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ARTICLE

Identification of Free Radicals in Pyrolysis Oil and Their Impact on Bio-oil Stability

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The existence of radicals in pyrolysis oil generated from loblolly pine in three different reactor systems was verified with electron-paramagnetic resonance (EPR) spectroscopy. Characterization of the bio-oil via its sub-fractions revealed the radicals were found to be preferentially located in the bio-oil lignin fraction, especially in the higher molecular weight lignin. Based on the observed EPR spectra (which lacked hyperfine structure) and low g-factors, the radicals are proposed to be stable carbon-centered and delocalized in a highly conjugated lignin π system. Furthermore, this study also examined the impact of radicals on bio-oil aging severity using an accelerated aging method and the addition of radical scavengers. Preliminary results support the hypothesis that bio-oil radicals are present in a stable state because radical scavengers showed negligible effects on controlling pyrolytic lignin condensation. Only a mild radical concentration reduction was observed after bio-oil accelerated aging.

Introduction

Bio-oil is a promising fuel intermediate that is produced from biomass pyrolysis at an elevated temperature (~500 °C) and inert environment. Typical crude bio-oil has poor fuel properties because it contains ~40% oxygen and ~25% water. Therefore, crude bio-oil needs to be hydrodeoxygenated before it can be used as a drop-in transportation fuel. Additionally, as a competitive fuel precursor, long-term storage and thermal stability is required before bio-oil can be used as a high-yield automobile fuel. However, bio-oil is not stable under regular storage conditions[1] not to mention under the high-temperatures often employed in the upgrading step. This long-term instability, often called aging, has been identified as a critical hurdle that prevents bio-oil from being commercialized. Common adverse aging behaviours include a viscosity increase and a phase separation (a sticky gum-phase separated from the liquid oil phase). In addition, catalyst coking during the bio-oil upgrading is also closely related to the high reactivity of bio-oil components towards polymerization.[2,3]

Bio-oil has more than 300 different chemical species and, under favourable conditions (e.g. heating with a strong acid catalyst), its components can polymerize and cause aging. However, due to bio-oils' complicated composition, the exact molecular-level polymerization mechanism is still unclear. Crude bio-oil, containing various carboxylic acids, is very acidic (pH~2) and could provide a good reaction medium for bio-oil components undergoing acid-catalyzed condensation. This particular polymerization mechanism may explain the aging phenomenon and it is currently under investigation in the authors' group. To date, the results indicate that acids do play an important catalytic role in condensing the bio-oil compounds; however the authors also observed mild condensation after acids were completely removed from the bio-oil. This finding implies that aging reactions may also be initiated by other condensation processes, i.e., from reactive radical species in the bio-oil. To verify this second possibility, a validation of the presence of radicals in the bio-oil is desired.

Previously, free radicals were found to exist in living plants and chemically extracted lignin.[4,5,6,7,8] Thermochemical conversion of biomass and its constituents also generate radical-possessed vapour intermediates,[9,10] and the stable radicals were eventually trapped in the solid products, i.e. biochar[11,12,13,14]. In view of these prior results, radicals may also exist in pyrolysis bio-oil; however, the authors found no study that has tested this hypothesis.

Electron paramagnetic resonance (EPR) spectroscopy is a useful analytical tool for studying materials with un-paired electrons, and, as such, is potentially an appropriate probe for

radicals in bio-oil. Although direct characterization of bio-oil using EPR spectroscopy has not been reported, Bayerbach and Meier[15] used it to characterize pyrolytic lignin (PL) isolated from bio-oil. However, no EPR data was included in their paper, which attempted to elucidate the structure of PL. Therefore in this paper, the authors, for the first time, report on the detailed EPR characterization of radicals in bio-oil and its sub-fractions. Additionally, the impact of radicals on bio-oil aging severity is preliminarily assessed using accelerated aging methodology with bio-oil and PL.

