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Ultrasound-Assisted Sequential Extraction for Lignocellulose Pyrolysis Bio-Oil Fractionation. Part I: Method Development

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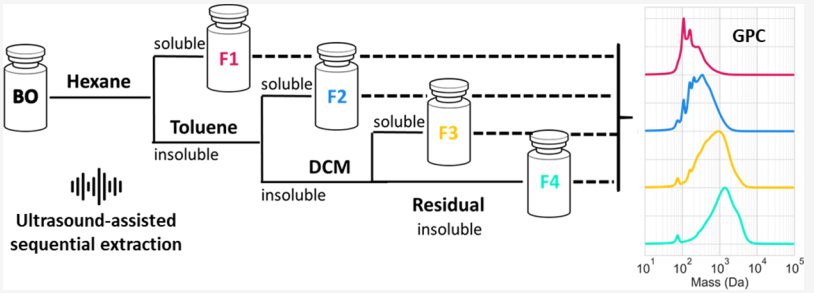

ABSTRACT: The work herein presents a novel ultrasound-assisted sequential extraction (UASE) method for fractionating bio-oils derived from lignocellulosic biomass pyrolysis. The fractionation method uses hexane, toluene, and dichloromethane, to produce fractions with distinctive composition with minimal sample loss as no stationary phases are used. Characterization of the fractions and the original bio-oil by gel permeation chromatography (GPC), proton nuclear magnetic resonance (^{1}H NMR), gas chromatography−mass spectrometry (GCMS), and pyrolysis-GCMS, revealed marked compositional differences between the fractions. Lighter fractions contained a high concentration of light aromatic compounds, ideal for hydrogenation into fuel, whereas heavier fractions showed similarity to lignin standards with an increased presence of sugar-like compounds. This study introduces a novel fractionation technique for bio-oil analysis and establishes a framework to standardize bio-oil quality assessment, with plans to apply these methods to various bio-oils in future research.

1. INTRODUCTION

Lignocellulosic biomass, such as forestry residues, can be transformed into valuable feedstocks for fuel applications through thermochemical processes such as fast pyrolysis.[1] This process breaks down macromolecules such as lignin, cellulose, and hemicellulose, and produces several products: a complex liquid mixture of organic compounds with high oxygen content (compared to petroleum-derived liquids), water, a solid phase known as char, and noncondensable gases.[2] The resulting liquid product, known as bio-oil, offers a lower environmental impact alternative to fossil fuels and can be further upgraded to advanced biofuels.[3] However, the wide boiling range and broad molecular weight distribution of these complex bio-oils, in conjunction with their high acidity and thermal instability, poses a significant challenge to advanced biofuel production. Consequently, comprehensive chemical characterization of bio-oils can provide critical information for developing and optimizing upgrading processes. The crucial role of these biofuel intermediates in the clean energy transition, has prompted analytical chemists to develop various approaches to understand their composition.[4,5]

One common analytical strategy to target the chemical complexity of bio-oils is fractionation, e.g., by chromatography and solvent extraction, followed by the characterization of the separate fractions.[6] For example, liquid−liquid extraction has been consistently reported by Oasmaa and co-workers since 1996.[7] The authors initially proposed a method focused on the water-soluble fraction, which was separated into ethyl-ether soluble and insoluble fractions. However, the water-insoluble fraction remained unexplored until later studies, in which the authors proposed a more comprehensive separation scheme.[8] This advanced approach began with *n*-hexane extraction, followed by fractionation by water solubility. However, it included further separation of water-insoluble fractions into dichloromethane (DCM) soluble and insoluble parts. These studies led to the definition of low molecular mass (LMM) lignin and high molecular mass (HMM) lignin, which correspond to the DCM soluble and insoluble fractions. This fractionation process was later applied to analyze hydrotreated

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bio-oil liquids in order to evaluate the hydrotreatment process performance,[9,10] and inspired further research based on the described fractionation method.[11] Recent reports have described other liquid−liquid extraction methods,[12] along with other approaches such as supercritical fluid extraction,[13] and chromatographic methods such as gel permeation chromatography (GPC),[14] silica-gel adsorption,[15] flash chromatography,[16] and centrifugal partition chromatography.[17]

The focus on lignin fractionation and characterization, given its central role for bio-oil production, has led to several studies based on size exclusion chromatography (SEC).[18,19] Several reports have shown that higher molecular weight (MW) components have higher oxygen content. Furthermore, recent studies by Mattsson et al.[20] have shown that lignin depolymerization in subcritical water results in oils whose fractionation suggests a strong correlation between polarity, or oxygen content, and MW, as pointed out by compositional analyses and SEC. Moreover, Van Aelst et al.[21] demonstrated a similar trend in six distinctive bio-oil fractions obtained from the reductive catalytic fractionation of pine wood. Their fractionation process used a heptane/ethyl acetate solvent mixture, mixed in various ratios and under controlled temperature. The authors demonstrated that cumulative GPC chromatograms of these fractions closely matched the size distribution of the original bio-oil. The role of SEC, particularly GPC, is central in bio-oil research, as it serves as a straightforward and effective analytical method for comparing bio-oil samples derived from various production processes. Such comparisons are important, as certain key physical properties of bio-oils (density, viscosity, etc.) are closely linked to their molecular structure/composition.[22−24] However, comprehensive characterization of bio-oils requires the use of complementary techniques such as nuclear magnetic resonance (NMR), gas chromatography mass spectrometry (GCMS), and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to analyze the samples at the molecular level.[25−29]

The work herein describes a novel fractionation method, based on the reported methods described above but enhanced by ultrasound-assisted sequential extraction (UASE). Pure solvents are used for each extraction step (hexane, toluene, and dichloromethane), and all the separation processes are carried out with small solvent and sample volumes (<30 mL). This novel approach minimizes sample loss and facilitates the concurrent efficient processing of multiple samples. Furthermore, by avoiding the use of water for fractionation, it significantly decreases solvent evaporation time. The use of high-energy ultrasound waves maximizes solubility for improved fractionation. The original sample, along with its resultant fractions, were characterized by GPC, ^{1}H NMR, GCMS, and pyrolysis-GCMS. This analytical strategy allows for an in-depth knowledge of bio-oil composition. This methodology represents a substantial progression in the field of lignocellulose pyrolysis bio-oil fractionation, offering a practical alternative to existing fractionation techniques.

