**Analysis of switchgrass-derived bio-oil and associated aqueous phase
generated in a semi-pilot scale auger pyrolyzer**

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Abstract: To efficiently utilize water-soluble compounds in bio-oil and evaluate the potential effects of these compounds on processes such as microbial electrolysis, this study investigated the physicochemical properties of bio-oil and the associated aqueous phase generated from switchgrass using a semi-pilot scale auger pyrolyzer. Combining separation and detection strategies with organic solvent extraction, an array of analytical instruments and methods were used to identify and quantify the chemical constituents. Separation of an aqueous phase from the crude bio-oil was achieved by adding water (water: crude bio-oil at 4:1 in weight), which resulted in a partition of 61 wt.% of the organic compounds into a bio-oil aqueous phase (BOAP). GC/MS analysis for BOAP identified over 40 compounds of which 16 were quantified. Acetic acid, propionic acid, and levoglucosan are the major components in BOAP. In addition, a significant portion of chemicals that have the potential to be upgraded to hydrocarbon fuels were extracted to BOAP (77 wt.% of the alcohols, 61 wt.% of the furans, and 52 wt.% of the phenolic compounds in crude bio-oil). Valorization of the BOAP may require conversion methods capable of accommodating a very broad substrate specificity. A better separation strategy is needed to selectively remove the acidic and polar components from crude bio-oil to improve economic feasibility of biorefinery operations.

Keywords: Bio-oil, Partition, Bio-oil aqueous phase, HPLC-PDA, GC/MS, GC-FID

1. Introduction

The depletion of fossil fuel reserves and the increasing concern over global warming have motivated scientists and researchers to look for alternative clean energy sources. Biomass is an important and plentiful renewable resource available to make clean fuels [1]. There are several technologies for converting biomass to advanced biofuels and chemicals, for examples, pyrolysis, gasification, fermentation, and liquefaction. Among these technologies, pyrolysis has high potential to produce drop-in, liquid hydrocarbon fuels. It is a thermochemical technology that generally conducted at temperatures ranging from 400 to 600 °C in the absence of oxygen, predominantly producing bio-oil with biochar and syngas as coproducts [2]. However, bio-oil produced from biomass pyrolysis is a carbon-based liquid product with poor physicochemical properties such as high content of water and oxygen, high acidity, and low heating value, requiring preprocessing and upgrading for fuel applications.

Due to the presence of multiple components in biomass, the pyrolysis reaction of biomass is extremely complicated involving the decomposition of cellulose, hemicellulose, lignin, and secondary decomposition with catalytic reactions of primary decomposition products catalyzed by inorganic minerals. This results in hundreds of compounds in bio-oil, which are classified as acids, alcohols, furans, aldehydes, esters, ethers, ketones, phenolics, and anhydrosugars, depending on their functional groups. Total carboxylic acids in the bio-oil were reported to be about 4–8 wt.%, with acetic acid being the most prevalent acid. High content of carboxylic acids renders corrosiveness of bio-oil, requiring special containers for bio-oil transportation and storage. Levoglucosan is a major compound in the group of anhydrosugars in crude bio-oil which contributes about 5–7 wt.% [3]. In addition to these compounds, a considerable amount of phenolics, which are decomposed from lignin, are also present in crude bio-oil. Bio-oil has been

considered as a promising chemical platform for producing renewable fuels and value-added chemicals for industrial applications [4].

Simply adding water to crude bio-oil is the first step to extract valuable chemicals by fractionating crude bio-oil into an organic and an aqueous phase [5-8]. Most polar compounds such as carboxylic acids and anhydrosugars are extracted into the aqueous phase, while phenolic compounds contribute to the organic phase [9-12]. Due to the high water content and low amount of organic compounds, the bio-oil aqueous phase fractionated by adding water is a low-value product compared to the crude bio-oil and organic phase, and thus has little value to be upgraded to fuels. To improve the economics of biomass pyrolysis, research strategies targeted at the production of hydrogen or other value-added chemicals from the bio-oil aqueous phase (BOAP) are being investigated [9,13-20]. Recently, a novel process of converting BOAP to hydrogen via microbial electrolysis has been reported [21]. However, compounds such as furan aldehydes and phenolics presented in BOAP might be inhibitory to microbes [17,21,22]. A better understanding of bio-oil and its aqueous phase composition is, therefore, necessary to evaluate and develop new conversion technologies.