Experimental

Bio-oil production and fractionation

The production and solvent fractionation of bio-oil pyrolyzed from loblolly pine has been described in detail elsewhere[16,17]. Briefly, the bio-oil was produced from a lab-scale fluidized-bed reactor at 500 °C under an inert environment using loblolly pine wood (particle size <0.5 mm). The pyrolysis vapour was collected as bio-oil using condensers and an electrostatic precipitator. For comparison purposes, bio-oils produced from loblolly pine at the University of Tennessee and Auburn University were also included in this study. The bio-oil solvent fractionation was performed according to the following procedures. The bio-oil was first extracted with excess DI water (bio-oil:water=1:30 w.t. ratio) and separated into water soluble (WS) and water insoluble (WIS) fractions. The water soluble fraction was further extracted with diethyl ether (v:v=1:1) and fractionated into an ether soluble (ES) and an ether insoluble (EIS) portion. The water insoluble fraction was extracted with dichloromethane (v:v=1:1) and fractionated into a low molecular mass lignin (LMM) portion and a high molecular mass (HMM) lignin portion. The solids contained in the HMM fraction were isolated using acetone. The pyrolytic lignin (PL) was prepared using ice-water precipitation on the whole bio-oil.[18]

Accelerated aging of bio-oil and pyrolytic lignin

Accelerated aging of bio-oil and PL was performed in glass reaction tubes (ACE, 8648-04-15mL) at 80±1 °C for 24 hours in a convection oven. As a comparison to regular aging without purging, raw bio-oils were also purged with 1.5 hour nitrogen before aging. The purging gas outlet was inserted below the bio-oil surface in order to replace the gas dissolved in the bio-oil as well as the gas contained in the headspace of the aging tube. In some aging experiments, 1 wt. % (based on PL) of radical scavengers, including eugenol, isoeugenol, guaiacol, ascorbic acid (Vc), 2,6-di-tert-butylphenol (DTBP), and butylated hydroxytoluene (BHT) were added to the PL acid solution (PL:acid=1:1 wt. ratio) before aging. In addition, PL diluted with methanol (30 wt.%) was also aged. After aging, the molecular weight of bio-oil was determined by Gel Permeation Chromatography (GPC) calibrated with twelve polystyrene standards (molecular weight ranging from 162 to 3,520,000 g/mol).

EPR characterization of bio-oil and its sub-fractions

Room-temperature continuous-wave EPR measurements of the raw bio-oil (FH), bio-oil fractions (WS, ES, EIS and WIS) and accelerated aged bio-oils (AA) were conducted on a Bruker ELEXSYS-II 500 CW EPR spectrometer operating at approximately 9.5 GHz (X-band). The PL and its sub-fractions (HMM, LMM) were diluted with methanol (0.3 g/mL) for the EPR measurements. Indulin AT lignin (MeadWestvaco), mill wood lignin (MWL, loblolly pine) and lignin acetone insoluble solids were measured in their solid states. For the measurements of the liquid samples, the bio-oil or its sub-fraction was drawn into a glass capillary (smi-micro/petto 1057-C for bio-oil; Drummond Microcaps 1-000-0250 for bio-oil fractions) to ensure a high Q-factor of the resonator. The capillary was sealed on both ends, placed into a standard quartz EPR tube (Wilmad-LabGlass, Vineland, NJ) and then inserted into the resonator. The modulation amplitude was 0.5 G and the modulation frequency was 100 kHz. The time constant was set at 20.48 ms with a 29.65 ms conversion time and a 30.36 s sweep time. 1024 point spectra were collected over a 100 G range. The incident microwave power was 2 mW. No EPR signal difference was found between the spectra obtained under air to those under nitrogen atmosphere. Therefore, all the EPR spectra reported here were collected under air atmosphere. The g-factor and peak-to-peak width (ΔH_{p-p}) was manually measured from the spectra using Bruker Xepr software. The Convx program[19] was used to determine the double integral of the EPR spectra. The relative radical concentration for raw and aged bio-oil was estimated by comparing their double integral to that of standard TEMPO radical.

Results and discussion

It is commonly agreed that radicals can be generated during biomass pyrolysis. For example, Zhang et al., found that cellulose favours a radical decomposition pathway forming levoglucosan via homolytic cleavage of the β-1,4-linkage.[20] Additionally, in-situ EPR characterization of lignin pyrolysis revealed the presence of free-radicals in the pyrolysis vapour phase.[9] These findings identified potential sources of radicals that could be delivered into the pyrolysis liquid product, bio-oil. However, radicals, by nature, are often extremely unstable which may make them less likely to exist in bio-oil, as extensive radical annihilation could take place quickly in the pyrolysis vapour phase. As such, the hypothesis that bio-oil contains free radicals has not been tested before.

