Oxidative Degradation of Biorefinery Lignin Obtained after Pretreatment of Forest Residues of Douglas Fir

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Biorefinery lignin stream obtained after wet explosion pretreatment of forest residues were further degraded using wet oxidation to produce high-value biochemicals. The biorefinery lignin stream was characterized to understand efficiency of oxidative degradation process.

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Abstract:

Harvested forest residues are usually considered a fire hazards and used as “hog-fuel” which results in air pollution. In this study, the biorefinery lignin stream obtained after wet explosion pretreatment and enzymatic hydrolysis of forestry residues of Douglas fir (FS-10) was characterized and further wet oxidized under alkaline conditions. The studies indicated that at 10% solids, 11.7 wt% alkali and 15 min residence time, maximum yields were obtained for glucose (12.9wt%), vanillin (0.4wt%) at 230°C; formic acid (11.6wt%) at 250°C; acetic acid (10.7wt%), hydroxybenzaldehyde (0.2wt%), syringaldehyde (0.13wt%) at 280°C; and lactic acid (12.4wt%) at 300°C. FTIR analysis of the solid residue after wet oxidation showed that the aromatic skeletal vibrations relating to lignin compounds increased with temperature indicating that higher severity could result in increased lignin oxidation products. The results obtained, as part of the study, is significant for understanding and optimizing processes for producing high-value bioproducts from forestry residues.

Keywords: Biochemical, Biorefinery, Douglas Fir, Lignin, Wet Oxidation.

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1 Introduction

One of the major problems affecting the forestry reserves in the Pacific Northwest region of the United States is the high-intensity wildfires caused as the result of residuals left behind after logging and harvesting of the forest. Removal of these residues is further of importance for overall insect, pest and disease resistance in the forests (Jurgensen et al., 1997). Official report, as of 2012, indicated an estimated amount of 75 million tons of harvest residues being produced every year (Perlack and Stokes, 2011). Studies have also indicated that thinning activities to avoid wild fires in the western United States alone can result in 155Mt of “waste” residual lignocellulosic biomass (Rummer et al., 2005). These forest residual wastes can be, instead, used as a low-cost raw material for the production of biofuels and biochemicals.

In biorefineries, the biomass will be ground and pretreated to break the acetate linkages between lignin and hemicellulose followed by enzymatic action on the cellulosic components to yield sugar monomers. These sugar monomers (eg. glucose/xylose) are then further fermented using microbes to produce biofuels such as ethanol or butanol. Forest residues might be low in cost but the high recalcitrance of the forest residuals usually results in a significant increase in the production costs (Cheng et al., 2015, Stephen et al., 2010). The recalcitrance of these forestry residues is due to the presence of recalcitrant wood materials that amounts to at least 15-25% of the whole tree mass (David and Atarbouch, 1987). These studies have indicated that the presence of bark material increases the amount of extractives, organic acids and lignin in the forestry residues resulting in decreased saccharification efficiency primarily through inhibition of the cellulolytic enzymatic activity. Recent studies have focused on the efficient pretreatment of these forestry residues using sulfite pretreatment or SPORL (Zhu et al., 2015) and wet explosion

pretreatment (Biswas et al., 2015). Both these pretreatments followed by enzymatic action resulted in effective biomass degradation to produce high sugar yields.

The focus of the current study is on the biorefinery lignin stream obtained from these forestry residues and its ability to be converted to high value bioproducts. Lignin is a highly branched amorphous heteropolymer connected through a system of C-C and phenylpropane linkages and is made up from oxidative coupling of hydroxyphenyl (H), guaiacyl (G) and syringyl (S) components (Himmel et al., 2007). While the SPORL pretreatment of the forest residues results in formation of lignosulfonates, studies have indicated that the lignin obtained from wet explosion pretreatment retained its native structure with limited modifications primarily since there were no harmful chemicals added during the pretreatment process (Rana et al., 2015). Briefly, wet explosion pretreatment is a combination of wet oxidation and steam explosion using oxygen as main oxidant to degrade lignocellulosic biomass structure (Biswas et al., 2015; Rana et al., 2012). Previous studies on wet exploded loblolly pine lignin at optimal conditions for sugar production indicated that structural modifications within the lignin molecule occurred in the form of methoxylation of the hydroxyphenyl components present in lignin (Rana et al., 2015). These studies indicated that an increase in the methoxylation of the lignin components resulted in a reduced recondensation and repolymerization of the lignin making it an ideal feedstock for further conversions, which potentially could lead to bio-products of high value, which could bring down the cost of biofuels production. Also in most studies on lignin, the sugars are separated from the lignin fraction using either alkali treatment (Rocha et al., 2012) or using organic solvents (Rana et al., 2015; Ke et al., 2011) resulting in a cleaner lignin fraction for further deconstruction to biochemicals. However, these purification/separation processes can

significantly affect process economics and the absence of data on the structure of lignin from forestry residues further complicates process design.