This study was to comprehensively characterize the switchgrass-derived bio-oil and its fractions, using multiple techniques, with the aim to evaluate the possibility and the potential effects of compounds presented in BOAP on new processes such as microbial electrolysis. Physicochemical properties of crude bio-oil and the associated aqueous phase were analyzed and the partition of chemicals into BOAP was evaluated.

2. Materials and methods

2.1 Materials

Air-dried switchgrass (*Panicum virgatum* L.) obtained from a local producer in eastern Tennessee was used for the bio-oil production. The water content of the biomass was 7–8 wt.%. Before pyrolysis, the material was ground to less than a 2 mm particle size. The switchgrass is composed of 34.1 wt.% cellulose, 25.7 wt.% hemicellulose, 18.8 wt.% lignin, 14.2 wt.% extractives, and 2.7 wt.% ash [23].

Ethyl acetate and chloroform purchased from Thermo Fisher Scientific (Waltham, MA) were used as organic solvents to extract organic compounds from BOAP for identifying the chemical composition of BOAP. These two chemicals were used as received. External standards used for quantifying compounds were purchased from Sigma-Aldrich (St. Louis, MO).

2.2 Bio-oil production

A semi-pilot scale auger pyrolysis system (Proton Power, Inc., Lenoir City, TN) equipped with a feeding system, a rectangular auger reactor, a pyrolysis vapor condensation section, and a biochar collector was used to pyrolyze switchgrass for bio-oil production (Figure 1). A detailed description of the pyrolysis system was provided elsewhere [23]. In brief, bio-oil was produced under the following operation conditions. Feedstock was transferred from the feeding hopper to the auger pyrolysis reactor by a single auger with a feeding rate of approximately 8.5 kg/h. The auger reactor (10 W×10 H×250 L cm) contained internal dual augers. The auger speed controlled the residence time of feedstock at 72 seconds. The heated zone was comprised of a 200 cm long electrical resistance furnace operating at 500 °C. The sweeping gas (nitrogen gas, 20 L/min) was introduced into the front of the auger reactor and moved with the evolved vapors to the condensation section. Before the vapors entered the condensers from the auger reactor, the particle chamber (20 cm in diameter and 100 cm long) precipitated fine particles from the vapors.

The biochar produced from the feedstock was collected into the biochar drum. The condensation section was comprised of three condensers in a series (10 cm in diameter and 200 cm long, each). The temperature of the three condensers were maintained between 10 and 15 °C using a circulation water cooling system. The bio-oils collected from the three condensers were immediately combined and mixed for homogeneity and stored in a walk-in freezer before fractionation and characterization. The non-condensable gases were burned at the end of the system. The experiment was performed in duplicate. After pyrolysis, the bio-oil and biochar were collected and weighted. The weight of non-condensable gases was calculated by difference.

Figure 1 Schematic diagram of semi pilot-scaled auger pyrolysis system

2.3 Bio-oil aqueous phase separation

The crude bio-oil obtained from the pyrolysis reactor was mixed by adding deionized water (DI water) in a ratio of 1:4 (wt.%) to separate into bio-oil aqueous phase (BOAP: water soluble fraction) and an organic phase (BOOP: water insoluble fraction). The mixture was shaken vigorously on a mini vortexer (Model: MS1 S7, Fisher Scientific) until forming homogeneous solution then stored at 4°C overnight. Then the mixture was centrifuged using an Iec Model 120

clinical centrifuge (International Equipment Company) at 5000 rpm for 30 minutes to accelerate phase separation. After separation, the BOAP and BOOP were collected and weighed.

2.4 Physical properties analysis

The properties of crude bio-oil and BOAP including density, pH, viscosity, water content, solid content, ash content, and total acid number were measured in triplicate. Density was measured according to the ASTM D1217 (2012) standard [24], and pH was measured with an Extech pH meter. A Schott TitroLine Karl Fischer volumetric titrator was used to measure water content according to ASTM D4377 (2011) standard [25]. Viscosity was measured at 40°C with serialized Schott Ubbelohde capillary viscometers according to ASTM D445 (2012) standard [26]. Ash content was measured according to ASTM D482 (2013) standard at 575 °C [27]. The solid content was determined according to Boucher et al. [28]. Total acid number (TAN) was measured by titrating bio-oil (0.1g) and BOAP (0.2g) in the solvent of water, isopropyl alcohol and toluene (volume ratio of water: isopropyl alcohol: toluene=1: 99:100) with 0.1M KOH isopropyl alcohol solution to an end point of pH 11 according to ASTM D664 (2011) standard [29]. The elemental analysis of crude bio-oil and BOAP were carried out at ALS Environmental-Analytical Laboratory (Tucson, AZ).