In order to test the above-stated hypothesis, bio-oils prepared from loblolly pine at three universities, i.e., NC State University (NC), University of Tennessee (TN) and Auburn University (AU), were characterized with EPR spectroscopy. Three bio-oil samples pyrolyzed from the same feedstock were included in this study to examine the influence of the pyrolysis reaction system on the results. Specifically, the NC and AU bio-oils were produced using a fluidized bed reactor while the AU bio-oil was obtained from an auger reactor. Also, the NC pyrolysis system has both condensers and an electrostatic precipitator (ESP) for oil collection while the AU and TN pyrolysis units collect the bio-oil using ice-cold condensers only. In addition, the AU pyrolysis system uses a vapour filter for char removal which reduces the amount of lignin aerosol in the bio-oil because these components are captured by the filter cake (the pyrolytic lignin in AU oil is only 0.3%).

As shown in Fig. 1, two broad EPR signals were observed for the NC and TN bio-oils; in contrast, the bio-oil collected at Auburn University showed a barely detectable signal indicating its low radical concentration. The spectral peak-to-peak widths (ΔH_{p-p}) and g-factors are between 3.2-5.2 G and 2.0026-2.0033, respectively (see Table 1). The g-factors for bio-oils made at NC and TN are very close to the g-factor of the free electron (2.0023). Additionally, no hyperfine splitting pattern was

observed for any of the spectra shown in Fig.1. A similar EPR signal with the same g-factor and peak shape was also observed after the bio-oil (NC) was stored in a refrigerator (-4 °C) for one month indicating its stable nature. These observations confirm that bio-oil contains unpaired electrons.

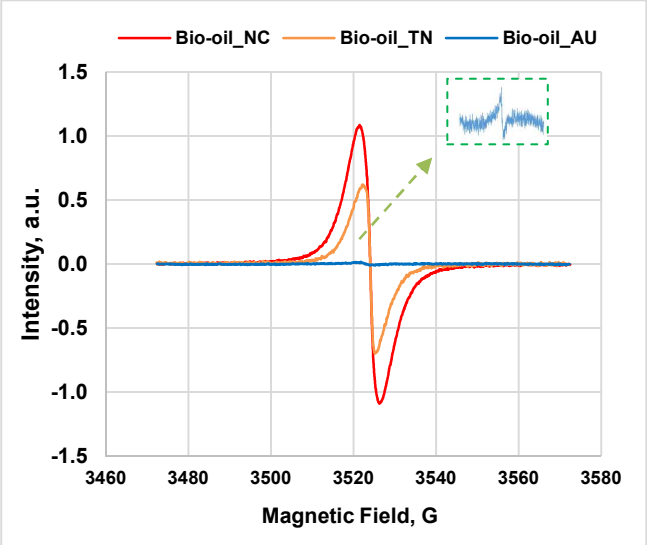

Fig.1 EPR spectra of bio-oils prepared from loblolly pine using pyrolysis reactors at North Carolina State University (Bio-oil_NC), University of Tennessee (Bio-oil_TN) and Auburn University (Bio-oil_AU).

Table 1 Features of the EPR spectra obtained from the bio-oils and lignins.

		ΔH_{p-p}, G	g-factor
Pyrolysis oils[a]	Bio-oil_NC	5.2	2.0026
	Bio-oil_TN	3.2	2.0026
	Bio-oil_AU	4.1	2.0033
Aged bio-oils[b]	Bio-oil_AA_unpurged	5.2	2.0026
	Bio-oil_AA_N_2	5.2	2.0026
	Bio-oil_AA_air	5.2	2.0026
Pyrolytic lignins	PL_NC	4.3	2.0027
	PL_TN	3.7	2.0026
	PL_AU	4.2	2.0029
Lignin fractions	LMM[c]	4.3	2.0033
	HMM[d]	4.8	2.0028
	Solids[e]	5.3	2.0027
Softwood lignins	Indulin AT[f]	8.0	2.0039
	MWL[g]	6.9	2.0039

a. Pyrolysis oil made from loblolly pine at NC State University (NC), University of Tennessee (TN), and Auburn University (AU).
b. Accelerated aged bio-oils using freshly prepared bio-oil at NC State University
c. LMM – low molecular mass lignin (Mw~400 g/mol)
d. HMM – high molecular mass lignin (Mw~1000g/mol)
e. Solids – acetone insoluble fractions of pyrolytic lignin
f. Indulin_AT – Kraft softwood lignin obtained from Meadwestvaco
g. MWL – mill wood lignin extracted from loblolly pine

Bio-oil can be separated into different fractions using water and organic solvents. The bio-oil water soluble fraction (WS) contains water, the ether insoluble sugar fraction (EIS) and the ether soluble fraction (ES). The water insoluble fraction (WIS) contains mainly lignin oligomers and is typically referred as pyrolytic lignin (PL). EPR characterization of these sub-fractions can help identify where the radical species is located in the bio-oil, and the WIS lignin fraction is a prime suspect as free radicals have already been identified in native lignin[21]. The fractionation yields of the various bio-oils generated for this study are listed in Table 2.