The aim of this study is to characterize the lignin stream obtained from a cellulosic biorefinery using Douglas fir forestry residues as feedstocks and evaluate potential oxidative degradation compounds that can be formed from the lignin side-stream residing after sugar removal. In this process, the biorefinery lignin stream is obtained after wet explosion pretreatment followed by enzymatic hydrolysis of the biomass. The lignin stream is washed to remove solubilised components, such as residual sugars and some extractives, and no further purification is performed, indicating that residual cellulose, some organic acids and some salt will also be present apart from lignin. In order to demonstrate the types of biochemicals that can be released, we used more extreme wet oxidation under alkaline conditions, high temperatures and low residence time. The residue left behind after wet oxidation of biorefinery lignin was also characterized using spectrophotometric techniques to evaluate the extent of oxidative degradation.

2 Materials and Methods

2.1 Chemicals

Reagent grade 35 wt% hydrogen peroxide in water, ethyl acetate (HPLC grade), 95 wt% sulfuric acid in water, 50 wt% sodium hydroxide in water, 1N hydrochloric acid concentrate and analytical standards for glucose, xylose, acetic acid, lactic acid, formic acid, vanillin, syringaldehyde, hydroxybenzaldehyde, cellobiose, furfural and hydroxymethyl furfural was obtained from Sigma Aldrich (St. Louis, MO, USA). Cellulase (Cellic Ctec2) and hemicellulase (Cellic Htec2) was obtained from Novozymes Inc. (Franklinton, NC, USA).

2.2 Feedstock and Composition

The Douglas Fir FS-10 sample used in the study is similar to that has been used previously (Biswas et al., 2015). The FS-10 sample was obtained from Weyerhaeuser (www.weyerhaeuser.com, Federal Way, WA, USA) and was mainly composed of Douglas Fir (64%), Hemlock (15%), Cedar (1%), Pine (1%), Spruce (3%), Balsam fir (1%) and Maple Hardwood (15%) residues (by weight basis). The dry biomass sample was milled using a Retsch cutting mill SM 200 (Retsch Inc. Newtown, PA, USA), screened and sieved through a 1.6 mm mesh. The FS-10 sample was then wet exploded according to the method previously described (Biswas et al., 2015; Rana et al., 2015), using a temperature of 185°C, 25 min residence time and 5% O₂ loading, followed by enzymatic hydrolysis using 40 mg enzyme /g cellulose with a 90/10 Ctec2/Htec2 (Novozyme) enzyme mixture. These conditions were selected based on preliminary experiments intended for maximum lignocellulosic biomass deconstruction with minimal effect on the lignin structure (Biswas et al., 2015). The wet explosion pretreatment of FS-10 was done under non-alkaline conditions with a measured reaction pH between 5 and 6. The hydrolysate is filtered and the solids left behind was washed with 3 bed volumes of water and dried in a convection oven at 40°C overnight. The dried solids were then powdered using a benchtop ball mill (3 lbs capacity; www.pyrocreations.com) for 8 hours and used as “biorefinery lignin” feed for further experiments. The carbohydrate, lignin and extractive compositional analysis of the dried and milled biorefinery lignin (moisture content <2%) and that of original FS-10 sample before pretreatment was done using Laboratory Analytical Procedures (LAPs) from National Renewable Energy Laboratory (Sluiter et al., 2011).

2.3 Alkaline Wet Oxidation

Alkaline wet oxidation of biorefinery lignin was carried out in 15 mL stainless steel tubes (6" length x 0.5" i.d, Swagelok, WA, USA). The biorefinery lignin (dried and milled) was mixed with a hydrogen peroxide solution in stainless steel tubes and placed in a preheated fluidized sand bath (Techne Model SBL-2D, VWR International, Batavia, IL, USA) controlled using Eurotherm TC-8D controller. The final volume in the tubes was maintained at 10.5 mL to allow sufficient headspace during the oxidations. Initial experiments were performed without addition of alkali and process conditions tested include 10% solids loading, temperatures from 180 to 300°C and 5 and 15 min residence times. Based on these preliminary tests (data not included), 1 min preheat time was allotted for stainless tubes to reach temperatures below 250°C and 1.5 min preheat time was used for temperatures between 250 and 300°C. The residence times used in experimental design does not include this preheat time. Second set of experiments was performed through addition of alkali at 1 g/g dry biorefinery lignin (11.7 wt% alkali) and 1.5 g/g dry biorefinery lignin (17.4 wt% alkali) at the similar experimental (temperature and residence time) conditions as specified previously. After wet oxidation, the stainless steel tubes were placed in an ice-water bath until the tubes were cooled to below room temperature. The wet oxidized samples from the reaction tubes were then transferred to centrifuge tubes and the reaction tubes were washed with known volume of water. The wash water was added to the centrifuge tubes and mixed before centrifuging at 10,000 rpm for 5 min to separate out the solids from the liquid. In case of alkaline wet oxidation, the samples were neutralized with hydrochloric acid (1N) before centrifugation. The liquid samples were appropriately diluted and analyzed using HPLC while the solids were analyzed using FTIR. All experiments were run in duplicate and the standard error bars are shown in the figures.

