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Characterization of biofuel refinery byproduct via selective electrospray ionization tandem mass spectrometry

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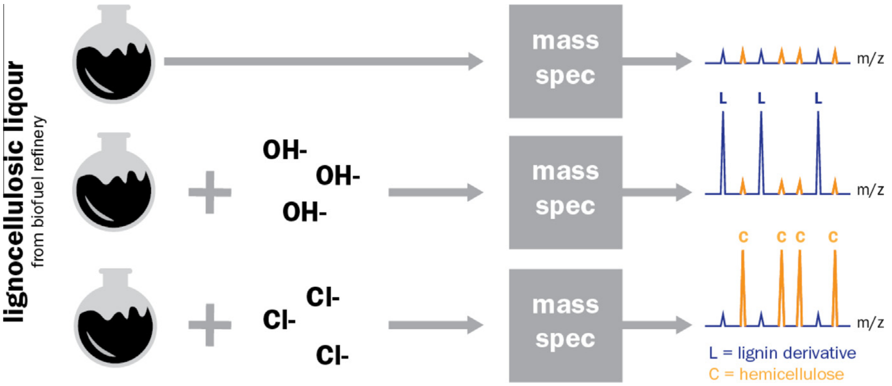
HIGHLIGHTS

- Electrospray provides soft ionization of autohydrolyzate from 100 to 3200 m/z.
- Ionization dopants allow discrete analysis of cellulose and lignin sans separation.
- Chloride attachment in electrospray ionization assists in ionizing hemicellulose.
- Deprotonation by hydroxide specifically enhances ionization of lignin derivatives.

GRAPHICAL ABSTRACT



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ABSTRACT

To achieve economic viability, biorefineries need to increase efficiency through characterization of byproducts for the purpose of valorization. One such byproduct is the liquid stream produced after autohydrolysis pretreatment, autohydrolyzate liquor, which contains valuable organic derivatives of hemicellulose and lignin from biomass. To characterize the autohydrolysis liquor, we employed a novel method for such liquor analysis that uses electrospray ionization and ion dopants in combination with tandem mass spectrometry using a quadrupole–time-of-flight mass spectrometer. Electrospray expands current analysis of such liquors through softer ionization. Ion dopants provide for differentiation of the complex mixture components without requiring derivatization or preliminary separation. The dopants—ammonium chloride and sodium hydroxide—primarily target and enhance ionization of hemicellulosic or lignin derivative species, respectively, based on the species' differing functionalities. Valuable structural information can be gleaned from these enhanced species by ion isolation and collision-activated dissociation (CAD), which reveals the presence of hemicellulosic or lignin derivative functionalities. These ionization techniques coupled with CAD enabled us to not only confirm the presence of low molecular weight ions, such as vanillin, as previously seen with gas chromatography-mass spectrometry but also expand the characterization to high molecular weight species. This expanded knowledge of the composition of autohydrolyzate liquor opens up the potential to develop lucrative co-products from this stream in a commercial biorefinery.

Abbreviations: HPAEC, high-performance anion-exchange chromatography; HRMS, high resolution mass spectrometry; CAD, collision-activated dissociation; AH-L, autohydrolyzate liquor.

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1. Introduction

Biomass feedstocks are becoming a more investigated source of renewable energy as an alternative to fossil fuels [1]. Biorefineries have the possibility to produce sustainable and environmentally friendly sources of energy from lignocellulosic biomass feedstocks. Unfortunately, biorefineries are not yet efficient. In the case of ethanologenic biorefinery processes, only a fraction of the structural carbohydrates are actually converted into the final product. The rest of the conglomeration—unhydrolzyed cellulose microfibrils bundled with lignin and residual hemicellulosic polysaccharides [2]—is typically burned for heat or electricity production [3].

This lignin-rich mixture could be a step toward economic salience for the biofuel refineries. If the complex mixture could be separated, it would become a valuable product of the refinery [4]. Instead of being burned, the lignin should be valorized into co-product streams for raising the economic feasibility of a biorefinery process [5]. In addition to having a higher energy content than ethanol, lignin has many applications outside of biofuels from adhesives to cement additives [3]. It is also the largest source of renewable material with an aromatic phenolic backbone and could be used for the production of polymeric building blocks and valuable phenolic compounds [3]. Unfortunately, separation of lignin from the lignocellulosic biomass mixture is challenging and inhibited by a lack of knowledge of the exact make-up of the complex mixture. Future uses of lignin rely on good characterization of lignin and tailoring of separation treatments to yield valuable lignin products [6].

One of the leading pretreatment methods in terms of investigated techno-economic feasibility is autohydrolysis [7]. Utilizing only pressure and hot deionized water, autohydrolysis is known to disrupt the structures of the hemicellulose and lignin, enabling greater accessibility for depolymerizing cellulolytic enzyme systems. Notable benefits of utilizing autohydrolysis pretreatment in a biorefinery process include (1) the absence of reagent and associated recycling costs, (2) existing industrial-scale infrastructure capable of housing pretreatment (pulp mill pre-hydrolysis reactors), and finally (3) the potential value of the generated autohydrolyzate liquor, obtainable by solid-liquid separation.