2. MATERIALS AND METHODS

2.1. Samples. The sample used for method development, referred to as BO, was a fast pyrolysis bio-oil produced from pinewood by BTG BTL (Enschede, Netherlands) company, that was provided by TotalEnergies Research and Technology (Gonfreville, France). The method was also applied to three distinct bio-oil samples provided by the Research Triangle Institute (RTI) International (North Carolina, USA), each produced via different fast pyrolysis processes. Bio-oils referred to as CFP-1 and CFP-2, were produced from Douglas Fir crumbles using the same catalytic fast pyrolysis (CFP) process in a 1-ton-per-day (TPD) pilot plant, but under different operational conditions. CFP-1 was processed at a temperature of 480 °C and a feed rate of 57.7 kg/h. Conversely, CFP-2 was produced in the same 1TPD pilot plant, but at a slightly lower temperature of 464 °C and a feed rate of 49.5 kg/h. Both samples utilized gamma alumina as a catalyst. The final sample, labeled RCFP, resulted from reactive catalytic fast pyrolysis (RCFP) of loblolly pine in a lab-scale fluidized bed reactor, 2.5 in. in diameter, under a hydrogen-rich atmosphere (80 vol %) and a molybdenum catalyst at a mean temperature of 500 °C, fed at a rate of 4−5 g per minute.[30,31]

2.2. Solvents and Standards. HPLC-grade solvents were used for fractionation and various chromatographic analyses. *n*-Hexane, toluene, dichloromethane stabilized with 50 ppm amylene, and THF, both nonstabilized and stabilized with 250 ppm dibutylhydroxytoluene (BHT), were purchased from Scharlab, Spain. Deuterated acetone-d_6 was obtained from Sigma-Aldrich, Germany, and served as the solvent for ^{1}H NMR analyses. Alkali lignin or Kraft lignin (Sigma-Aldrich, Germany) was used as a standard to compare with the BTG BTL pyrolysis bio-oil. This standard is a conjugated base of lignin. A dispersion was formed by mixing 200 mg of alkali lignin in 3 mL of THF. However, it only partially dissolved after adding 2 drops of concentrated HCl (Sigma-Aldrich, Germany). Following this, the THF-solubilized, regenerated lignin was separated from the resultant solids alkaline chloride salts and the excess of unreacted alkali lignin. This separation was achieved through filtration using a PTFE (polytetrafluoroethylene) filter with a 0.45 μm pore size purchased from VWR (Pennsylvania, USA).

2.3. Fractionation Methodology and Sample Characterization. *2.3.1. Bio-Oil Fractionation by Ultrasound Assisted Sequential Extraction.* Approximately 1 g of bio-oil was placed in a 30 mL glass vial and mixed with 5 mL of *n*-hexane. Sonication was carried out for 10 min at room temperature using a BANDELIN electronic GmbH & Co. KG (Berlin, Germany) ultrasound bath device, operating at a frequency of 35 kHz and with a power 40/160 W. The ultrasound parameters mentioned above have previously been used to dissolve complex mixtures, such as petroleum asphaltene fractions dispersed in toluene, without disrupting their aggregates, as seen through GPC.[32] This indicates that the energy provided by ultrasound waves enhances the interaction between the solvent and analyte while maintaining the chemical integrity of the analyte. The resulting mixture was subsequently centrifuged at 1500 rpm for 5 min. The supernatant was collected in a preweighed vial. The extraction with n-hexane was repeated ten times on the remaining insoluble fraction, and the collected supernatants were combined in the same preweighed vial. The remaining insoluble bio-oil underwent the same fractionation process ten times, first with toluene and then with dichloromethane (DCM) for another ten times.Each extracted fraction, as well as the residual insoluble bio-oil, was dried under N_2 until weight stabilization. The procedure yielded four fractions: hexane-soluble (F1), toluene-soluble (F2), dichloromethane-soluble (F3), and the residual bio-oil (F4). Each fraction was weighed to determine its mass percentage in the original sample, and the percentage of light volatiles and water lost during drying under N_2 was calculated gravimetrically. This method primarily results in the recovery and analysis of nonvolatile compounds. This process was performed in triplicate for the BO sample to evaluate its repeatability. For the other samples (CFP-1, CFP-2, and RCFP), it was carried out once to assess the method's applicability across different bio-oils. Samples produced through different industrial pyrolysis processes were chosen to examine how variations in production methods affect bio-oil composition.

The solvents hexane, toluene, and dichloromethane (DCM) were selected after initial solubility tests. Lignocellulose pyrolysis bio-oils, which have a high oxygen content, exhibit strong intermolecular interactions. Nonpolar solvents like hexane and toluene are not effective at breaking these interactions through simple stirring. However, polar solvents like methanol can easily dissolve bio-oils.

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The use of ultrasound enhances the process by creating cavitation, which partially disrupts the intermolecular interactions of the bio-oils, allowing each solvent to dissolve specific molecules. Preliminary ultrasound tests revealed that the proportion of bio-oil extracted using a single solvent increased along with the polarity of the solvent. For instance, when utilizing toluene for direct extraction, both the hexane-soluble (F1) and toluene-soluble (F2) fractions were dissolved, as toluene could effectively dissolve the hexane-soluble part. Therefore, to achieve a selective separation, a sequential fractionation was arranged in a specified order, starting from the less polar bio-oil fraction and progressing to the most polar portion.

