Biomass and Bioenergy 108 (2018) 198–206

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Research paper

Determining aromatic and aliphatic carboxylic acids in biomass-derived oil samples using 2,4-dinitrophenylhydrazine and liquid chromatography-electrospray injection-mass spectrometry/mass spectrometry☆

Samuel A. Lewis Sr.[a], Raynella M. Connatser[a,*], Mariefel V. Olarte[b], James R. Keiser[a]

[a] Oak Ridge National Laboratory, 1 Bethel Valley Rd., Oak Ridge, TN 37830, USA
[b] Pacific Northwest National Laboratory, 902 Battelle Boulevard, Richland, WA 99352, USA

ARTICLE INFO

Keywords:
Biomass
Pyrolysis oil
Derivatization of carboxylic acids
2,4-Dinitrophenylhydrazine (DNPH)
Carbonyl speciation
Tandem mass spectrometry

ABSTRACT

Converting biomass to a useful fuel commonly incorporates the pyrolysis of the biomass feed stock. The base liquid fraction usually contains high concentrations of ketones, aldehydes and carboxylic acids, of which each can cause detrimental issues related to the storage and upgrading process. Knowing the carbonyl species and the concentration of each will provide value information to the pyrolysis researchers, specifically as that community branches into more targeted end-products such as jet fuel or biogenic-derived oxygenate-containing fuel products. The analysis of aldehydes, ketones and small alkyl carboxylic acids using 2,4-dinitrophenylhydrazine (DNPH) derivation method has been well documented and the method is commonly used the analytical community. By using liquid chromatograph coupled to tandem mass spectrometry, biomass sample analysis can be complete with identification of most carbonyl species. The issue of identifying isobaric ketone and aldehyde compounds can be resolved by utilizing differences in retention time or characteristic fragment ions of ketones and aldehydes. One issue which could not resolved using published methods was identifying aromatic or large non-aromatic carboxylic acids from their corresponding hydroxyl aldehyde or ketone analogs. By modifying the current method for determining carbonyls in biomass samples, carboxylic and hydroxyl-carbonyl can be determined. A careful adjustment of the pH during the extraction procedure and extended heating time of the DNPH solution allowed for the successful derivation of aromatic carboxylic acids. Like other dinitrophenylhydrazones, carboxylic acid derivatives also produce a unique secondary ion pattern, which was useful to distinguish these species from the non-acid analogs.

With the concerns surrounding the limited supply of fossil fuel and its adverse effects on the environment, the need to develop a sustainable bio-derived fuel becomes more pronounced [1–3]. Sources as diverse as non-food grasses, hard and softwood trees, shrubs, algae, and even agricultural and municipal waste streams are being investigated as sources of biologically renewable fuels and chemicals [4–6]. Several issues have arisen during the development of processes to convert biomass to fuels, including accelerated corrosion of processing and storage materials of construction and instability of intermediate products such as polymerization and phase separation [7–10]. One widely researched method of breaking down the biomass, which is composed mainly of lignin and cellulose, to more fuel-like molecules is via pyrolyzing the biomass and then upgrading the pyrolysis oil catalytically and/or via hydrothermal liquefaction [11,12]. Pyrolysis is the intense heating of biomass in a dearth of oxygen to produce energetically valuable but compositionally complex liquids and off-gasses [13]. During catalytic upgrading of the pyrolysis liquids, the aim is to minimize acidity and reduce the prevalence of oxygen using a supported metal catalyst and hydrogen under some pressure; hydrothermal liquefaction reduces oxygen level and acidity [14]. By examining the chemical structures of lignin and cellulose, it is easy to see how carbonyls and carboxylic acids can be formed during the pyrolysis process. Such potentially reactive species often create obstacles to upgrading chemistries via fouling, catalytic deactivation, corrosion, or product destabilization [15].

One issue lacking adequate redress involves determining the

☆ Notice: This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/downloads/doe-public-access-plan).

* Corresponding author. Oak Ridge National Laboratory, The NTRC Bldg., 2360 Cherahala Blvd., Knoxville, TN 37932, USA.
E-mail address: connatserrm@ornl.gov (R.M. Connatser).

https://doi.org/10.1016/j.biombioe.2017.10.043
Received 31 July 2017; Received in revised form 26 October 2017; Accepted 29 October 2017

carbonyl species which are formed and the rate of deoxygenation of these species. There are analytical methods already in place to determine bulk properties such total aldehyde and ketones, and total acid concentration [16–23]. These methods give good guidance and simple conclusions regarding the pyrolysis chemistry and upgrading process. However, disadvantages are associated with each of these methods, and no single approach can describe even the majority of the 300 + constituents of most biomass-derived liquids, especially pyrolysis oils. With the advance of the pyrolysis-to-fuel process, there is a need to know how individual compounds behave during the process. The structural elucidation of larger carboxylic acids will also become increasingly important as upgrading processes evolve to remove the smaller but ubiquitous acetic and formic acids in pyrolysis oils. What's more, as this work demonstrates, a significant portion of the acid content could actually arise from molecules such as aliphatic acids. These energetically valuable compounds, were they accurately quantified, could be better targeted for upgrading to fuel-like liquids via traditional acid catalyzed esterification rather than the cost and heat intensive catalytic hydrotreating currently utilized on the whole pyrolysis oils.

