl (OH) Group Analysis of Bio-oil: ^{31}P -NMR

The OHNs of all bio-oils produced at different HTL conditions are illustrated in Fig. 3. A typical ^{31}P -NMR spectrum of a bio-oil is shown in Fig. S2 (see supporting information). The type of OH groups including aliphatic, phenolic (guaiacyl, catechol, C_5 substituted condensed phenolic types) and acidic type of OH were identified according to ^{31}P -NMR chemical shifts reported by Ben and Ragauskas (2011). High OHN (5.35–10.70 mmol/g) is required for a bio-oil to be considered as a high quality biopolyol (Zou et al. 2012). All the bio-oils, except the bio-oils produced from W-HTL at 300 °C and 350 °C, exhibited higher OHN than 5.35 mmol/g; therefore, could thus be considered as a good candidates as biopolyols.

3.2.1. Effect of Temperature on Hydroxyl Groups

ANOVA analysis showed that temperature had a significant effect on the total OHN of bio-oils produced by both W-HTL (p -value=0.0007) and W/E-HTL (p -value=0.0015). In the case of W-HTL, the total OHN first decreased (p -value=0.0142) as the temperature was increased from 250 to 300 °C, and then increased (p -value=0.0873 and 0.0088 for 300-350 °C and 350-390 °C, respectively) with the increasing temperature to 390 °C. On the other hand, the total OHN of bio-oils from W/E-HTL experiments decreased steadily (p -value=0.0782 and 0.0285 for 250-300 °C and 300-350 °C, respectively) till 350 °C, and then started to increase (p -

value=0.3759) as the temperature reached to 390 °C. A similar trend in the OHN with temperature was observed for the bio-oils produced by organic solvent liquefaction of biomass where authors attributed this trend to condensation reactions that occurred between degradation fragments of biomass (Wei et al. 2014). It is noteworthy to mention that for the both W-HTL and W/E-HTL experiments, aliphatic OH decreased while phenolic OH increased with increasing HTL temperature from 250 to 390 °C. Aliphatic OH was the dominant hydroxyl type at the lower temperature; however, at higher temperature, phenolic type OH was observed to be the major hydroxyl type. For instance, at 250 °C HTL, the aliphatic OH accounted for 50.3% and 62.7% of the total OHN of W-HTL and W/E-HTL, respectively. On the other hand, when the HTL temperature reached to 390 °C, phenolic OH accounted for 91% and 75.6% of the total OHN of W-HTL and W/E-HTL, respectively. Statistical analysis also showed that HTL temperature had significant effect on aliphatic and phenolic OH (p -value<0.0001 for both aliphatic and phenolic OH at W-HTL and W/E-HTL). This trend (decrease in the aliphatic OH and increase in the phenolic OH with increasing HTL temperature) could be due to the thermal stabilities of wood polymers (cellulose, hemicellulose and lignin). The higher concentration of aliphatic OH at lower temperature may be attributable to mainly hemicellulose degradation since (i) hemicellulose is the first depolymerized wood polymer under heat (Via et al. 2013), and (ii) the degradation route of hemicellulose in water yields sugar derivatives containing aliphatic OH groups (Changi et al. 2015). Lignin is the main source of the phenolic compounds in a bio-oil (Cheng et al. 2010), and degradation of lignin occurs at temperature above 260 °C (Yuan et al. 2010). Thus, the increase in the amount of phenolic OH with increasing HTL temperature could mostly be due to the lignin degradation. The acidic OH did not significantly changed with temperature (p -value=0.2980 and 0.0462 for W-HTL and W/E-HTL, respectively), and stayed at

a concentration of 0.66 ± 0.06 and 0.72 ± 0.16 mmol/g for W-HTL and W/E-HTL, respectively. Changi et al. (2015) reviewed the degradation of cellulose and hemicellulose in water at sub- and super-critical conditions. Authors reported that at sub-critical conditions, hemicellulose degradation occurs much faster than cellulose while cellulose degradation increases when the HTL conditions are at the near- or super-critical conditions. It was also reported that their degradation products contain acidic components such as formic, acetic and lactic acid. Therefore, degradation products of hemicellulose and cellulose could be attributable to the source of acidic OH at subcritical (250 – 350 °C) and super-critical (390 °C) conditions, respectively.