Over the course of four years, the UASE method was developed through extensive testing of various approaches aimed at optimizing bio-oil fractionation. Several strategies, including liquid−liquid extraction, acid−base extraction, column chromatography, and extrography, encountered significant challenges. For example, liquid−liquid extraction faced difficulties with solvent miscibility, making biphasic separation difficult and limiting fractionation possibilities. Acid−base extraction effectively separated phenolic compounds but resulted in significant losses during acid regeneration due to high solubility in water. Preparative column chromatography was impractical due to uncontrolled elution from highly polar solvents required for loading the sample on the column. After evaluating these methods, UASE emerged as the most effective choice. This process maintains sample integrity by avoiding interactions with solids and acid−base reactions. Importantly, ten extraction iterations per solvent were conducted to optimize UASE; additional extractions did not yield more material.

2.3.2. Gel Permeation Chromatography (GPC). The GPC analysis were conducted using an UltiMate 3000 Dionex high-performance liquid chromatography (HPLC) system (Amsterdam, Netherlands). This instrumentation included an UltiMate 3000 microflow pump, an autosampler, a low dead-volume port-to-port microinjection valve, and an ultraviolet diode array detector (UV DAD) set at 254 nm. The separation columns consisted of four styrene-divinylbenzene gel permeation columns, purchased from Waters Corporation (Milford, MA, USA) and with the following features: HR4 (5 μm particle size; 600,000 Da polystyrene equivalent exclusion limit), HR2 (5 μm particle size; 20,000 Da exclusion limit), and two HR0.5 columns (5 μm particle size; 1000 Da exclusion limit). Additionally, a Styragel guard column (4.6 mm i.d. × 30 mm) was incorporated to extend the operational life of the series columns. Chromatographic analysis was performed through isocratic elution, employing stabilized THF as the solvent. The procedure involved a consistent flow rate of 0.8 mL/min and a total run time of 65 min. For this process, the sample, diluted 100-fold by weight in stabilized THF, was introduced with an injection volume of 20 μL. The system was calibrated using polystyrene (PS) standards with a molecular weight from 3,152,000 to 162 Da. This broader range was used in previous studies for crude oil samples where significant aggregation phenomena were observed.[33] This calibration yielded a polynomial correlation that links molecular weight (MW) and chromatographic retention time (t) as shown by eq 1 (see calibration curve in Figure S1).

$$\log(\mathrm{MW}) = -1.22 \times 10^{-4} t^3 + 1.90 \times 10^{-2} t^2 - 1.05t + 22.1 \quad (1)$$

For data visualization, homemade Python scripts Jupyter Notebooks were used.

2.3.3. Gas Chromatography and Pyrolysis Gas Chromatography Mass Spectrometry (GCMS and Py-GCMS). 2.3.3.1. Analysis of Original Samples and Recovered Fractions. GCMS analyses were performed with a Shimadzu GCMS-QP2010 SE (Kyoto, Japan). Chromatographic separation occurred on a DB-5HT column (30 m length, 0.25 μm film thickness, 0.25 mm diameter). The temperature program began at 45 °C and increased at a rate of 10 °C/min until 280 °C; the final temperature was held for 10 min. The injector was maintained at 300 °C. Samples were diluted 50-fold by weight and injected onto the column with a split ratio of 100. Helium carrier gas was used at a flow rate of 1 mL/min. The mass spectrometer detector collected data up to m/z 500 with a set temperature of 250 °C. The Py-GCMS analysis mirrored the GCMS conditions, as it used the same MS instrument. The temperature program, however, initiated with a 4 min hold at 45 °C before increasing to 280 °C and then held for another 4 min. For pyrolysis, samples, < 100 μg of each of the samples were placed in the sample holder and subjected to a single-shot pyrolysis at 500 °C for 1 min. Compounds were identified using the NIST library with a minimum match factor of 90%.

2.3.3.2. Analysis of Lost Volatiles. The same GCMS setup was used to study the chemical composition of compounds that were lost during the solvent evaporation process. In this experiment, 0.5 g of bio-oil was placed in a 20 mL vial,[34] which was sealed with a septum. This vial was then heated to 70 °C for an hour. The resulting clear vapor condensate on the lid was analyzed using the temperature ramp parameters from Rahman et al.[35] The GC oven settings were as follows: it held at 35 °C for 3 min to capture and concentrate volatile compounds, increased the temperature at a rate of 6 °C/min to 100 °C, then at 15 °C/min to 260 °C, and maintained this temperature for 4 min.[35] Compounds were identified using the NIST library with a minimum match factor of 90%.

2.3.4. Proton Nuclear Magnetic Resonance (^{1}H NMR). ^{1}H NMR analyses were conducted at 25 °C using a Bruker AVANCE 400 MHz spectrometer (Massachusetts, USA) with Acetone-d_6 as solvent. Chemical shift values were corrected to the residual solvent peak (2.04 ppm). Ten mg of each of the samples were diluted in 650 μL of deuterated acetone. Experimental NMR parameters included a zg pulse program, a 4.2 s acquisition time, and a 5 s relaxation delay. The spectral bandwidth was established at 19.5 ppm (7812.5 Hz), with a total of 32 scans, similar to the conditions used elsewhere.[36]

3. RESULTS AND DISCUSSION

3.1. Method Development and Repeatability. Table 1 presents the calculated mass percentage of each fraction derived from BTG BTL bio-oil (BO) relative to the initial mass of the whole sample. Table 1 indicates that the recovery, or gravimetric sum, of all fractions adds up to only ∼70% of the starting mass. This could result from the loss of water, known to be 22−23% in weight for this commercial sample,[16] and volatile compounds during the drying process of the fractions. Interestingly, volatile species are responsible for the BO distinctive smoky scent. To determine the amount of water and volatiles in the original sample, the whole BO was heated at 70 °C for 1 h. Triplicate tests demonstrated that ∼68.7% of the BO mass consisted of nonvolatile components (data shown in Supporting Information, Table S1), which is slightly lower compared to the recovery, likely due to heat application. Therefore, the recovery or gravimetric sum of nonvolatiles could be considered quantitative. To better understand the chemical composition of volatiles lost during solvent evaporation, the method described in section 2.3.3.2 was