Table 2 Solvent fractionation yields of bio-oil samples

	Bio-oil_NC	Bio-oil_TN[c]	Bio-oil_AU[c]
WS[a], %	82.6	80.9	99.7
ES[a], %	28.8		
EIS[a], %	34.1		
H_2O, %	19.7		
WIS[b] (PL)	17.4	15.1	0.3
LMM[b], %	8.8		
HMM[b], %	8.5		
Char, %	0.1		

a. WS – water soluble; ES – ether soluble; EIS – ether insoluble; WS=ES+EIS+H_2O
b. WIS – water insoluble; LMM – low molecular mass lignin; HMM – high molecular mass lignin; WIS=LMM+HMM+Char
c. ES, EIS, LMM, HMM and char fraction of TN and AU bio-oil were not quantified as they were not subjected to EPR study

Fig. 2 presents the spectra of fractionated NC bio-oil. As shown in this figure, the WS, ES and EIS fractions produced no EPR signals at the applied testing conditions. In contrast, an unstructured EPR peak was detected for the WIS lignin fraction (30 wt.% in methanol). Its peak width of 4.3 G and g-factor of 2.0027 is similar to the one from the whole bio-oil (NC) suggesting that the WIS layer is the main fraction that is responsible for the bio-oil radicals. This finding also explains why the AU bio-oil displayed a weak EPR response (Fig.1) as it is nearly WIS free (~0.3 wt.%). In addition, the PL fractions from TN and AU (30 wt.% in methanol) are also EPR active as shown in Fig. 3; however, their signal intensities are significantly lower than that of PL extracted from NC oil. These findings revealed that PL is the only fraction that holds bio-oil radicals.

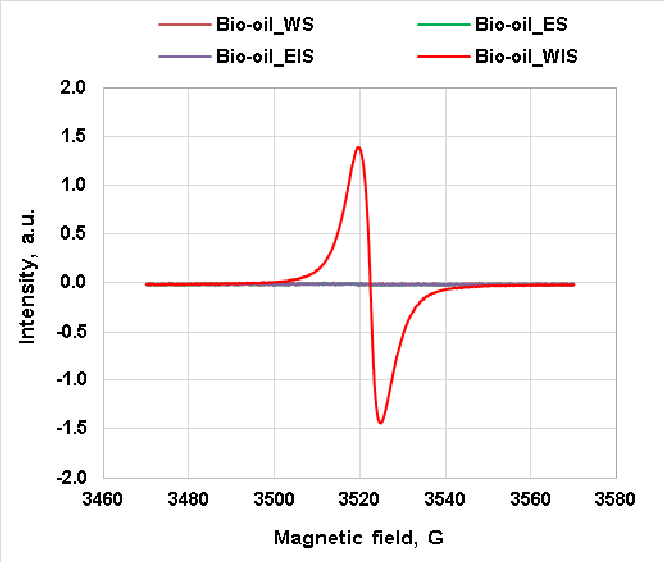

Fig. 2 EPR spectra of solvent fractionated bio-oil (Bio-oil_NC). The WS, ES and EIS fractions had no EPR response and their signal overlapped; the WIS fraction was dissolved in methanol (30 wt.%) for EPR characterization. The bio-oil used for these spectra contained 82.6 wt. % WS (including water, ES and EIS), 17.4 wt. % WIS, 28.8 wt. % ES and 34.1 wt. % EIS.