3.2.2. *Effect of Ethanol on Hydroxyl Groups*

All of the bio-oils obtained from W/E-HTL experiments at each temperature exhibited higher total OHN than W-HTL except at 390 °C. Ethanol was found to have a significant effect on the total OHN of the bio-oils produced at 250, 300 and 350 °C (p -value=0.0123, 0.0052 and 0.0193, respectively); however, no significant effect was observed for the experiment at 390 °C (p -value=0.1082). The high OHN at W/E-HTL experiments could be attributed to the synergy effect of water and ethanol as discussed in the section 3.1.2. Since ethanol is a good hydrogen donor solvent and effectively inhibits the re-condensation of biomass degradation products, the bio-oil obtained from W/E-HTL had a lower molecular weight and a higher polydispersity index (Cheng et al. 2010) suggesting that the bio-oil mainly contains monomers and oligomers from decomposition of biomass. Therefore it is conjectured that the higher amount of monomers and oligomers provided more side groups such as hydroxyls in the bio-oil.

One major result found in this study was that the bio-oils produced by W/E-HTL at each temperature contain more aliphatic and less phenolic OH than the bio-oils obtained from W-HTL. This finding suggests that ethanol significantly contributed the aliphatic type OH in the bio-oils (p -value=0.0050, 0.0003, 0.0005 and 0.0107 for 250, 300, 350 and 390 °C, respectively). Our lab has previously studied the source and variation of hydroxyl groups of the bio-oil produced by liquefaction of loblolly pine with ethylene glycol (EG), and found that lignin fragments undergo condensation reaction with EG which results in less phenolic and more aliphatic type of hydroxyl groups (Celikbag et al. 2014). The same rational can be applied to the W/E-HTL in which ethanol may undergo condensation reactions with the phenolic OH groups of the lignin fragments. This finding is also in agreement with previous GC/MS studies of cornstalk lignin in W/E-HTL where the yields of phenolic compounds (phenol, ethylphenol, guaiacol) were reported to be lower when the ethanol concentration in the water/ethanol mixture was increased (Ye et al. 2012). ANOVA analysis of phenolic OH showed that ethanol had significant effect at 390 °C only (p -value=0.0033). For below supercritical conditions, no significant effect of ethanol was observed on phenolic OH (p -value=0.5963, 0.1555 and 0.2712 for 250, 300 and 350 °C, respectively).

These findings suggest that OHN of a bio-oil could be adjusted by HTL temperature and ethanol. For example, W/E-HTL at lower temperature seems to constitute optimum HTL conditions for the production of biopolyol with high concentration of aliphatic OH. Such biopolyols could be utilized in polyester production. On the other hand, bio-oil produced from high temperature W-HTL offers high concentration of phenolic OH which could be used as a biopolyol for the production of rigid polymers such as epoxy. However, the bio-oil yield should

be taken into account as well because it is an important consideration for the economic viability of the process.

3.3. Carbonyl (C=O) Group Analysis of Bio-oil: ^{19}F -NMR

3.3.1. Effect of Temperature on Carbonyl (C=O) Groups

The carbonyl group concentration of bio-oil derivatized with 4-(tri-fluoromethyl)phenylhydrazine was calculated by quantitative ^{19}F -NMR and results are illustrated in Fig. 4. A typical ^{19}F -NMR spectra is shown in Fig. S3 (see supporting information). ANOVA analysis showed that temperature had no statistically significant effect on the carbonyl concentration in the bio-oil produced from W-HTL (p -value=0.2985); however, a significant temperature effect was found in the bio-oil produced from W/E-HTL (p -value=0.0001). The carbonyl concentration of bio-oils produced from W-HTL and W/E-HTL followed the same pattern under the temperature treatment: first increased (p -value=0.1326 and 0.0002 for W-HTL and W/E-HTL, respectively) as the HTL temperature was increased from 250 °C to 300 °C, and then decreased (p -value= 0.0022 and 0.0099 for W-HTL and W/E-HTL, respectively) with increasing temperature from 300 to 350 °C. A slight increase in the carbonyl concentration (p -value=0.6216 and 0.5658 for W-HTL and W/E-HTL, respectively) was observed when the HTL temperature had reached to 390 °C. The first increase in the carbonyl concentration could indicate the enhanced degradation of hemicellulose at 300 °C since the major degradation product of hemicellulose is xylose which is a 5-carbon sugar aldehyde (Changi et al. 2015). Moreover, cleavage of ester bonds in lignin could result in the increase of carbonyl concentration (Huang et al. 2014) as lignin degradation mostly occurs at temperature above 260 °C (Yuan et al. 2010). Decrease in the carbonyl concentration with increasingly severe HTL conditions is more

likely due to the dehydration (Changi et al. 2015) and re-condensation of carbonyl groups with other degradation products.