Table 1. Mass Percentage Values for Fractions F1, F2, F3, F4, and Overall Recovery in Three Fractionation Replicates, along with Their Calculated Mean, Standard Deviation (SD), and Coefficient of Variation (CV)

	gravimetric proportion of fractions original sample (%)				
replicate	F1 (n-hexane)	F2 (toluene)	F3 (DCM)	F4 (residual)	recovery (%)
1	6.5	14.9	20.6	27.5	69.5
2	6.9	14.5	19.2	27.5	68.0
3	6.7	16.0	21.3	27.6	71.6
mean	6.7	15.1	20.4	27.5	69.7
SD	0.2	0.8	1.1	0.1	1.8
CV (%)	2.9	5.1	5.3	0.3	2.6

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employed using GCMS. It was determined that the light volatiles, excluding water, consisted of methanol, acetic acid, and acetol, as well as some aromatic compounds such as guaiacol and its derivatives (see Figure S2) as observed by Rahman et al.[35]

The fractionation process exhibited optimal repeatability, as shown by a coefficient of variation (CV) of <6% for all the fractions. Moreover, the gravimetric ratios indicate that the BTG BTL BO is rich in highly polar species. Given the high oxygen content of the compounds typically produced in wood pyrolysis, it is reasonable that the BTG BTL sample features a limited content of F1 (~6.7%), which was extracted with *n*-hexane (dipole moment, μ = 0.08). In contrast, fraction F2, extracted with toluene (μ = 0.31) — a nonpolar yet aromatic solvent — accounts for about ~15.1% of the sample. The use of a more polar aprotic solvent, dichloromethane (μ = 1.14), yields a higher amount of material, ~ 20.4%, extracted in fraction F3. Lastly, the dried residue corresponds to ~27.5% of the sample and it can be solubilized in polar solvents capable of hydrogen bonding, e.g., tetrahydrofuran and methanol.

It is important to point out that the BO studied here is insoluble in nonpolar solvents like *n*-hexane and toluene when simply stirred at room temperature; however, it becomes partially soluble upon applying ultrasound. It should be highlighted that ultrasound creates cavitation bubbles, which release nonpolar molecules from the BO's "sticky" polar organic matrix.[37] Such nonpolar species can then be completely solubilized in solvents such as *n*-hexane and toluene. As a result, the fractionation process can be highly selective as it takes advantage of the intermolecular solvation interaction between the analytes and the chosen solvents, which is enabled by cavitation. This method eliminates the need for specific consumables and complex instruments, such as chromatography columns and high-performance liquid chromatography (HPLC) equipment. This simplification of the experimental setup helps avoid issues related to the irreversible adsorption of highly polar molecules in standard liquid chromatography (LC) columns.[15,16] To evaluate the method's effectiveness, alkali lignin underwent acid base regeneration via $HCl_{(aq)}$ addition. Subsequently, the solubility of the regenerated solid was tested in the extraction solvents under sonication. The solid remained insoluble in *n*-hexane, toluene, and DCM, however, it was fully solubilized in THF. The solubility behavior of regenerated lignin is consistent with that of the residual fraction F4 of the BTG BTL bio-oil. The results indicate that the bio-oil's most polar fraction features compositional properties similar to those of lignin. Lignin, a polyphenolic macromolecule, features phenolic hydroxyls, methoxy, and aldehyde groups, contributing to its complex structure. Essential for plant cell wall rigidity and environmental stress protection, its extraction, primarily used in the pulp industry, alters its structure and properties. Extraction methods, either chemical, biological, or physical, affect the resulting form, such as kraft lignin (KL), soda lignin, organosolv lignin, lignosulfonates, dissolved lignin, enzymatic hydrolysis lignin, and milled lignin. The structure of lignin varies with the lignocellulosic source and extraction method, typically exhibiting high polarity due to numerous oxygenated groups per molecule.[38] Throughout the development of this method, we conducted repeated tests using the BTG BTL sample at different time points. These tests consistently showed results that suggested good reproducibility within the same sample setup. While these internal tests indicate the method's robustness, validating its performance across different laboratories would be valuable.

Table 2 presents the mass percentage distribution of four distinct fractions (F1, F2, F3, and F4) obtained from the fractionation of various bio-oil samples: BO, CFP-1, CFP-2, and RCFP. A detailed comparison of these samples will be discussed in Part II of this paper, but some preliminary observations can be noted. First, the method is applicable to all types of lignocellulosic pyrolysis bio-oils. Second, the reactive catalytic process (RCFP) produces a bio-oil with a higher content of nonpolar products, where fractions F1 and F2 together make up nearly 70% of the total mass. This process also results in a lower proportion of fractions F3 and F4. Notably, the CFP-2 process yields the highest polar fraction (F4), exceeding that of its counterpart, CFP-1, which is produced at a higher temperature and feed rate, as well as surpassing the outputs from the noncatalyzed processes like BO.