2.4 Liquid Fraction Analysis

132 The liquid samples were filtered through a 0.2 mm 45-micron syringe filters and run through a
133 Dionex Ultimate 3000 (Sunnyvale, CA, USA) high performance liquid chromatography (HPLC)
134 system equipped with Aminex 87H column 250 X 4.6 mm (Biorad, CA, USA) using the
135 following conditions: eluent (4mM sulfuric acid in water) flowing through column (maintained
136 at 60°C) at 0.6 mL/min for 120 minutes.

137 The samples were further analyzed using gas chromatograph fitted with a mass spectrometer
138 (GC-MS) to determine the amount of lignin-based phenolic compounds produced during wet
139 oxidation. 4 mL of ethyl acetate was added to 2 mL of sample and placed in an ultrasonic water
140 bath (Bransonic 3510R-DTH, CT, USA) preheated to 65°C and sonicated for 1h at 40kHz.
141 During this process, all the lignin-derived organic compounds will be selectively extracted into
142 the ethyl acetate phase and can be recovered by properly pipetting out the supernatant after
143 cooling to room temperature conditions. The GC-MS conditions for analysis were similar to
144 described in previous work (Rana et al., 2015). Briefly, 1 µL sample was injected into helium
145 (used as carrier gas) flowing at 60 mL/min which then passes through a DB-5 (30m length X
146 250µm I.D. X 0.25µm thickness, J&W Scientific, Folsom, CA, USA) capillary column fitted in
147 an Agilent 7890A GC (Santa Clara, CA, USA). The eluting compounds were detected using an
148 Agilent 5975C inert XL EI/CI mass spectrometer with triple axis and characterized using
149 existing NIST libraries. The GC conditions using split-less injection to separate the compounds
150 were: 100°C for 5 min; 270°C at 5°C/min, hold for 5 min with a total run time of 44 min. The
151 injector was maintained at 300°C. The MS conditions for analysis were 5 min solvent delay,
152 230°C source temperature, 150°C quad temperature, mass fragments collected between 25 and
153 1000 a.m.u. and gain factor of 1. The lignin-based components present in the liquid samples
154 were characterized using the GC-MS and expressed in terms of % peak area as follows:

$$\% \text{ Area} = \frac{\text{Sum of peak areas of G/H/S type compounds from GC - MS}}{\text{Sum of all peak areas of G, H \& S compounds from GC - MS}} \times 100 \quad (1)$$

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2.5 Solid Fraction Analysis

2.5.1 Solid-State Nuclear Magnetic Resonance (NMR) Spectrometry

Solid-state NMR data for the biorefinery lignin was collected on an Agilent DD2 600 MHz console (Agilent, CA, USA) with an Agilent 4 mm double-resonance probe. The spectrometer frequency for collection of C^{13} was 150.808 MHz. The data were collected with CP-MAS with and without TOSS-4 for suppression of the sidebands. The spinning rate was 6 KHz, the initial 1H 90 degree pulse was 2.2 μs and the cross polarization contact time was 1 ms using a 50% linear ramped 1H spin lock. 1H decoupling was accomplished with SPINAL-64 modulation at a decoupling field of 94 KHz. The acquisition time was 20 ms and 1024 scans were acquired per sample with a relaxation delay of 5 seconds. TOSS-4 was used for suppression of sidebands and was optimized using a sample of glycine spinning at 6 KHz and chemical shifts were referenced using the glycine carboxyl carbon at 173.03 ppm. Data were anodized with 20 Hz of exponential line broadening, zero filled to 4096 points and Fourier Transformed.

The resonances were broadly assigned as follows: CH_3 in acetyl groups of hemicellulose (21-24 ppm), alkyl CH and CH_2 in lignin subunits (30-40 ppm), methoxy groups in lignin G and S units (56 ppm), C_6 carbon atoms in carbohydrates (63 ppm), C_2 , C_3 and C_5 carbon atoms in carbohydrates (73 ppm), sugars in hemicellulose (82 ppm), C_4 carbon atoms in carbohydrates (89 ppm), C_1 carbon atoms in carbohydrates (105 ppm), unsubstituted olefinic or aromatic carbon atoms (110–127 ppm), quaternary olefinic or aromatic carbon atoms (127–143 ppm), olefinic or

aromatic carbon atoms with OH or RO substituents (143–167 ppm), esters and carboxylic acids (169–195 ppm) at 173 ppm, carbonyl groups in lignin (195–225 ppm) (Capanema et al, 2005).

2.5.2 Fourier Transform Infrared (FTIR) Spectroscopy

For analysis in the infrared region, samples containing 1 mg of either the biorefinery lignin or residual solids after wet oxidation were dried for 24 h in a constant temperature oven and placed in a desiccator. The sample was then mixed with 250 mg of potassium bromide (KBr) and processed into tablets by compression and subjected to analysis in the infrared region (400–4000 cm^{-1}) using Perkin Elmer Spectrum GX (Waltham, MA, USA). The lignin spectra were normalized using spectral data at band 1510–1513 cm^{-1} , corresponding to aromatic ring vibrations (Faix et al., 1994). The assignment of FTIR bands are shown in Table S1 (Kubo and Kadla, 2005; Pandey, 1999).