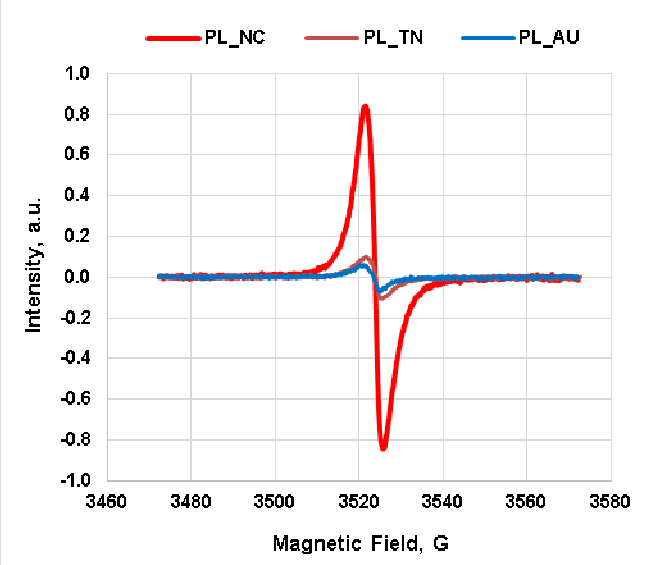

Fig. 3 EPR spectra of isolated pyrolytic lignin (PL) from bio-oils prepared at North Carolina State University (PL_NC), University of Tennessee (PL_TN) and Auburn University (PL_AU).

It is well known that bio-oil contains a small amount of suspended char due to reactor cyclone inefficiency, and a previous study identified free radicals in the pyrolysis char[12]. Thus, there is a possibility that the bio-oil/PL EPR signals shown in Figs. 1 to 3 may only arise from the char radicals. If this is correct, then aging through radicals will not occur as char radicals are typically very stable. Thus, the further fractionation of the WIS layer into LMM, HMM and solid char (acetone insoluble of HMM) fractions followed by EPR characterization could provide an answer to the above inference.

As shown in the spectra of Fig. 4, the LMM, HMM and solid fractions derived from NC lignin (yields shown in Table 2) are all EPR active which, in turn, suggests that these bio-oil liquid components also contribute to the overall EPR signal of the PL. Their ΔH_{p-p} and g-factors, summarized in Table 1, are similar to that obtained from PL_NC while the LMM have slightly higher g-factors. When considering the signal intensities shown in Fig. 4, the signal from HMM is stronger than that from LMM (8:1, double integral) but weaker than that from the solid fraction (1:33, double integral). The equal amounts of LMM and HMM in the methanol solution for the EPR test allow for the quantitative comparison, and their concentration ratio is approximately 8:1. Since the weight ratio of HMM and LMM in the PL is close to 1:1, 8:1 can also represent the radical concentration of HMM:LMM in the PL. On the other hand, since the char sample was measured in the solid state, its comparison to HMM/LMM in PL needs to consider a dilution factor. To compare the radical content in solid to that in HMM and LMM, the solid EPR signal was normalized based on its concentration in PL (~1.5%) and the comparison showed the solids hold approximately 25% of equivalent radical content in the HMM. Based on the above analyses, the PL radicals were found to preferentially reside in the HMM fraction. Additionally, the ratio of radical content in HMM, LMM and solids when presented in PL are estimated to be 8:1:2.

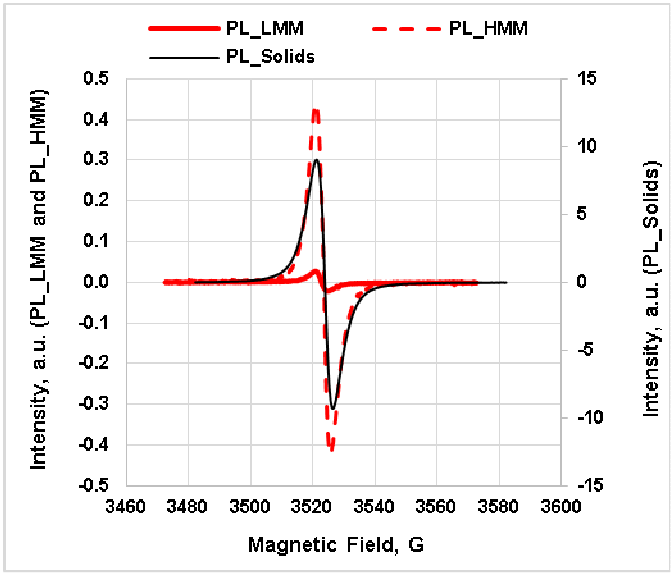

Fig. 4 EPR spectra of isolated pyrolytic lignin fractions (NC). PL_LMM and PL_HMM spectra are referred to left axis; the PL-solids is referred to right axis.