Table 2. Mass Percentage Distribution of Bio-Oil Samples (BO, CFP-1, CFP-2, and RCFP) across Fractions F1, F2, F3, and F4, as Well as the Mass Lost during the Fractionation Process due to Volatilization

	mass percentage (%)			
fractions	BO	FP	CFP	RCFP
F1	6.5	12.3	8.8	50.7
F2	14.9	12.8	13.7	16.5
F3	20.6	14.7	12.7	5.2
F4	27.5	32.3	42.2	6.0
lost volatiles	30.5	27.9	22.6	21.6

3.2. Gel Permeation Chromatography. The samples were analyzed by gel permeation chromatography to evaluate the differences in hydrodynamic volumes and estimated molecular weights between the original bio-oil and its fractions. A UV-DAD detector set at 254 nm was used as most of the samples contain aromatic molecules. The chosen wavelength has been shown to be representative for qualitative analyses of bio-oils.[23] In GPC, the weight fraction (w_i) of each polymer chain is determined by measuring their absorbance (A_i) (see eq 2a). This reveals the proportion of different polymers in the sample. Two types of average molecular weights are then calculated. The number average molecular weight (M_n) uses w_i and reflects the average based on the frequency of each polymer chain's molecular weight (M_i) (see eq 2b). The weight-average molecular weight (M_w) emphasizes the impact of larger polymer chains due to their greater mass (see eq 2).

$$\text{(a) } w_i = \frac{A_i}{\sum A_i}\text{; (b) } M_n = \frac{\sum (w_i \cdot M_i)}{\sum w_i}\text{; (c) } M_w = \frac{\sum (w_i \cdot M_i^2)}{\sum (w_i \cdot M_i)} \quad (2)$$

Figure 1a presents the BO GPC chromatogram. The results indicate that the BO features a wide range of molecular weight, from 40 to 6000 Da. In the field of bio-oil (BO), specific ranges for categorizing molecular weights into low (LMW), medium (MMW), and high (HMW) have not been established. In contrast, in the case of crude oil, Desprez et

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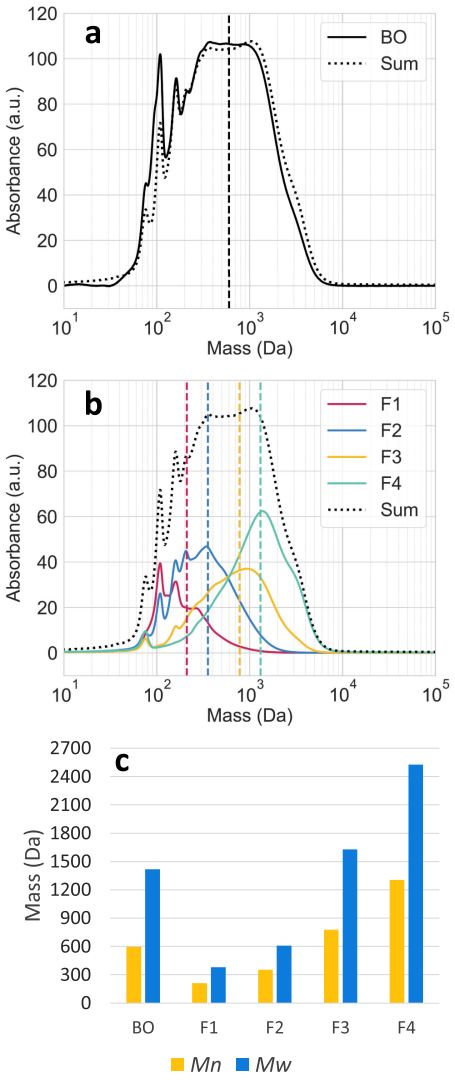

Figure 1. GPC-UV-DAD chromatograms of (a) bio-oil sample (BO) with M_n 597 Da (dotted vertical line). (b) Chromatograms of fractions F1, F2, F3, and F4, each adjusted for gravimetric factors, with their respective *Mn* values represented by dotted vertical lines at 210, 354, 776, and 1303 Da. The combined reconstructed signal at 254 nm is shown by the 'Sum' line. (c) Bar graph illustrating the values of M_n and M_w of each sample. Retention time converted in molecular weights using a calibration curve derived from polystyrene standards (see original chromatogram Figure S3).

al. defined these categories based on a calibration curve and retention times.[33] They identified LMW as being equal to or less than 180 Da, MMW ranging from 180 to 2,740 Da, and HMW from 2,740 to 40,000 Da. However, the molecular weights in BO are generally not as large as those in crude oil. Therefore, for the purposes of this paper, the molecular weight ranges for BO are defined as follows: LMW as 250 Da or less, MMW between 250 and 1,000 Da, and HMW between 1,000 and 6,000 Da. Two relative maxima are observed between 100 and 200 Da, which might be associated with monomer products of pyrolysis, i.e., aromatics and phenolics. High-molecular-weight species are also prevalent in the GPC chromatogram, which leads to values of number-average molecular weight (M_n), and weight-average molecular weight (M_w) of 597 and 1,418, as shown in Figure 1c.

Figure 1b indicates that the fractions reveal marked differences in their molecular weight distribution. Clearly, from F1 to F4, there is shift to higher molecular weights as shown in the GPC chromatograms, which translates into progressively higher M_n and M_w values (Figure 1c). Therefore, the hydrodynamic volume or aggregation state seems to correlate with the fraction's polarity. Previous studies have shown that this trend has also been observed in lignin fractions.[18,19] The GPC chromatograms of the fractions were "scaled" according to their gravimetric yields and summed in order to reconstruct a total GPC chromatogram. Figure 1b (black dotted line) shows that the reconstructed chromatogram from the sum of the fractions is very similar to that of the original BO (Figure 1a).[20,21] Similar results have been observed in previous studies on pyrolysis bio-oils,[21] crude oils,[39] maltenes,[40] and asphaltenes.[41] This suggests that the developed separation strategy is primarily physical and quantitative, without altering bio-oil chemical composition.[40]

Figure 2 presents the normalized GPC UV-DAD chromatograms for fraction F4 and regenerated alkali lignin standard.