3 Results and Discussion

3.1 Compositional Analysis and Characterization of Biorefinery Lignin

The sugar and lignin composition of the original FS-10 and the biorefinery lignin (after wet explosion and enzymatic hydrolysis of FS-10) are shown in Table 1. It can be seen from Table 1 that the biorefinery lignin still contained about 23 wt% cellulose while most of the hemicellulose was dissolved after wet explosion pretreatment.

Solid-state NMR spectra for the original FS-10 and biorefinery lignin is shown in Figure S1 and discussed in detail in the supplementary section of this manuscript. Due to the presence of sugar components in the biorefinery lignin, there was no specific trends observed between FS-10 and biorefinery lignin except for a significant reduction in the acetate methyl linkages in the biorefinery lignin after wet explosion. The NMR spectra also indicated a slight increase in the

methoxy linkages associated with G- & S- units of lignin at 56 ppm. Similar trends were previously observed with wet explosion pretreatment of loblolly pine and was postulated to be a result of in-situ methoxylation of the hydroxyphenyl- (H-) components during wet explosion pretreatment (Rana et al., 2015).

Fourier Transform Infrared (FTIR) spectroscopy is commonly used for the characterization of the functional groups of the lignocellulosic biomass and changes caused by different treatments (Sim et al., 2012). While softwood contains predominantly only G- components, the FS-10 biomass contains both softwood and hardwood residues which results in the presence of some S- components too. The biorefinery lignin obtained from wet explosion pretreatment of FS-10 was found to have both softwood and hardwood properties and showed an uncharacteristic peak (not usually seen in FTIR spectra of softwood such as Douglas Fir) (Dence and Lin, 1992) representing C-O stretching of syringyl group at 1318 cm^{-1} . Previous studies have indicated that the G-type lignin absorbs in both 1270 and 1230 cm^{-1} bands, S-type lignin components absorbed mostly in the 1230 cm^{-1} bands (Pandey, 1999). The FTIR spectra of the biorefinery lignin is shown in Figure S2 of the supplementary information section attached to this manuscript. The S/G ratio of the biorefinery lignin was found to be 0.88 by comparing the FTIR absorbance at 1270 cm^{-1} and 1225 cm^{-1} respectively. The small peak shift for the syringyl units was caused due to the mixed nature of biomass. Further trends observed in the FTIR spectra of biorefinery lignin is discussed in detail in the supplementary section.

Based on the solid-state NMR and FTIR data, it can be hypothesized that further wet oxidation of the biorefinery lignin obtained from FS-10 will result in production of greater quantities of sugar oxidation products due to the higher percentage of cellulosic moieties still left behind. It

can also be predicted that among the lignin oxidation products, G-type units will be significantly higher.

3.2 Effect of temperature, residence time and alkali loading on yield of sugar oxidation products

The yields of sugar oxidation and degradation products as a function of temperature, residence time and alkali loading at 10% solids is shown in Table 2 and the trends for glucose, lactic acid and formic acid along with the standard deviations are shown in Figure 1. While glucose and formic acid can be produced as a result of thermal degradation of cellulose, the primary oxidation product from cellulosic component in the biorefinery lignin would be lactic acid (and acetic acid which will be discussed separately). It can be seen from Figure 1 that wet oxidation under alkaline conditions resulted in greater yields for glucose, lactic acid and formic acid with maximum yields of 129 ± 6.49 mg glucose/g dry biorefinery lignin at 230°C ; 116 ± 1.71 mg formic acid/g dry biorefinery lignin at 250°C ; and 124 ± 0.01 mg lactic acid/g dry biorefinery lignin at 300°C with an alkali loading of 1 g/g biomass (11.7 wt% alkali) and residence time of 15 min. Such trends have been previously observed especially for lactic acid where the lactic acid yield increased with an increase in temperature up to 300°C followed by a decrease due to significant thermal degradation (Jin et al., 2005). These studies had indicated maximum yields for lactic acid of around 100 mg/g dry feed from pure cellulose within two minutes of reaction at 300°C at an oxygen loading as high as 70%. Compared to the literature that uses higher oxygen loading than 7.5% used in our experiments, the results showed slightly higher lactic acid yields at similar temperature but higher residence times. No significant difference in the yields of glucose, formic acid and lactic acid could be observed in Figure 1 as a function of alkali loading. Few anomalies to this conclusion can be seen at 250°C and 11.7 wt% alkali where there was a significant difference in the glucose and lactic acid yields at the different alkali loadings. However, this can

be attributed to the thermal degradation of glucose and lactic acid as shown by an increase in the formic acid yields at 250°C. Previous study (Demesa et al., 2015) have shown significant production of formic acid from oxidation of technical lignin but in the current study, formic acid yields are commensurate with thermal degradation of glucose and hence, has been considered as primarily being obtained from sugar components present in the biorefinery lignin.