Unfortunately, autohydrolyzate liquor (AH-L) is complex in nature and challenging to analyze fully with one instrument. Gas chromatography-mass spectrometry (GC-MS) analysis of ethyl acetate extract from dilute-acid hydrolyzate was shown to be an effective means of quantifying monomeric lignin fragments in hydrolyzate [8]. Despite this method's success, it is intrinsically limited by soluble-lignin's volatility within the applied column, hindering analysis of higher mass lignin fragments. Concerning hemicellulose-derived carbohydrates, GC-MS requires derivatization in the form of permethylation or acetylation to facilitate analysis [9]. Such derivatization, intended to simplify analysis, instead increases the variety of compounds present, making the mixture more complex and the spectrum more convoluted. The spectrum was made even more complex by the electron ionization (EI) coupled to the GC-MS. EI is a harsh ionization technique that typically fragments compounds, turning a single peak into several and rendering the molecular ions unobservable. A system utilizing high-performance anion-exchange chromatography (HPAEC) coupled with mass-spectrometry (MS) was capable of quantifying a variety of carbohydrates containing up to six residues in length [10]. However, the presence of unidentifiable peaks within the HPAEC-MS chromatograms again alludes to the less-than-complete characterization of pretreatment hydrolyzates that continues to occur in spite of great effort.

High resolution mass spectrometry (HRMS) and tandem mass spectrometry (MSMS) in concert with electrospray ionization (ESI) and selective ionization dopants provide a means of successive characterization of both lignin- and hemicellulose-derived fragments soluble within AH-L. ESI, a soft ionization technique, enables ionization of higher mass compounds than ionizable with GC-EI-MS. GC-MS is limited by the volatility of the compounds and decomposition resulting from the temperature of the GC oven, which lowers its mass range; EI further encourages degradation of the compounds via fragmentation. The soft ionization of ESI overcomes the previously discussed challenges associated with complete characterization of AH-L.

Although HRMS is capable of differentiating thousands of components in an individual sample, there remain some hurdles to full analysis. One of the issues associated with such complex mixture analysis via mass spectrometry is ion suppression—a phenomenon where certain ions are less efficiently ionized and falsely appear at lower abundances in mass spectra or disappear altogether. It becomes necessary to tailor ionization in order to separately enhance the ionization efficiency of certain mixture components for several stages of analysis. Ammonium chloride (NH_4Cl) and sodium hydroxide (NaOH) have been demonstrated to enhance ionization efficiency for hemicellulosic and lignin derivative species, respectively, in primarily model compound mixtures [11–13]. We combined these two techniques successively to analyze the wide variety of hemicellulosic species and then lignin derivative species present in AH-L, thereby minimizing the need for lengthy separation and extraction steps.

Once the targeted ions have been selectively enhanced, collision-activated dissociation (CAD) can be utilized to gain structural information. All ions except the target ions are filtered out by the quadrupole mass analyzer. Then the target ions are accelerated and collided with nitrogen gas to dissociate, or fragment, the ions. The resulting fragment ions—known as product ions—are recorded by the mass analyzer and reported in the CAD spectrum. With the higher resolving power afforded by the quadrupole time-of-flight (QTOF), we can determine the elemental composition of the fragments for structure elucidation.

In this study, ionization dopants were used to selectively enhance ionization of mixture components through gas phase basicity matching for simpler characterization of hardwood and non-wood AH-L. The resulting enhanced ionization—exhibiting up to a 30-fold increase in ion abundance for the targeted compounds—allowed for separate analysis of carbohydrates and lignin, respectively, without extraction or derivatization of the sample.

2. Experimental

2.1. Raw materials

All raw materials were air-dried at room temperature for two weeks to reach constant moisture. After air-drying, the moisture content of each biomass was determined by the mass loss after oven-drying overnight at 105 °C. Hardwood chips from red maple (*A. rubrum*) were obtained from the North Carolina State University Cooperative Tree Improvement Program and hand-cut to a size of 2 cm × 1 cm × 0.5 cm (length, width, thickness). The non-woody biomass, sugarcane bagasse, was kindly donated to the Department of Forest Biomaterials by a cooperating Brazilian bioenergy research institute. Before experimentation, both feedstocks were subjected to Soxhlet extraction utilizing an organic extraction solvent composed of 2:1 (v/v) benzene/ethanol. The purpose of this is to remove non-structural extractives that can hamper accurate analysis of results from the perspective of lignin. After 48 h of extraction, biomasses were air-dried under a fume hood and then stored in plastic bags at room temperature prior to pretreatment. In addition, part of the extractive-free feedstocks was ground by