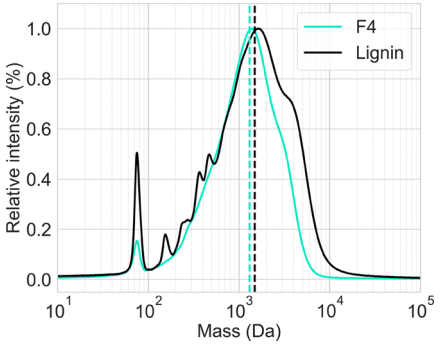

Figure 2. GPC-UV-DAD chromatograms normalized with intensity for BTG BTL fraction F4 and regenerated alkali lignin. The retention time was converted to molecular weights using a calibration curve derived from polystyrene standards.

The results indicate a remarkable similarity between both samples, which reveal close Mn values, i.e., ~1,300 Da for F4 and ~1,500 Da for the lignin standard. Similarities in solubility and molecular weight of fraction F4 with lignin suggests that species in F4 represent the molecules that were not efficiently degraded by the pyrolysis process, and are then the most challenging for biofuel/refinery applications. Yet, their potential conversion into fuel molecules depends on their thermal stability and reactivity, suggesting that with proper processing, such as hydrocracking and hydrotreating, they might still be valuable for biofuel production. In a previous work, Oasmaa et al.[8] found that a fraction from a pyrolysis bio-oil, soluble in dichloromethane, consisted of low-molecular-mass (LMM) compounds, whereas the insoluble fraction comprised high-molecular-weight (HMM) lignin-like species.[8] Those findings agree with the results presented in Figure 2. To the best of our knowledge, this is the first time that the most polar/high-MW fraction from a bio-oil is compared with a lignin standard through GPC. We hypothesize that the method presented herein can be used to assess bio-oils quality, whether for selection of optimal feedstocks or evaluation of production/upgrading processes, through bio-oil's solubility behavior and GPC UV. The GPC data indicate higher molecular weights, especially in the more polar fractions of the studied bio-oils. This suggests aggregation behavior similar

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to that of the most polar petroleum fraction, known as asphaltenes.[42,43] However, confirming the presence of aggregates in these samples requires further investigation. Notably, the molecular weight profile of the most polar fraction (F4) aligns with that of lignin standards, supporting the aggregation hypothesis. However, additional research will be necessary to definitively determine whether the observed molecular weights represent aggregates or individual molecular entities. The discrepancy in molecular weights determined by negative-ion electrospray ionization coupled with high-resolution mass spectrometry (as detailed in Part II of this study) and GPC indicates that polar bio-oil fractions tend to self-aggregate, similar to the behavior observed in asphaltenes. It is important to note that the system was calibrated using polystyrene (PS) standards with a molecular weight range from 3,152,000 to 162 Da. This broader range was employed in previous studies of crude oil samples where significant aggregation phenomena were observed.[44−46] Thus, the detection of low molecular weights by mass spectrometry, although limited by ion transfer optics, the physics of the mass analyzer, and ionization efficiencies, suggests that the polar fractions, as identified by GPC, are highly aggregated. The following section focuses on the molecular composition of the light components in F1−F4 fractions accessed by GCMS analysis.

3.3. Gas Chromatography and Pyrolysis Gas Chromatography Mass Spectrometry (GCMS and Py-GCMS). The GCMS analysis presented in Figure 3 offers a comparative qualitative assessment of volatile compounds in the BTG BTL BO and its fractions. The Total Ion Current (TIC) chromatograms shown in Figure 3a reveal that the majority of volatile compounds are extracted in F1 and F2, with a reduced amount in F3, and even a lower quantity in F4. It is not surprising that the fractions with smaller molecular weights or molecular size (as revealed by GPC) featured the highest amounts of volatile compounds. The results indicate that the species detected by GCMS comprise a mixture of products commonly reported for the pyrolysis of lignin, cellulose, and hemicellulose, e.g., aromatic molecules with O-containing functional groups, furanols, and sugars.[29] Selective ion mode GCMS analyses were focused on the ion with m/z 60 (shown in Figure 3b), which is a fragment characteristic of sugar-like structures ($^{+}C_2H_4O_2$).[47,48] This ion is derived from a dominant chromatographic peak that corresponds to levoglucosan, with a retention time (r.t.) between 14 and 15 min.[7] It is interesting to note that levoglucosan is not present in early fractions, F1 and F2; however, it is distinctly visible in F3 and is most prominent in F4. Collectively, GPC and GCMS results indicate that F4 contains high-molecular-weight species highly similar to lignin and the highest content of sugar derivatives.

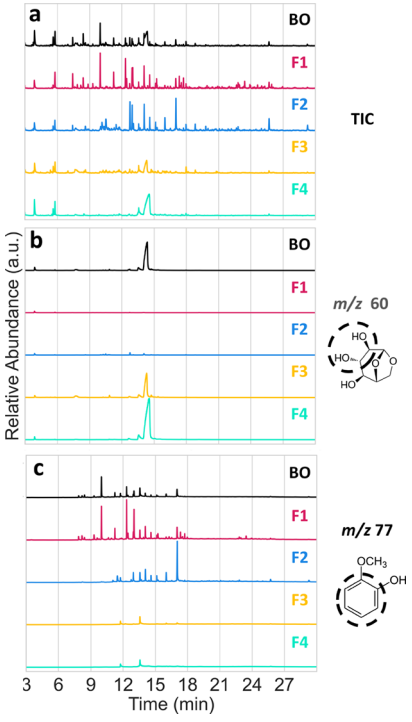

Figure 3. GCMS Chromatograms, the data was normalized using the sample with the highest abundance. (a) TIC, (b) peaks that feature the fragment ion with m/z 60 ($^{+}C_2H_4O_2$), and (c) peaks that reveal the fragment ion with m/z 77 ($^{+}C_6H_5$).