3.3 Effect of temperature, residence time and alkali loading on yield of lignin oxidation products

The yields of lignin oxidation products (vanillin, syringaldehyde and hydroxybenzaldehyde) as a function of temperature, residence time and alkali loading at 10% solids are shown in Table 2 and Figure 2. As can be seen from Figure 2(a) (i) & (ii), there was no significant effect of residence time and alkali loading on the vanillin yield from the alkaline wet oxidation of biorefinery lignin. The insignificant effect of alkali loading on vanillin yield above pH of 11.5 has been previously seen where a two-fold increase in alkali concentration above 1.2 g/g lignin resulted only in a marginal increase in vanillin yields (Mathias and Rodrigues, 1995). There was, however, a significant effect of temperature with maximum yield of 3.85 ± 0.24 mg vanillin/g dry biorefinery lignin at 230°C, 11.7 wt% alkali loading and 15 min residence time. It was also seen that there was no significant difference between vanillin yields at 230 and 250°C and different residence times when alkali loading was 11.7 wt%. However, there was a significant effect of residence time and temperature on the hydroxybenzaldehyde and syringaldehyde yields with maximum values of 2.01 ± 0.02 mg/g dry biorefinery lignin and 1.32 ± 0.02 mg/g dry biorefinery lignin respectively at 280°C, 11.7 wt% alkali loading and 15 min residence time. Since there is only around 56.8 wt% of lignin in the biorefinery lignin feed, the theoretical maximum yields of vanillin, syringaldehyde and hydroxybenzaldehyde was calculated as 6.79, 2.32 and 3.54 mg/g

dry pure lignin respectively. While vanillin yields as high as 12 wt% has been shown from softwood lignin (Fache et al., 2016), the recalcitrance of the biorefinery lignin from mixed forestry residues should be taken into consideration along with the high temperatures tested in the study, which could lead to a decrease in expected vanillin yields. Another reason for lower yields of lignin oxidation products when compared to the afore-mentioned studies is that they have used a lower solids loading (≤ 6 wt%) when compared to the current study.

As can be seen from Figures 2(b) (ii) and (c) (ii), the hydroxybenzaldehyde and syringaldehyde yields increased significantly above 250°C followed by a decrease at 300°C probably due to thermal degradation. This sudden increase in yields above 250°C was also accompanied by a significant decrease in the vanillin yields (shown in Figure 2(a) (ii)). The thermal degradation of vanillin above 250°C can be attributed to the increased severity of alkaline wet oxidation conditions. An increase in the alkali loading to 17.4 wt% showed a significant decrease in vanillin yields as a function of temperature above 210°C and residence time of 15 mins, which can again be attributed to the increased severity of the alkaline wet oxidation. Such thermal degradation of vanillin during alkaline wet oxidation has been previously observed at temperatures above 150°C (Araujo et al., 2010). Hence, reduced temperatures and higher residence times (above 15 min) would have sufficiently favored lignin conversion to vanillin in the current study. However, in absence of alkaline conditions, vanillin yields from wet oxidation of biorefinery lignin increased with an increase in temperature until 280°C but the yields, as expected, were considerably lower than that obtained under alkaline conditions. The selectivity toward oxidation of biorefinery lignin to vanillin can be considerably improved through the optimizing temperature, residence time and the use of a catalyst (Fargues et al., 1996; Pinto et

al., 2011; Wong et al., 2010). Since hardwood content in the forestry residue was lower, significant syringaldehyde yields were not expected using the current biorefinery lignin. Apart from vanillin, syringaldehyde and hydroxybenzaldehyde, other G-, S- and H-type lignin oxidation products was also found to be produced and was characterized through GC-MS analysis. Some of these products were identified as catechol (H), 4-ethyl guaiacol (G), 4-propyl guaiacol (G), homovanillyl alcohol (G), homovanillic acid (G), syringol (S) and propylveratrole (S). The GC-MS analysis also showed that the most predominant G-type lignin oxidation product was vanillin/iso-vanillin and S-type lignin oxidation product was syringaldehyde. Since it was difficult to obtain standards for some of the afore-mentioned compounds, the % peak area ratio was used to understand the effect of oxidation variables on the G-, S-, and H-type lignin components (Figure 3). Such comparisons have been done before (del Rio et al., 2007) and have been successfully used to compare the distribution of lignin components at different experimental conditions. It was found that no H-type lignin oxidation products were seen when alkali was added to wet oxidation of biorefinery lignin. However, it is evident from Figure 3 that predominantly G-type units were produced through wet oxidation of biorefinery lignin. Some S-type units were also produced at temperatures below 280°C. Most of these compounds identified through GC-MS analysis are either intermediates or degradation products of vanillin, syringaldehyde and hydroxybenzaldehyde and the trends seen in Figure 3 is similar to that observed from liquid phase analysis shown in Figure 2.