2.5 Molecular weight and distribution analysis

Bio-oils are made up of molecules of different sizes resulting from fragmentation of cellulose, hemicelluloses, and lignin, constituting mixtures of compounds with a range of molecular weights. The number average molecular weight (M_n), the mass average molecular weight (M_w), and molecular weight distribution ($MWD = M_w/M_n$) of crude bio-oil, BOAP, and

BOOP were determined on an EcoSEC Gel Permeation Chromatography (GPC) system (TOSOH Bioscience LLC) equipped with one TSKgel SuperMultipore HZ-M guard column, two Tosoh TSKgel SuperMultipore HZ-M columns (4.6×150 mm; $4 \mu\text{m}$), and an RI detector. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 0.35 mL/min. The bio-oil samples were diluted about 1000 times by adding THF and then filtered through a $0.2 \mu\text{m}$ filter. The filtered samples were analyzed using GPC at 40°C . The elution time was converted to molecular weight by calibration with polystyrene standards. All of the experiments were performed in duplicate.

2.6 Organic extraction from BOAP and chemical identification by GC/MS

Due to the high water content and low concentration of organic compounds in BOAP, direct analysis of BOAP using GC/MS could be of low resolution and harmful to the instrument. For identifying the organic compounds, two organic solvents with relatively high polarity, namely ethyl acetate and chloroform, were used for the chemical extraction of BOAP. The organic solvents were first mixed with BOAP at different volume ratios (organic solvent: BOAP = 1:1 and 2:1) in a separating funnel and shaken vigorously. Then the separating funnel was left undisturbed for 24 hours to allow phase separation.

Chemical compounds in crude bio-oil and extracted chemicals from BOAP were identified by gas chromatography/mass spectrometry (GC/MS). A Shimadzu GC–MS (QP2010S) with a Restek Rtx-5MS capillary column was used. The column temperature was programmed at 45°C for 3 min followed by increase to 150°C at $5^\circ\text{C}/\text{min}$; then the temperature was further increased to 260°C at $10^\circ\text{C}/\text{min}$ and held for 7 min at the final temperature. The inlet was set at 240°C , and sample injection was made in a split mode (1:20). The compounds were identified by

comparing their mass spectra with those from the National Institute of Standards and Technology (NIST) mass spectral data library.

2.7 Quantification of major compounds by GC-FID, HPLC-RI, and HPLC-PDA

BOAP contains many oxygenated compounds, which might have different absorptions in the ultraviolet or visible region. Traditional high performance liquid chromatography with ultraviolet /ultraviolet-visible spectroscopic detection (HPLC-UV/UV-VIS) can only be performed at a certain fixed wavelength, which may not give a significant absorption signal for all the chemicals. In this study, we employed a high performance liquid chromatography system (Jasco 2000Plus) from Jasco Analytical Instruments (Easton, MD) equipped with a PU-2089S plus pump, a MD-2018 plus photodiode array detector (PAD), a RI-2031 Plus intelligent RI detector, and an AS-2055 plus auto sampler to analyze BOAP. The measurement of BOAP by HPLC-PDA was performed over a wide range of wavelength (190 nm to 338 nm). The maximum absorption wavelength and retention time were used to identify and calibrate the compounds. The liquid chromatography was conducted at 50 °C using a Bio-Rad column HPX-87H (300 x 8 mm) column. The injected sample size was 20 µl. The mobile phase was 5 mM H₂SO₄ in deionized water with a flow rate of 0.6 mL/min.

A gas chromatography-flame ionization detector (GC-FID) with a HP-5 column (30 m x 0.32 mm, 0.25 µm film thickness) was used for quantifying major compounds in crude bio-oil and volatile compounds in BOAP. The same temperature program with the GC/MS was used in the GC-FID. Compounds were quantified using external standards in both the HPLC and GC-FID analysis.

3. Results and discussion

3.1 Bio-oil production

Liquid fractions from pyrolysis were condensed and collected from three condensers. All collected fractions were combined to obtain the crude bio-oil. The details of product yields from two runs are shown in Table 1. The process yielded 50–54 wt.% bio-oil, 29 wt.% biochar, and 17–21 wt.% non-condensable gases. The product yields from the two runs were similar. The bio-oil yield obtained in this study is lower and the biochar yield is higher comparing with those in another study where a fluidized bed pyrolysis was used to produce switchgrass bio-oil [30]. The main reason for these differences in yield is the longer residence time in our auger pyrolysis (72 seconds) versus a few seconds in fluidized bed reactors, which slows the pyrolysis and generates more biochar.