Conversely, selective ion mode based on benzene fragment ion (m/z 77) (Figure 3c), highlights which fractions are rich in aromatic compounds. In this case, F1 reveals the most dominant aromatic profile, followed by F2. Such species are scarcely present in F3 and F4. Focusing on the guaiacol derivates, the results indicate that *n*-hexane is particularly useful for the selective extraction of m-cresol (retention time or r.t. 8.18 min), guaiacol (r.t. 8.40 min), 2,5-dimethylphenol (r.t. 9.30 min), and high amounts of *p*-creosol (r.t. 9.99 min). Compounds with ketone and aldehyde groups were partially extracted with *n*-hexane; however, extraction with toluene yielded a fraction dominated by such species. For instance, F2 reveals vanillin (r.t. 12.94 min), acetoguaiacone (r.t. 14.07 min), guaiacylacetone (r.t. 14.60 min), and notably, a highly abundant peak for coniferyl aldehyde (r.t. 17.02 min). Interestingly, isoeugenol (r.t. 13.57 min) is detected across all fractions, and 2-methoxy-4-vinylphenol (r.t. 11.74 min) is observed in F2, F3, and F4, which indicates a strong interaction of these isomers with heavier molecular structures. Molecular structures are included in Figure 4.

The results from Py-GCMS analysis revealed a chromatographic profile that was largely consistent with that obtained by GCMS for most samples. The primary distinction between GCMS and Py-GCMS for all samples lies in the relative intensity of the peaks, as shown in Figure 5. However, a more pronounced difference was noted for the F4 fraction, where certain aromatic compounds that were not revealed in the GCMS, were identified by Py-GCMS. These findings suggest that while the high temperatures of the Py-GCMS's pyrolysis furnace may only facilitate the volatilization of light molecules already present in all fractions, in the case of the F4 fraction, the heat additionally aids in the pyrolysis of the refractory components of lignin that may not have been completely broken down during the bio-oil production process. To further investigate the structural characteristics of these fractions, proton NMR was employed.

3.4. Proton Nuclear Magnetic Resonance Analysis (^{1}H NMR). The application of ^{1}H NMR spectroscopy in the analysis of complex matrices facilitates a comparative evaluation of the presence and proportions of various functional groups. In ^{1}H NMR spectra, peak intensities directly correlate with the prevalence of protons in a particular chemical environment, as illustrated in the highlighted regions from 0 to 10 ppm in Figure 6a. The accompanying bar chart (Figure 6b) quantifies these observations and presents the area

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Label	Retention time (min)	Compound
1	8.18	meta-Cresol
2	8.40	Guaiacol
3	9.30	2,5-Xylenol
4	9.99	p-Creosol
5	11.24	p-Ethylguaiacol
6	11.75	2-methoxy-4-vinylphenol
7	12.33	Eugenol
8	12.47	p-Propylguaiacol
9	12.94	Vanillin
10	13.57	Isoeugenol
11	14.07	Acetoguaiacone
12	14.37	Levoglucosan
13	14.60	Guaiacylacetone
14	15.14	1'-Hydroxyeugenol
15	15.25	4-Hydroxy-3-methoxypropiophenone
16	16.00	4-hydroxy-3-methoxy-benzopropanol
17	17.02	Coniferyl aldehyde

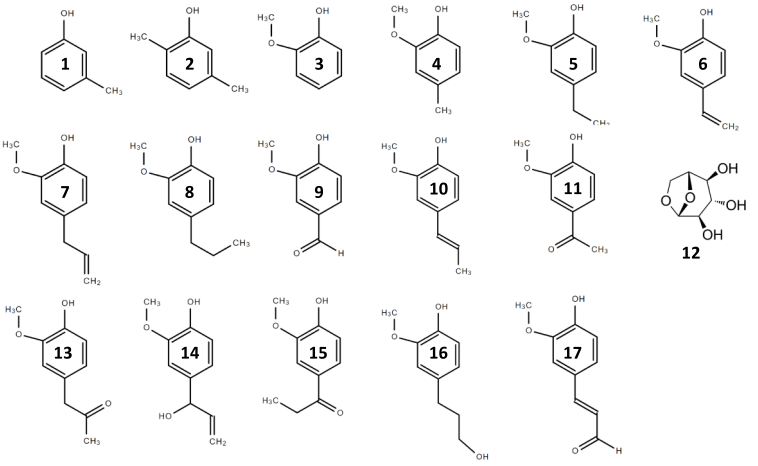

Figure 4. Identification of significant compounds present in bio-oil sample and fractions by GC−MS. Compounds were identified using the NIST library with a minimum match factor of 90%).

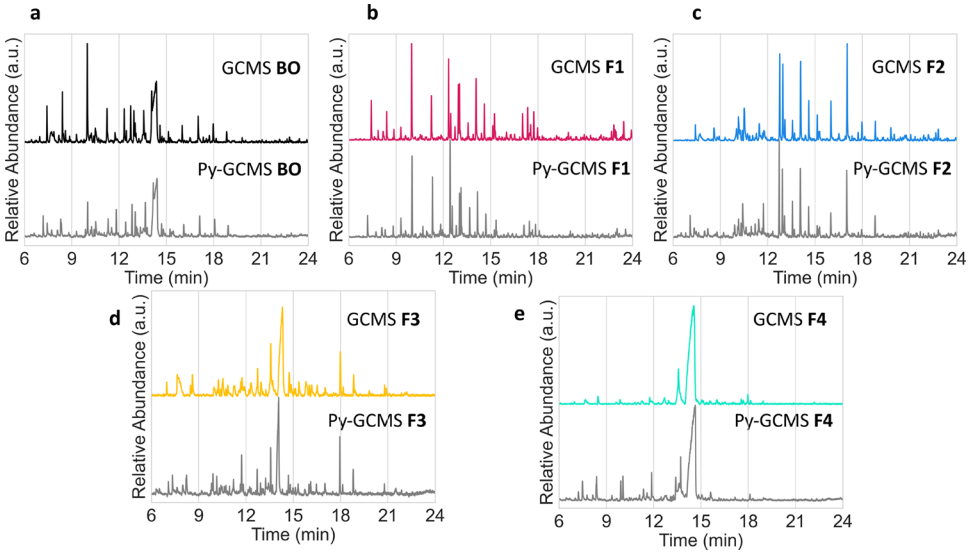

Figure 5. GCMS (shown in different colors) and Py-GCMS (shown in gray) TIC normalized chromatograms for all the samples: (a) whole BO, (b) F1, (c) F2, (d) F3, and (e) F4.