3.4 Effect of temperature, residence time and alkali loading on yield of acetic acid

While there have been studies that have discussed the production of acetic acid primarily from the oxidation of cellulosic components (Jin et al., 2005), there has also been other studies that show the wet oxidation of lignin compounds resulting in acetic acid. Previous studies have

shown that at high oxygen loadings (50-100%), temperature of 300°C and 1 minute residence time, maximum acetic acid yield of 9% was obtained from lignin model compounds (Suzuki et al., 2006). Other studies (Klinke et al., 2002) have indicated that at lower temperatures of 185-195°C and 6-12 bar O₂ pressure, maximum acetic + formic acid yield of 14.2 wt% (or 142 mg/g dry biomass) was obtained from alkaline wet oxidation of wheat straw. In this study, a maximum acetic acid yield of 107 mg/g dry biorefinery lignin was obtained at optimal conditions of 11.7 wt% alkali loading, 280°C and residence time of 15 minutes at an O₂ loading of 7.5%. It can be seen from Figure 2 (d) that the acetic acid yield increased with an increase in temperature up to 280°C with no statistical significance between yield obtained at 280 and 300°C. However, when alkali loading was increased to 17.4 wt%, there was significant decrease in the acetic acid yield above 280°C, which could be a result of thermal degradation. At a residence time of 15 mins, the acetic acid yields showed similar trends as lignin oxidation products. Previous study (Demesa et al., 2015) have shown an increase in acetic acid yield from lignin at temperatures above 200°C until a residence time of around 20 min followed by saturation until a total residence time of 60 min. This study indicated that the decrease in carboxylic acid at high temperatures could be due to condensation reactions between the lignin and carboxylic acids resulting in repolymerization of the lignin components which, in turn, inhibits partial oxidation processes.

Previous studies (Jin et al., 2005; Fang et al., 2012; Wang et al., 2014) have indicated that acetic acid can be produced from wet oxidation of glucose through 2 parallel pathways, one with lactic acid as intermediate and the other with HMF and levulinic acid as intermediates. According to these pathways, 1g of glucose can theoretically produce 1g lactic acid or 0.7g HMF or 0.645g levulinic acid eventually leading to 0.667g acetic acid. The thermal degradation of 1g of glucose can also theoretically result in 1g of formic acid. Hence, overall theoretical acetic acid yield from

cellulosic components of biorefinery lignin can be estimated as 425 mg/g dry biorefinery lignin assuming complete oxidation of cellulose to acetic acid. However, due to the partial oxidation, as can be seen from Table 3, the concentrations of lactic acid, levulinic acid and HMF produced during wet oxidation was also taken into account and calculations predicted that, especially under alkaline conditions, greater amount of acetic acid was produced (at the optimal conditions) when compared to the maximum theoretical yield estimated from cellulose. For example, under optimized conditions (for acetic acid yield) of 11.7 wt% alkali, 280°C and 15 min residence time, approximately 243.23 mg glucose/g dry biorefinery lignin (not taking into account the C₅ sugars) was either left behind or reacted to produce formic acid, lactic acid, levulinic acid and HMF. This amounted to around 11.8 mg glucose/g dry biorefinery lignin left behind that could have resulted in, theoretically, 7.85 mg acetic acid/g dry biorefinery lignin. However, under these conditions, liquid phase analysis of the wet oxidized biorefinery lignin indicated almost 107 mg acetic acid/g dry biorefinery lignin. The excess acetic acid would have to be produced from the wet oxidation of the lignin components since all the water-soluble extractives present in the biorefinery lignin was sufficiently removed during water washing before wet oxidation and the concentration of hemicellulosic components was found to be minimal in the biorefinery lignin. Incorporating the small amount of hemicellulosic components such as xylose in the calculations did not significantly alter the amount of acetic acid produced from the lignin components. Due to the significant differences in the chemical reactivity of the components present in biorefinery lignin, the theoretical calculations are only an approximate qualification to indicate that under the optimal conditions in this study, significant amounts of lignin oxidation products produced acetic acid at higher temperatures (>250°C). However, these theoretical calculations also showed that the cellulosic components present in the biorefinery lignin were the primary sources for acetic

acid production when compared to lignin components. Since acetic acid has significant applications in chemical and fuel sectors, an increased acetic acid yield from the biorefinery lignin (from both sugar and lignin components) is more desirable when compared to a pure lignin stream. Based on the data obtained from this study, the overall process reaction scheme for production of bioproducts from alkaline wet oxidation of biorefinery lignin is shown in Figure 4.

3.5 Comparison between FTIR spectra of residual solids after wet oxidation of biorefinery lignin

The residual solids left behind after alkaline wet oxidation of biorefinery lignin was used to determine the extent of deconstruction of cellulosic and lignin linkages. The variation of FTIR absorbance over the entire spectrum as a function of temperature, residence time and alkali loading is shown in Figure 5 (The assignment of FTIR bands can be found in Table S1 in the supplementary section). It can be seen from Figure 5(a) that in absence of alkali, there was no significant effect of temperature on the cellulosic and lignin based structures in the residual solids after wet oxidation when residence time was 5 mins. However, there was a significant reduction in –OH based cellulosic linkages ($>3300\text{ cm}^{-1}$) and an increase in the primary and secondary alcoholic linkages ($900\text{-}1200\text{ cm}^{-1}$) with an increase in temperature, when residence time was 15 mins. This could be attributed to the primary oxidation of cellulosic moieties present in biorefinery lignin and can be attributed to the low severity of the wet oxidation treatment (due to absence of alkali) since a complete oxidation was not seen. This observation can be further corroborated by a negligible change in the aromatic structural vibrations ($1200\text{-}1500\text{ cm}^{-1}$) attributed to lignin components. The variation in FTIR absorbance as a function of residence time and temperature was significantly lower during non-alkaline wet oxidation when compared to 11.7 wt% alkali, 15 min residence time (Figure 5(b)) and 17.4 wt% alkali, 5 and 15 min residence time (Figure 5(c)).