Table 1 Comparison of products of 1st and 2nd runs switchgrass pyrolysis

Bio-oil production	Pyrolysis temperature (°C)	Bio-oil yield (wt.%)	Biochar yield (wt.%)	Non-condensable gas yield (wt.%)
1st batch	500	50	29	21
2nd batch	500	54	29	17

3.2 Bio-oil aqueous phase separation

The crude bio-oil obtained from switchgrass pyrolysis contained about 42.3 wt.% water. The crude bio-oil resulting from mixing of the liquids from the three condensers was homogenous initially. However, when the crude bio-oil was stored at 4°C in a refrigerator for several days, a dark and sticky fraction precipitated at the bottom of the container. To achieve phase separation and obtain BOAP, DI water at 4:1 weight ratio of water to bio-oil was added to the crude bio-oil.

Due to the difference of solubility and polarity distribution of organic compounds, polar compounds were extracted to BOAP. Table 2 shows the partition distributions of crude bio-oil, organics and water to BOAP and BOOP after separation.

Bio-oil is mainly composed of oxygenated compounds and these compounds have a wide range of polarity. The bio-oil separation caused by adding water resulted in over 60 wt.% of these compounds in the crude bio-oil being extracted to BOAP, and for lower organics (about 39 wt.%) from crude bio-oil being extracted to BOOP. During the aqueous phase separation, most water (about 87 wt.% of the total water in crude bio-oil) was partitioned to BOAP; only about 13 wt.% water in crude bio-oil was partitioned into BOOP. After aqueous phase separation, the organics in BOOP were concentrated because of low water content. However, a large fraction of organics in the crude bio-oil was extracted to BOAP in aqueous phase separation. To utilize BOAP efficiently, comprehensive characterization for BOAP is necessary.

Table 2 Partition distribution of crude bio-oil, organics, and water after aqueous phase separation

	Crude bio-oil (wt.%)	Organics (wt.%)	Water (wt.%)
BOAP	72	61	87
BOOP	28	39	13

3.3 Physical properties analysis of crude bio-oil and BOAP

Table 3 shows physical properties of crude bio-oil and BOAP. The water in the crude bio-oil is from the moisture of biomass and the production of water via reactions such as dehydration of cellulose, hemicellulose, and lignin during biomass pyrolysis. Fast pyrolysis such as fluidized bed pyrolysis generally generates less water which is about 15–25 wt.% of crude bio-oil due to the fast heating rate [30]. In this study, the heating rate of our augur reactor was slower than that

of the fluidized bed. Therefore the water content in the crude bio-oil was 42.3 wt.% . The density of crude bio-oil was about 1.13 g/mL, which was similar to the previously reported data [30]. Due to the DI water added to crude bio-oil, BOAP contained more water, measured at about 91.7 wt.%. The density of BOAP was about 1.01 g/mL. The pH value, solid content, and ash content of observed crude bio-oil were about 2.84, 1.70 g/mL, and 0.31 wt.%, respectively, which are within the range of reported data [30].

Table 3 Physical properties of crude bio-oil and BOAP

Properties	Crude bio-oil	BOAP derived from crude bio-oil with 4:1 water addition
Water content (wt.%)	42.3±0.7	91.7±1.1
Total solid (wt.%)	1.70±0.25	Not detected
pH value	2.84±0.07	3.02±0.01
Density (g/mL)	1.13±0.01	1.01±0.01
Ash (wt.%)	0.31±0.04	0.09±0.01
Viscosity at 40 °C centistokes (cSt)	6.50±0.82	0.75±0.01
TAN, mg KOH/g	137.4±3.0	30.1±1.3

Table 4 shows the elemental analysis of the crude bio-oil and its fractions. BOOP contains higher carbon content than crude bio-oil and BOAP because organics were concentrated during aqueous phase separation. About 55 wt.% of the original carbon in crude bio-oil was extracted to BOAP, although the carbon content in BOAP was about 4.7 wt.%, and about 45 wt.% of the original carbon remained in BOOP. During the bio-oil aqueous phase separation, oxygenated compounds with high polarity were extracted to BOAP. The elemental analysis showed that over