under the curve for the regions associated with specific functional groups.[49]

The ^{1}H NMR spectra reveal distinct patterns in the downfield integrated region I (9.5 to 10 ppm), which is diagnostic for aldehydes. These volatile molecules were also detected by GCMS, but with a low abundance in all samples (<1.1%). The content of species within the aromatic and heteroaromatic region II (6 to 8.5 ppm) decreases from F1 to F4; the most polar fraction reveals a ∼ 2-fold lower concentration of aromatic species compared to F1. It is important to highlight that F4 features a higher molecular weight as determined by GPC, which suggests a greater degree of molecular conjugation (i.e., lignin-like oligomers), which affects the shape of the signal of these protons.[50]

Region III (2.7−6.0 ppm) is associated with ethers, alcohols, methoxy groups, and carbohydrates, that exhibit a notable increase in relative area from F1 to F4. This trend is consistent with the continuous increase in polarity and molecular weight from F1 to F4. Conversely, the areas for regions IV and V, which correspond to aliphatic protons adjacent to heteroatoms or unsaturation (1.5 to 2.7 ppm) and alkanes (0.5 to 1.5 ppm), decrease from F1 to F4. This trend agrees with the nature and polarity of the solvents used for fractionation. The pronounced content of aromatics and saturated hydrocarbons in F1 highlights its potential for energy applications or chemical feedstock.

4. CONCLUSIONS

An innovative and straightforward fractionation technique has been developed for analyzing bio-oils from pyrolysis of lignocellulosic feedstocks. The methodology, which involves ultrasound-assisted sequential extractions (UASE) with pure solvents, results in four distinct fractions, each with specific chemical properties and compositions. These fractions, along with the original bio-oil sample, were examined in detail by GPC, proton NMR, and analysis of volatile components by GCMS and Py-GCMS. This fractionation approach is applicable across all types of pyrolysis oils, and enables

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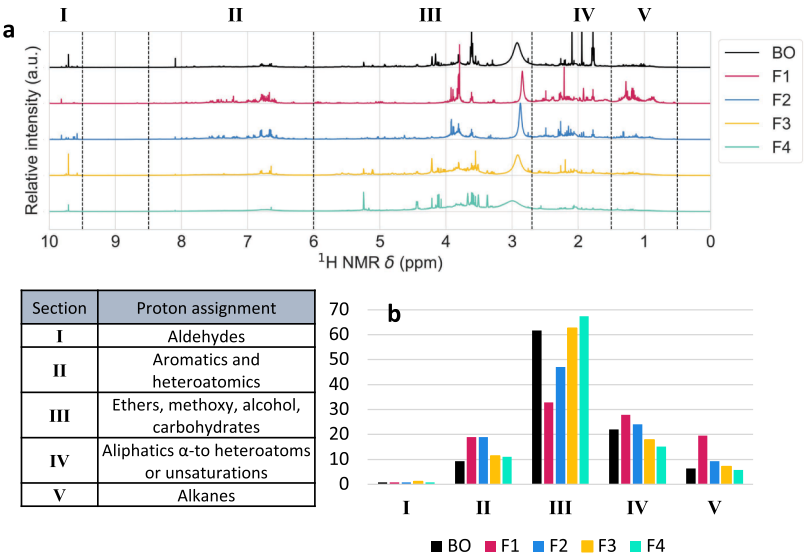

Section	Proton assignment
I	Aldehydes
II	Aromatics and heteroatomics
III	Ethers, methoxy, alcohol, carbohydrates
IV	Aliphatics α-to heteroatoms or unsaturations
V	Alkanes

Figure 6. Proton NMR (a) spectra for the samples, after removal of the signals attributed to deuterated acetone 2.04 ppm, and (b) bar chart of relative integration results in the sections of interest.

detailed insights into their chemical makeup and quality. The analysis indicates that lighter fractions, namely F1 and F2, are rich in light aromatic compounds suitable for hydrogenation into fuel. In contrast, fraction F3, which has a higher molecular weight, contains aromatic species in smaller amounts, along with sugar-like molecules, indicating the need for a more complex hydrogenation process. The heaviest fraction, F4, has the lowest quantity of aromatic volatiles and a molecular profile akin to lignin standards, along with a higher concentration of sugar-like compounds. This fraction is the most resistant to pyrolysis, as confirmed through the comparison of GCMS and Py-GCMS data. Given these findings, the fractionation method described in this study provides a reliable standard for evaluating bio-oil quality. A greater proportion of F1 and F2 relative to F4 indicates superior bio-oil quality, suggesting more efficient production methodologies or feedstock. Thus, this fractionation technique could become a standardized metric for evaluating bio-oil production methods. Consequently, the second part of this study will apply this fractionation methodology to various types of bio-oils and characterize them at the molecular level to evaluate the method's advantages.[51]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.energyfuels.4c01959.

Proportion of volatiles and nonvolatiles of the bio-oil (BO) sample in three replicates (Table S1); calibration curve of polystyrene standards in the GPC columns (Figure S1); GCMS analysis of light volatiles potentially lost during solvent evaporation process of the UASE method (Figure S2); and original GPC-UV-DAD chromatograms of (a) bio-oil sample (BO) and its fractions corrected with their respective gravimetric factors (Figure S3) (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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