Increasing alkali loading to 11.7 wt% alkali while maintaining residence time of 5 mins did not show any significant effect of temperature on linkages present in biorefinery lignin. Partial oxidation of biomass has been previously shown to occur at low residence times and insufficiently high temperatures resulting in formation of low molecular weight organic products from polymeric substrates (Bhargava et al., 2006). However, when residence time was increased to 15 mins, there was initially a decrease in the –OH based cellulosic linkages with increase in temperature until around 250°C followed by a significant increase at 300°C. The aromatic skeletal vibrations related to the lignin components increased with an increase in temperature to 300°C. It can also be seen that the sudden increase in the –OH based cellulosic linkages at 300°C was accompanied by a significant reduction in the alcoholic groups. A possible reason for this trend could be a secondary oxidation reaction for cellulosic components which has been previously shown (Jin et al., 2005; Jin et al., 2006; Zhou et al., 2006) for increased carboxylic acid production such as lactic acid (shown in Table 2).

Similar trends were seen when the alkali loading was increased to 17.4 wt% (Figure 5(c)). It can be seen from both the liquid phase data as well as FTIR analysis on the residual solids that 5 min residence time was insufficient for complete oxidation of biorefinery lignin resulting in poor yields for biochemicals tested as part of the study. While alkaline wet oxidation at higher temperatures (>180°C) and residence time of 15 mins resulted in increased sugar oxidation or degradation, the consistent increase in the aromatic skeletal vibrations in the residual solids that usually attribute to the lignin structures, could indicate that further oxidation to increase yield of lignin oxidation products such as vanillin, syringaldehyde and hydroxybenzaldehyde could be achieved with a further increase in residence time. An increase in temperature would, however,

result in thermal degradation of these lignin oxidation products resulting in organic acids such as formic and acetic acid as can be seen in this study.

4 Conclusions

The current study examined the applicability of biorefinery lignin from forestry residues to produce high value products. Alkaline wet oxidation at higher temperatures was found to favor formation of oxidation products such as lactic, acetic and formic acids and partial oxidation of lignin to vanillin. This was confirmed through FTIR analysis on residual solids that showed an increase in the aromatic skeletal vibrations as function of residence time between 180 and 250°C. Due to the low feedstock cost, further process optimization (primarily residence time) to efficiently convert biorefinery lignin stream to bio-based chemicals could potentially create more value into future biorefineries.

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Figure Caption List

Figure 1. Variation in concentration (mg/g dry biomass) of sugars and sugar oxidation products including (a) glucose, (b) formic acid, (c) lactic acid as a function of temperature at different NaOH loadings and residence time of (i) 5 mins and (ii) 15 mins. (● – 0wt% NaOH; ▲ – 11.7wt% NaOH; ■ – 17.4wt% NaOH)

Figure 2. Variation in concentration (mg/g dry biomass) of lignin oxidation products (a) vanillin, (b) hydroxybenzaldehyde, (c) syringaldehyde, and (d) acetic acid as a function of temperature at different NaOH loadings and residence time of (i) 5 mins and (ii) 15 mins. (● – 0wt% NaOH; ▲ – 11.7wt% NaOH; ■ – 17.4wt% NaOH)

Figure 3. Variation in % lignin oxidation products in form of H-, S- and G-type compounds obtained at 10% solids and (A) 0% NaOH loading; (B) 11.7% NaOH loading; (C) 17.4% NaOH loading as a function of temperature at different reaction times (i) 5 min; and (ii) 15 mins.

Figure 4. Overall process reaction scheme showing the alkaline wet oxidation of biorefinery lignin to bioproducts.

Figure 5. Variation in FTIR absorbance of residual solids obtained at 10% solids loading and (a) 0% NaOH loading; (b) 11.7% NaOH loading; (c) 17.4% NaOH loading as a function of wavelength at different temperatures and reaction times of (i) 5 min; and (ii) 15 mins

Table Caption List

Table 1: Compositional analysis of biorefinery (FS-10) lignin

Table 2: Bioproducts obtained from wet oxidation of biorefinery lignin (FS-10) as a function of alkali loading, time and temperature

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1: Compositional analysis of biorefinery (FS-10) lignin

Component	Composition of FS-10(%)	Composition of Wet-Exploded FS-10 (%)
Cellulose	30.3	23.1
Xylan	5.40	1.84
Galactan	2.20	0.19
Arabinan	1.40	0.00
Mannan	8.80	2.21
Ash	0.70	0.30
Lignin	46.2	56.8
Extractives	2.80	11.8