One proven method for the speciation of aldehydes and ketones is by derivation with DNPH and analysis by liquid chromatography with on-column ultraviolet–visible spectroscopy (HPLC-UVvis) [17,22,24,25]. In fact, this important quantitative and qualitative analytical method, first published by Allen and Brady, has been widely used for physiological, biological, and environmental samples, as well as automotive exhaust and water samples specifically [26–37]. Most literature states that DNPH can be selective to only ketones and aldehydes, which was also found to be true with our bio-oil samples if the sample is not pH adjusted, properly extracted, and heated in a prescribed fashion. In these literature works, the generalized explanation for the resistance to DNPH addition reaction with carboxylic acids, amides and esters, focuses on the fact that these moieties exhibit resonance-associated stability as a lone-pair of electrons interacts with the p-orbital of the carbonyl carbon. This leads to enhanced charge delocalization in the molecule that would be lost upon addition of a reagent to the carbonyl group [7,38]. Also with carboxylic acids, there is the effect of the DNPH acting as a base, leaving the resulting carboxylate negatively charged and unable to be attacked by the nitrogen nucleophile [38,39]. These general energetic barriers are defeated by adding heat to enhance the addition reaction and keeping pH low to facilitate nucleophilic attack on the carboxylic acid carbonyl carbon by the DNPH. Despite the noted resistance to DNPH addition for carboxylic acids, it has been published previously that small carboxylic acids (not from bio-oil) can also be derivativatized by heating the sample in the in the acidified DNPH solution [4].

When analyzing the complex oxygenated products of pyrolysis derived from the cellulose and lignin components of biomass, several groups of compounds can be made, including aldehydes, ketones, carboxylic acids, hydroxyl aldehydes, hydroxyl ketones, hydroxyl carboxylic acids with a number of isomerization possibilities. Using only HPLC-UVvis instrument can lead to numerous unknown peaks and misidentified peaks. The modification of sample preparation to facilitate co-derivatization of carboxylic acids along with the more traditionally derivatized carbonyls combined with the careful use of known standards for the classes of acids to allow discernment between the carboxylic acids and their isobaric ketone or hydroxyaldehyde analogs.

1. Experimental

1.1. Chemicals

All solvents were HPLC-grade and at least 99.9% pure. All reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA, except DNPH cartridges, Catalog #, which were purchased from Waters, Inc., Taunton, MA, USA.

Bio-oil tested for this work were produced from a The feedstock for this study is composed of a mix of 45 wt% pine, 25 wt% forest residue and 30% industrial construction waste streams that were composited and pelletized at the Idaho National Laboratory (INL) in Idaho Falls, ID, USA. The blended feedstock was then pyrolyzed at the National Renewable Energy Laboratory (NREL) in Golden, CO, USA, using their Thermochemical Process and Development Unit (TCPDU). The entrained flow reactor used nine zones set at 500 °C with a residence time of 2.9 s. The Pacific Northwest National Laboratory (PNNL) in Richland, WA, USA upgraded the bio-oil. Stabilization was done at 140 °C and 8273.7 kPa H_2 with in-house synthesized ruthenium carbon using a continuous trickle bed reactor. Catalyst synthesis was previously described [40]. Some properties of the starting pyrolysis oil and the stabilized oil collected after 12.5 h time-on-stream that was analyzed in this paper are presented in Table 1. Duplicate samples were tested. Elemental, Karl-Fisher and Total Acid Number analyses were performed by ALS Laboratory (Tucson, AZ). The carbonyl content was determined at PNNL following NREL TP-5100-65888, as described previously [20].

Table 1
Bio-oil and stabilized oil composition.

	Amount	
	Pyrolysis Oil	Stabilized Oil (TOS = 12.5 h)
C, wt% (as received)	40.21	39.97
H, wt% (as received)	7.92	8.8
O, wt% (by difference)	51.76	51.11
N, wt% (as received)	0.12	0.12
S, ppm (as received)	< 0.02	< 0.02
Water Content (Karl Fisher), wt%	28.8	32.4
Total Acid Number, mg KOH/g bio-oil	91	83.1
Carbonyl Content, mmol C=O/g bio-oil	5.1	2.2

1.2. Sample preparation and derivatization

In preparation for separation and detection, 0.1 gram (g) of each bio-oil sample was weighed into a clean borosilicate glass vial and 1 cm^3 of deionized (D.I.) water (Elix 5 by Millipore, Inc., Burlington, MA, USA) was added. The sample was placed into an ultrasonic bath (Branson 2510, Danbury, CT, USA) for 1 min (min.) and then brought to pH 6 with 10–90 mm^3 of 10% NH_4OH in D.I. water. Because of the small sample volume, pH strips were used to measure the pH ("pH-indicator strips pH 0–14 Universal indicator"; EMD Millipore, Billerica, MA, USA; catalog number 1095350007). A 1 cm^3 methylene chloride rinse (vortex then centrifuge, 1 min each) is then carried out and the water phase is withdrawn. This phase is then brought to pH 3 with 200–700 mm^3 of hydrochloric acid (0.01 g cm^{-3}) in D.I. water. Separately, 5 cm^3 methanol is flushed across a SepPak® DNPH-Silica Cartridge Plus-Short Body (360 mg), catalog number WAT037500. These two solutions, the withdrawn water phase and the flushed methanol now containing DNPH, are combined and incubated at 80 °C for 5 h. The solution is then allowed to return to room temperature and sequential methylene chloride rinses totaling at least 5–7 cm^3 are carried out. The methylene chloride is evaporated to dryness under gentle heating (40 °C) and nitrogen flush (3 dm^3 per minute), and the derivatized sample is brought back up in 1 cm^3 acetonitrile for analysis.

1.3. Instrumental

The analysis was carried out using a Hewlett Parkard 1100 liquid chromatograph and Bruker Esquire 6000 ion trap mass spectrometer. The separation was completed using a Waters X-Bridge Phenyl BEH column (3 × 100 mm) with 3.5 mm^3 particle size, catalog number 186003328. The liquid chromatograph parameters were as follows. The flowrate was 0.7 cm^3 per minute and the column temperature was