The above experiments confirmed the existence of radicals in the bio-oil and PL; however the EPR spectra did not provide enough information to elucidate their origin(s). Typically, the chemical environment surrounding the radical has an effect on the value of the g-factor and hyperfine coupling constant. The radicals in native lignin and various lignin preparations have been proposed to have semiquinone architecture.[22] Also, gas-phase pyrolysis of a lignin model compound, catechol, yields semiquinone radicals.[23] These findings serve as a foundation

for hypothesizing a radical structure presented in the pyrolytic lignin. Specifically, it is believed that phenoxyl radicals could represent the PL's radical structure. However, as shown in Table 1, the PLs' g-factors are substantially lower than those reported for native pine lignin of 2.0037 [24] and lignin pyrolysis vapour of 2.0072 [9]. The g-factors and ΔH_{p-p} measured for mill wood lignin (MWL) and Kraft lignin from the same pyrolysis raw materials are significantly higher than that of PL and suggest different radical environments in the PL. Since the g-factors of carbon-centered radicals (close to 2.0023) are lower than those of oxygen-centered radicals (g-factor close to 2.0040)[25], these PL's radicals are tentatively concluded to be mainly associated with carbon rather than with oxygen. Additionally, the low g-factor may also result from the spin-orbital interaction between free electrons with metal species contained in the bio-oil,[24] however ICP metal content analyses performed on the bio-oil showed only trace amount of these susceptible metals rendering this possibility less likely. This suggests that radicals in pyrolytic lignin do not favour the formation of phenoxyl radicals which is predominantly favourable in other type of lignins.

The chemical nature of the radical carbon can often be revealed by studying the hyperfine structure presented in the EPR spectrum. However, similar to the EPR signal obtained from isolated lignin, no hyperfine structure was observed for the signal obtained from PL. In an effort to reveal the hyperfine structure, the bio-oil and PL were diluted by a factor of 10, and EPR spectra were acquired under an inert atmosphere to resolve the broad peak. Unfortunately, spectra with peak splitting were not observed. In addition, because oxygen (O_2) is paramagnetic and results in exchange broadening when it collides with another paramagnetic species, an experiment was conducted that involved vacuum degassing the bio-oil and PL methanol solutions before the acquisition of EPR spectra. However, similarly as before, no hyperfine splitting was observed in them.

Because hyperfine splitting patterns were not observed in our EPR experiments, the authors believe that this "hyperfine-absent" phenomenon may be explained by the fact that either a similar chemical environment surrounds the unpaired free electrons or that the acquired EPR spectra are simply averages of signals from groups of highly complex free-radical species. The former hypothesis is less realistic as the lignin structures, even after pyrolysis treatment, are highly complicated, and the analogous chemical environments close to the radicals are less likely to repeat in the PL. The latter explanation, however, may be plausible because radicals resonating in a highly conjugated structure may also provide a reasonable explanation as to why radicals can be stabilized in PL. This radical resonating model can be indirectly supported by two facts. First, PL has already been found to be an effective scavenger targeting DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radicals[26] and its scavenger capability may be attributed to the delocalization effect. As discussed before, the HMM fraction contains significantly more radicals than the LMM fraction. GPC analyses of these two lignin fractions found that the average MW for HMM is ~1000 g/mol (equivalent to eight guaiacol units), while the LMM is ~400 g/mol (equivalent to three guaiacol unites). The high molecular weight HMM fraction will clearly provide higher conjugation degrees than LMM as more aromatic rings can be interconnected. The radicals will prefer to stay in a bigger π system as a result of stabilization. Secondly, in Bayerbach and Meier's most recent publication,[15] pyrolytic lignin (Mw close to HMM) was proposed to stay in a conjugated structure in which aromatic rings are connected either directly or by alternating double bonds which may support our radical resonating model and, in return, our finding indicates that their PL structure model is highly credible.

After confirming radical existence in bio-oil and PL, its impact on bio-oil aging severity was investigated. Radicals in the bio-oil may lead to condensation, and, if this occurs, the radical concentration would decrease significantly due to annihilation and the bio-oil aging severity (determined by average molecular weight) would increase accordingly. However, as discussed earlier, these bio-oil radicals could be very stable and the authors do not expect that they play a detrimental role in aging reactions, especially under low temperature heating.