Table 2: Bioproducts obtained from wet oxidation of biorefinery lignin (FS-10) as a function of alkali loading, time and temperature

NaOH Concentration (g/g biomass)	% Solids	Time (min)	Temperature (°C)	Glucose	Xylose	FA ^a	AA ^b	LA ^c	LeA ^d	Furfural	HMF ^e	HBA ^f	Vanillin	SA ^g
0.0 (0% Loading)	9.95	5	180	12.5	4.50	16.7	3.82	0.971	0.495	0.733	1.64	0.168	0.254	0.0591
			210	20.4	10.7	25.8	6.88	2.07	1.20	0.750	0.538	0.134	0.353	0.0802
			230	18.0	14.9	18.3	5.96	1.39	0.718	3.64	0.957	0.0697	0.339	0.0606
			250	17.9	10.5	15.1	7.41	1.93	1.09	8.73	3.30	0.144	0.446	0.0811
			280	7.05	3.53	11.2	1.94	5.11	1.09	9.92	1.82	0.222	0.661	0.114
			300	1.15	0.404	0.956	0.711	0.410	0.132	1.11	0.0921	0.0466	0.116	0.0721
		15	180	14.8	7.90	16.9	4.29	1.33	0.601	1.21	1.69	0.0778	0.150	0.0423
			210	26.7	13.3	23.2	6.74	1.89	0.690	5.28	1.95	0.182	0.502	0.0878
			230	13.5	6.86	16.1	10.0	3.96	1.09	11.6	3.47	0.214	0.660	0.129
			250	7.68	3.62	14.1	13.1	6.81	2.08	20.7	5.75	0.282	0.872	0.203
			280	1.73	1.04	8.81	23.5	11.8	2.25	11.2	4.54	0.370	1.142	0.227
			300	1.25	1.22	4.10	26.2	14.3	3.12	2.74	0.723	0.459	1.402	0.254
1.0 (11.7% Loading)	10.0	5	180	10.75	5.86	33.5	23.6	30.3	3.40	0.00	0.00	0.240	1.54	0.204
			210	32.9	6.73	44.0	23.9	43.2	2.64	0.00	0.00	0.339	2.84	0.223
			230	70.4	9.81	57.7	27.6	49.6	2.36	1.14	3.05	0.515	3.38	0.270
			250	32.0	6.09	90.9	47.7	46.7	4.26	4.11	3.83	0.681	3.79	0.262
			280	70.7	11.3	98.0	56.4	120	5.21	2.40	0.00	0.919	3.22	0.283
			300	56.5	6.06	101.0	65.8	84.6	5.34	3.54	0.00	0.482	0.459	0.161
		15	180	25.3	6.20	33.3	23.6	38.9	3.28	0.00	0.00	0.345	2.63	0.293
			210	47.8	8.65	44.5	22.1	85.4	2.01	0.00	0.00	0.346	2.71	0.175
			230	129	10.29	92.1	40.4	51.4	3.97	2.55	3.43	0.693	3.85	0.285
			250	48.9	8.36	116	56.7	62.8	6.49	1.95	2.07	0.767	3.79	0.228
			280	61.4	9.34	63.4	107	109	5.83	3.22	0.00	2.01	2.34	1.32
			300	67.4	4.52	100.0	61.2	124	5.49	5.85	0.00	0.819	2.10	0.185
1.5 (17.4% Loading)	10.0	5	180	11.9	5.64	18.4	10.67	12.9	2.02	0.00	0.00	0.111	0.981	0.174
			210	25.4	6.07	37.3	20.5	31.8	2.66	0.00	0.00	0.343	2.63	0.266
			230	57.9	10.9	65.6	34.9	71.0	3.03	4.51	0.00	0.466	3.67	0.259

			250	77.6	12.3	92.2	51.9	109	3.67	3.29	0.457	0.557	2.87	0.191
			280	93.8	25.3	101.0	66.2	118	5.81	8.07	3.10	0.708	2.01	0.216
			300	8.16	3.12	15.9	10.9	6.59	1.45	0.00	0.00	0.094	0.218	0.0378
		15	180	29.4	7.16	41.9	25.4	36.5	3.79	0.00	0.00	0.341	2.37	0.218
			210	68.7	13.0	72.4	46.8	85.4	5.18	0.00	0.00	0.425	2.31	0.200
			230	80.3	11.9	93.7	56.2	111	5.79	0.998	0.387	0.639	2.09	0.194
			250	92.0	12.4	110	65.0	119	4.87	3.47	3.56	0.836	1.71	0.1035
			280	76.9	8.22	23.2	65.0	22.4	6.12	3.50	1.64	0.320	0.388	0.1068
			300	3.11	3.13	17.8	28.3	10.4	1.48	0.00	0.00	0.171	0.0760	0.0570

All concentrations are in mg/g dry biomass; ^aFA-Formic acid; ^bAA-Acetic acid; ^cLA-Lactic acid; ^dLeA-Levulinic acid; ^eHMF-5-Hydroxymethyl furfural; ^fHBA-Hydroxybenzaldehyde; ^gSA-Syringaldehyde