3.3.2. Effect of Ethanol on Carbonyl (C=O) Groups

ANOVA analysis showed that addition of ethanol had significant effect on carbonyl concentration at each HTL temperature (p -value=0.0030, 0.0020, 0.0031 and 0.0055 for 250, 300, 350 and 390 °C, respectively). Compared to W-HTL, a lower carbonyl concentration was found in the W/E-HTL bio-oils at each temperature (Fig. 4). This is in agreement with research by Kosinkova et al. (2015) who pursued the HTL of bagasse using water/ethanol solvent. In that study, the GC/MS analysis of bio-oil showed that the amount of ketones in the bio-oil decreased as the amount of ethanol was increased in the water/ethanol mixture. Acetalisation studies of bio-oil also showed that highly reactive carbonyl groups could be converted into more stable acetal and hemiacetal groups by low molecular weight alcohols such as methanol and ethanol (Li et al. 2011, Ye et al. 2014). Eckert and Chandler (1998) investigated the tautomeric equilibrium of Schiff base 4-(methoxy)-1-(N-phenylforminidoyl)-2-naphthol in the supercritical ethane in the presence of ethanol as the co-solvent, and reported that ethanol could shift the keto-enol equilibrium toward the enol form with increasing density which suggests that ethanol played a role in the conversion of ketone carbonyls to hydroxyl groups. Similar transformation of ketones to enols was also observed by Nazari et al. (2015) where birchwood sawdust was liquefied in the presence of KOH in a HTL process. Taking the reported observations into account, it is concluded that the lower carbonyl and higher hydroxyl concentration in bio-oil produced via W/E- HTL in this study could be attributed to (i) the stabilization of carbonyl groups to acetal groups by ethanol, and (ii) the shift of keto-enol tautomerism toward the enol form.

Compounds containing carbonyl groups are considered undesirable for the utilization of bio-oil due to their high reactivity which leads to unstable, highly acidic bio-oil (Huang et al. 2014). The results of this study suggest that, especially at lower temperatures, carbonyl concentration could be significantly reduced by addition of ethanol to the HTL process. Such bio-oil with improved properties may open venues to increased utilization.

4. Conclusion

The effect of ethanol on hydroxyl and carbonyl groups in bio-oil was studied. The highest bio-oil yield was obtained from W/E-HTL at 300 °C. ³¹P-NMR and ¹⁹F-NMR showed that addition of ethanol had a significant effect on the total hydroxyl and carbonyl concentration of the bio-oils. It was found that addition of ethanol increased the total OHN and aliphatic type OH; however, decreased the phenolic OH concentration. Moreover, lower carbonyl concentration was observed when ethanol was introduced to the HTL process. With optimization of the HTL reaction conditions the properties of bio-oils might be tailored towards their area of application.

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Figure Captions

Fig. 1 Product separation procedure of HTL of loblolly pine.

Fig. 2 Product yields at different HTL conditions (W=water, W/E=water/ethanol solvent).

Fig. 3 OHN of bio-oils produced by HTL of loblolly pine at different conditions derivatized with TMDP.

Fig. 4 Carbonyl group concentration of bio-oils produced by HTL of loblolly pine at different conditions derivatized with 4-(tri-fluoromethyl)phenylhydrazine (W=water, W/E=water/ethanol solvent).

Table 1. The initial and final pressure in the reactor at each temperature in HTL process.

Temperature (°C)	Initial N ₂ Pressure (MPa)		Final Pressure (MPa)	
	H ₂ O	H ₂ O/EtOH	H ₂ O	H ₂ O/EtOH
250	2	2	7	9
300	2	2	11	14
350	2	2	19	22
390	0	0	24	24

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Highlights

- For the first time, ^{31}P -NMR and ^{19}F -NMR were employed to understand the effect of ethanol on the formation bio-oil.
- Ethanol significantly increased the bio-oil and decreased the residue yield.
- Addition of ethanol increased the hydroxyl concentration while decreased the carbonyl concentration.
- More aliphatic and less phenolic type OH was obtained when ethanol was used as a co-solvent.