To elucidate the relationship between radical presence and aging severity, accelerated aged bio-oils were characterized with EPR spectroscopy and their average molecular weights were determined by GPC analyses. To ensure that oxygen dissolved in bio-oil will not affect the detected signals, a nitrogen pre-purged bio-oil was also included. Fig. 5 presents the comparative EPR spectra from raw (fresh) and aged bio-oils. The aged bio-oils (AA_unpurged and AA_N_2) showed slightly lower EPR intensities than the raw bio-oil indicating minor radical annihilation may have occurred during the aging process. As shown in Table 1, the g-factors and peak widths of aged bio-oils remained unchanged after aging. The estimated radical concentration slightly decreased from 185.3 μM (FH) to 150.8 μM (AA-unpurged) and 157.0 μM (AA-N_2), respectively, which is not statistically significant in a typical EPR quantification experiment. Thus, significantly reduced radical concentration likely has not been observed and this confirms the above inference that bio-oil radicals are persistently stable. Also, as shown in Table 3, the two aged bio-oils showed similar molecular weights indicating their similar aging severity. These results indicate that bio-oil radicals may not have a critical effect on aging severity at the applied heating conditions.

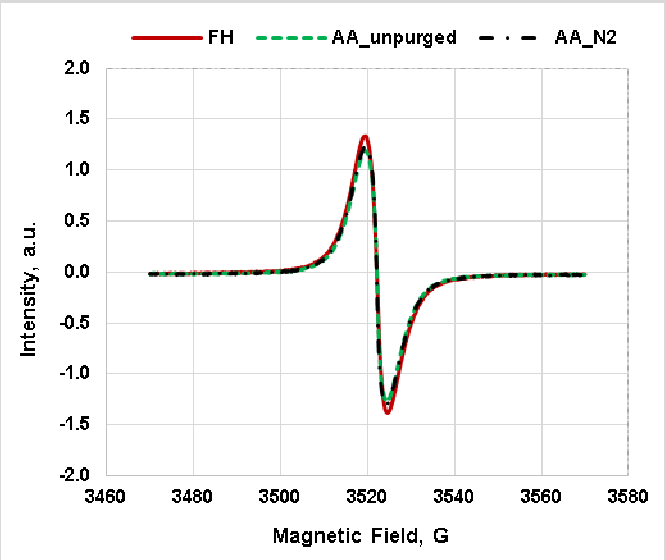

Fig. 5 EPR spectra of fresh and accelerated aged bio-oils. FH: fresh bio-oil; AA_unpurged: aged bio-oil without pre-purging; AA_N_2: aged bio-oil with nitrogen pre-purging.

Table 3 Average molecular weight of accelerated aged bio-oil.

	FH[a]	AA_unpurged[b]	AA_N_2[c]
Mw - RI[d], g/mol	352	705	707
Mn - RI[d], g/mol	263	371	373
Mw – UV[d], g/mol	357	831	841
Mn – UV[d], g/mol	177	226	232

a. Fresh bio-oil
b. Accelerated aged bio-oil without pre-purging before heating
c. Accelerated aged bio-oil with nitrogen pre-purging before heating
d. RI, refractive index detector; UV, ultraviolet detector

Another manner to assess the radical effect on bio-oil aging is through the use of radical scavengers. Radical scavengers remove and stabilize free radicals by being oxidized themselves, and they are often added in fuel products for stabilization during storage and application. If radicals contained in the bio-oil could be stabilized by a scavenger, the bio-oil aging severity represented by a molecular weight increase potentially could be controlled. Alternately, the use of organic solvents such as methanol to dilute the radical concentration may also reduce the radical-radical coupling potential and achieve the same goal.

Since radicals were only found in the bio-oil lignin fraction, accelerated aging with direct addition of radical scavengers or methanol into this fraction was studied. Also, using PL rather than the whole bio-oil excludes the interference from the bio-oil WS fraction that could react and consume the added additives. After fractionation, the PL is essentially acid-free and uncontrolled lignin condensation initiated by protons is less likely to occur; the radical condensation becomes the only path that can increase the molecular weight of aged PL. For example, in Table 4, mild condensation occurred to PL-AA (51% Mw increase) that could be attributed solely to the radical condensation reactions.

Experimentally, to ensure good mixing of the scavengers and the lignin during aging, acetic acid (pKa~4.75) was used to fully dissolve the PL and the scavengers. Although lignin can condense under acidic conditions, as shown in Table 4 acetic acid did not effectively catalyze such condensation because similar aging indexes (51.2% and 71.6%) were observed for aged PL with and without acetic acid. In this sense, the molecular weight increase after PL aging may still result solely from radical condensation. In addition to acetic acid, formic acid was also used to dissolve the PL. The formic acid (pKa~3.75), however, was found to be an effective catalyst on condensing the PL as the Mw of aged lignin increased by 405.9 % when compared to the starting lignin. Therefore, with the presence of formic acid, radical scavenger capability on inhibiting lignin condensation can be assessed under a background where acid condensation of PL could readily occur.

