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Molecular characterization of nitrogen-containing species in switchgrass bio-oils at various harvest times

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HIGHLIGHTS

- Molecular composition of nitrogen species was determined in switchgrass bio-oils.
- The decrease of nitrogen compounds is monitored by harvest month.
- Pyridine and imidazole are proposed as the major structural motifs.

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ABSTRACT

Nitrogen-containing species in bio-oils obtained from fast pyrolysis of switchgrass were studied using high resolution mass spectrometry at various harvest times throughout the year. Almost three hundred chemical compositions of nitrogen species were determined through efficient ionization and accurate mass information. N_2 is the most abundant heteroatom class, followed by NO, N_2O, NO_2, and N_1 compounds. Nitrogen species, especially N_2 compounds, dominate the bio-oil spectra in early summer, but decrease significantly in later harvest times. From the contour plots of double bond equivalent versus carbon number and tandem mass spectrometric analysis, the major structural motif for N_1 and NO class compounds are assigned as pyridine and that of N_2 class compounds as imidazole. The dramatic decrease of N_2 class compounds in delayed harvest bio-oils is well correlated with the decomposition of proteins, represented by imidazole as a pyrolysis product of histidine, as the senescence of the perennial plant proceeds. Some of the heterocyclic aromatic compounds are also found in gas chromatography–mass spectrometry, further supporting our analysis.

1. Introduction

Thermochemical conversion of biomass to biofuel offers a promising biorenewable energy alternative in transportation fuels. In particular, bio-oils produced from fast pyrolysis of lignocellulosic biomass could be used for transportation needs after upstream refining [1]. Fast pyrolysis involves the rapid heating of biomass at temperatures near 500 °C without oxygen to produce biochar, syngas (CO and H_2), and bio-oil [2,3]. Bio-oil is a liquid fraction that contains an aqueous phase and an oily, water-insoluble phase. The physical properties of bio-oil resemble that of petroleum crude oils. However, their chemical compositions are quite different (bio-oil contains up to 50 wt% of oxygen and petroleum crude is almost completely oxygen free) mostly due to the difference in the processes involved; i.e., petroleum crudes are believed to be produced at moderate temperatures but through a very long process under high pressure [4].

Current bio-oil characterization is mostly focused on bulk property measurements such as pH, water content, ash content, viscosity, and elemental composition (CHNO analysis) [5,6]. Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR), and gas chromatography–mass spectrometry (GC–MS) are commonly utilized analytical methods to provide molecular details of bio-oils [7,8]. However, FTIR and NMR are unable to differentiate individual molecules and only provide average functional group information in the mixture. GC–MS is able to characterize individual molecules after GC separation, but identification is limited to volatile compounds present in the database.

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High resolution mass spectrometry (HRMS) combined with soft ionization is a powerful tool for complex mixture analysis and is utilized for direct chemical composition analysis of thousands of molecular compounds in crude oils [9]. We have adapted this approach and successfully demonstrated its application for bio-oil analysis [10,11]. Over 800 chemical compositions were characterized in red oak bio-oil using negative electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) [11]. In our previous studies, we have not detected any nitrogen-containing compounds, mostly because of the low nitrogen content in the biomass feedstock.

Switchgrass (*Panicum virgatum* L.) is a perennial, warm-season grass native to the midwestern U.S. that begins growth in early May, peaks in July, and senesces in the fall. Switchgrass has been described as a "model" biomass crop for bioenergy purposes [12]. Unlike woody biomass feedstock that typically has very low nitrogen content, switchgrass harvested prior to senescence has relatively high nitrogen content that could adversely affect its thermochemical conversion into biofuel due to its pollution effects and catalysis poisoning [13,14]. A recent study by Wilson et al. shows a promising result that late harvested switchgrass leads to low nitrogen content in both biomass feedstock and bio-oil products [5]. Yet, nitrogen still remains even in the latest harvest sample (0.1–0.2 wt% in April harvest bio-oil), which should be removed in the subsequent upgrading process. Unknown molecular characteristics of these nitrogen species could be a hurdle in designing appropriate chemical processes in the upgrading process.

HRMS analysis of nitrogen-containing compounds is routinely reported in petroleum oils [15,16]. There have been a few studies reporting nitrogen compounds in bio-oils [17,18]; however, there is no such study that investigates the structural details at a molecular level. In the current study, we have taken a systematic approach to study the molecular details of nitrogen species in switchgrass bio-oils. First, we compared several ionization methods for the analysis of nitrogen compounds in bio-oils. Second, we have performed a petroleomic analysis of HRMS data, particularly contour plots of double bond equivalent (DBE) versus carbon number, to infer their structural motifs. Third, we performed tandem mass spectrometry (MS/MS) of a few target compounds to obtain structural details from their fragmentation patterns. In parallel, GC–MS analysis was performed to confirm the structural motifs of nitrogen species. Last, we applied this approach for the analysis of bio-oils from various harvest times to infer their molecular changes through a complete growth cycle.