Table 4 Average molecular weight of accelerated aged pyrolytic lignin (PL) with addition of radical scavengers and methanol.

	Mw, g/mol	Aging index[a], %	Mw, g/mol	Aging index[a], %
PL-FH	455			
PL-AA[b]	688	51.2		
PL-FH-MeOH	428			
PL-AA-MeOH[c]	516	20.6		
	Acetic acid		Formic acid	
PL-AA-control[d]	781	71.6	2302	405.9
PL-AA-Eugenol	717	57.6	1630	258.2
PL-AA-Isoeugenol	779	71.2	1577	246.6
PL-AA-Guaiacol	715	57.1	1786	292.5
PL-AA-Vc[e]	722	58.7	1578	246.8
PL-AA-DTBP[f]	810	78.0	1532	236.7
PL-AA-BHT[g]	792	74.1	1592	249.9

a. Aging index was calculated according to (Mw_{aged}-$Mw_{initial}$)/$Mw_{initial}$*100%
b. Aged pyrolytic lignin without addition acid, radical scavenger and methanol
c. Aged pyrolytic lignin with addition of methanol but without addition of radical scavenger and acid
d. Aged pyrolytic lignin with addition of carboxylic acid but without radical scavenger
e. Vc – vitamin c, ascorbic acid
f. DTBP – 2,6-Di-tert-butylphenol
g. BHT - Butylated hydroxytoluene

The average molecular weights of aged PL with the presence of radical scavengers under acidic conditions are summarized in Table 4. According to the aging index, only eugenol, guaiacol, and vitamin C showed minor effects on controlling the lignin condensation in acetic acid solution. As stated earlier, under weak acidic conditions, radicals may be the only initiator to crosslink lignin units. However, using radical scavengers did not show distinct stabilization effects. This again supports the hypothesis that these radicals are already in a stable state; therefore the scavengers cannot provide further stabilization effect to inhibit its kinetically slow polymerization.

In contrast to the situation with acetic acid, when radical scavengers were added to PL in formic acid, the PL's aging indexes were about half of those without scavenger addition. This suggests a positive effect on the scavenger's ability to inhibit lignin crosslinking. However, it is not clear if such an effect can be attributed to their radical stabilization capability as they did not show similar inhibition efficiency when added with acetic acid. With formic acid, radical scavengers may have participated in the acid-catalyzed condensation reactions and

interrupted severe condensation between lignin macromolecules. For example, the radical scavengers' aromatic ring could attack the α-carbocation in the lignin side chain and seal this reaction site for further condensation with other lignin molecules. Therefore, aged PL did not show similar condensation degrees to which scavengers were not added. Besides, the condensation degree is significantly higher with formic acid addition than that with acetic acid and this suggests that acid-condensation is a more predominant reaction than radical condensation.

In addition to using radical scavengers, PL may also be stabilized when its radical concentration can be effectively diluted with an organic solvent such as methanol. Although the PL's radicals are already in a stable state, kinetically they can still engage in a mild polymerization when two free-radicals meet simultaneously. This is why PL had mild molecular weight increase after accelerated aging. However, when the radicals were effectively diluted, the above mentioned radical coupling probability will be reduced and the PL will become more stable. As shown in Table 4, the aging index for aged lignin with methanol dilution is only half of that for the aged raw lignin. Compared to stabilizing radicals with the use of scavengers, particularly in acetic acid solutions where acid-catalyzed lignin condensation is inhibited, diluting the radical concentration showed a more pronounced effect on controlling the lignin condensation severity.

Conclusions

The EPR characterization of bio-oil revealed the existence of free radicals in this bio-derived pyrolysis liquid. The radicals were found to be preferentially located in the bio-oil lignin fraction. Based on the observed EPR spectra (without hyperfine structure) and low g-factors, the radicals are proposed to be stable carbon-centered and delocalized in a highly conjugated π system. Due to their stable nature, the bio-oil radical concentration did not decrease significantly after accelerated aging. The addition of radical scavengers to the bio-oil also showed negligible effects on inhibiting the lignin radical condensation, which further confirmed their stable nature. In comparison to the use of radical scavengers, diluting the radical concentration with methanol showed a more profound effect on controlling the lignin condensation.

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