2. Experimental section

2.1. Materials

Switchgrass biomass and resultant bio-oils are essentially the same as previously reported [5]. Briefly, switchgrass trials were established in Boone County, IA, USA (41°55′N, 93°44′W) in spring 2008. Biomass was harvested from replicated plots ($n = 4$) at five different time points during the 2010 growing season (21 June, 20 July, 30 August, and 8 November in 2010, and 4 April in 2011), then dried to a constant weight, ground and sieved using the screen size of 200–700 μm. Bio-oil was produced in a free fall reactor at 550 °C by fast pyrolysis [5,19]. The bio-oils undergo a complex recovery system that fractionates the samples in order to reduce water content and acidity. In the present study, we used samples recovered from the third stage fraction (SF3). This fraction represents electrostatically precipitated aerosol droplets and typically contains the highest nitrogen content according to elemental analysis [5,19].

2.2. GC–MS analysis

The June bio-oil sample was used for GC–MS analysis because of its high nitrogen content. After dilution in methanol to 20% (by weight), 1 μL of sample was injected into a Varian (Walnut Creek, CA, USA) 320-MS system coupled with a 450-GC. The 320-MS is a triple quadrupole mass spectrometer operated in electron ionization (EI) mode and scanned for m/z range of 35–650. The GC column was ZB-1701 (60 m × 250 μm, 0.25 μm film thickness; Phenomenex, Torrance, CA, USA) with 1 μL sample injection (275 °C) at a split ratio of 1:30. The temperature programming of the GC oven started at 35 °C for 3 min followed by a ramp of 3 °C min^{-1} to a final temperature of 280 °C, where it is held for 4 min.

The analysis of GC–MS data was performed using AMDIS software (NIST, v2.69) for automatic deconvolution and database search. The NIST08 EI-MS spectral library was used with a minimum match score of 750.

2.3. High resolution mass spectrometry

One representative bio-oil sample from each different harvest time was dissolved in methanol to 1 mg mL^{-1} to minimize chemical change during storage and stored in Nalgene bottles at 4 °C until analysis. Stock solutions were diluted to a final concentration of 0.1 mg mL^{-1} in 50:50 (v/v) methanol and water for electrospray ionization or 85:15 (v/v) methanol and toluene (Fisher Scientific, Fair Lawn, NJ, USA) for atmospheric pressure photoionization (APPI). A 50:50 (v/v) methanol and water solvent system was also used for APPI, but without much difference except lower ion counts and fewer low-mass ions (data not shown). Pyridine-d_5 was purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada) and added as an internal standard to a final concentration of 1 μM for semi-quantification of nitrogen-containing species.

A majority of HRMS data acquisition was made using a linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap Discovery; Thermo Scientific, San Jose, CA, USA). The orbitrap MS data was acquired at the mass resolving power of 30,000 at m/z 400 (transient of 0.4 s). A 5 kV source voltage was used for positive-ion ESI and −4.5 kV for negative-ion ESI. A vacuum ultraviolet (UV) lamp (PhotoMate, 10.0/10.2 eV; Syagen, Tustin, CA, USA) was used for APPI. For MS/MS analysis, isolation and fragmentation were performed using the linear ion trap of the instrument and mass spectral data acquisition was made using the orbitrap. Collision energies of 35–50% and precursor isolation width of ±1.0 Da were used for MS/MS. FT-ICR (7T Solarix, Bruker, Billerica, MA, USA) was also used for some of the initial experiments with positive ESI at 4.5 kV and with a time-of-flight of 0.4 and 0.6 ms and at the mass resolving power of 280,000 at m/z 400 (transient of 0.9 s).

2.4. Data analysis

Orbitrap MS data was exported to a text file using QualBrowser (Thermo Scientific) for all the peaks with their relative intensities above 1%, which is well above six times the baseline noise. The text file was imported to Composer (Sierra Analytics, Modesto, CA, USA) for calibration, chemical composition assignment, and visualization. Five-point internal calibration was performed by Composer using the exact masses of known peaks (e.g. pyridine-d_5 at m/z 85.0807 and levoglucosan at m/z 185.0420). The possible number of each element in chemical composition analysis was limited to 30 carbons, 60 hydrogens, 15 oxygens, and 5 nitrogens. Chemical compositions were assigned within a mass tolerance of 3 ppm in positive ion mode and 5 ppm in negative ion mode. Dopant peaks in APPI, mostly toluene and its oxidation products, are not included in the chemical composition analysis.