73 wt.% of the original oxygen in crude bio-oil was extracted to BOAP while only 27 wt.% remained in BOOP. Moreover, 78 wt.% of the original hydrogen in crude bio-oil was extracted to BOAP. The crude bio-oil also contained about 0.4 wt.% nitrogen, which is in the same range as that for the crude bio-oil from switchgrass pyrolysis reported previously [3]. The nitrogen content in BOOP was about 0.6 wt.%, which accounts for about 44 wt.% of the original nitrogen in the crude bio-oil; about 56 wt.% of the original nitrogen in crude bio-oil was extracted to BOAP. Crude bio-oil contains a high oxygen content and the hydrotreating of crude bio-oil consumes a large amount of hydrogen. The aqueous phase separation in this study showed that BOOP contains low water and oxygen content, and high carbon content. This advantage gives BOOP a high heating value, requiring less hydrogen in hydrotreating. In contrast to BOOP, BOAP contains higher water content and about 78 wt.% of the original hydrogen and 73 wt.% of the original oxygen of the crude bio-oil. While BOAP is not suitable for hydrotreating, it could be used as a feedstock for hydrogen production via processes such as microbial electrolysis [21].

Table 4 Elemental analysis of crude bio-oil, BOAP and BOOP

Element	Crude bio-oil (wt.%)	Percentage of the original to BOAP (wt. %)	Percentage of the original to BOOP (wt. %)
C	44.4	54.6	45.4
H	8.1	78.4	22.6
O	47.1	73.0	27.0
N	0.4	55.9	44.1

3.4 Gel permeation chromatography (GPC) analysis for crude bio-oils, BOAP and BOOP

The number average molecular weight (M_n), the mass average molecular weight (M_w), and molecular weight distribution ($MWD = M_w/M_n$) of bio-oils, BOAP, and BOOP are showed in

Table 5. BOOP is mainly composed of phenolics and oligomers from the lignin decomposition [31]. As expected, the mass average molecular weight of BOOP is significantly higher than that of crude bio-oil and BOAP. Compared to crude bio-oil and BOOP, the mass average molecular weight of BOAP is lower, demonstrating that smaller molecular compounds were extracted in to BOAP.

Table 5 The number average molecular weight (M_n), the mass average molecular weight (M_w), and molecular weight distribution ($MWD=M_w/M_n$) of bio-oils

Bio-oil	M_n (g/mol)	M_w (g/mol)	MWD (M_w/M_n)
Crude bio-oil	222±3	284±2	1.28±0.01
BOOP	223±7	443±20	1.99±0.03
BOAP	134±4	217±8	1.52±0.12

M_n : the number average molecular weight

M_w : the mass average molecular weight

$MWD=M_w/M_n$: molecular weight distribution

3.5 Chemical extraction of BOAP by organic solvents and analysis by GC/MS

After the extraction by organic solvents, the solvents were evaporated using a rotary evaporator and the organics extracted from BOAP were collected and weighted. Around 57 wt.% and 70 wt.% of organic compounds in BOAP were extracted by ethyl acetate at the volume ratio of ethyl acetate to BOAP at 1:1 and 2:1, respectively (Figure 2). It is also observed that about 46 wt.% and 54 wt.% of organic compounds in BOAP were extracted by chloroform at the volume ratio of chloroform to BOAP at 1:1 and 2:1, respectively. These results indicate that ethyl acetate at the volume ratio of 2:1 performed the best to extract chemicals from the bio-oil aqueous phase. It is worthy to note that during the organic solvent extraction, acetic acid, propionic acid, and

levoglucosan were not easily extracted into the organic phase due to their polarity and high solubility in water.

Figure 2 Organic distribution in solvent phase and water phase

Table 6 shows the compounds identified in crude bio-oil and organics extracted from BOAP by organic solvents. The compounds were categorized to nine groups, including acids, alcohols, aldehydes, esters, furans, ketones, phenolics, sugars, and PAHs (poly aromatic hydrocarbons). The GC/MS analysis indicates that a considerable amount of alcohol, furans, ketones, and phenolics in BOAP was extracted by organic solvents. Acids and sugars were also detected by GC/MS of the extracted fractions at however much lower levels. Ethyl acetate extracted more organic chemicals from BOAP than chloroform. Furthermore, almost all chemicals extracted by chloroform were also extracted by ethyl lactate. Therefore, to identify the chemical compounds in BOAP as much as possible, ethyl acetate was used as the organic solvent for organic compound extraction at the volume ratio of solvent to BOAP at 2:1, which was determined to be the best choice.

310 Table 6 Identified compounds in crude oil and extracted organics from BOAP by GC/MS

Categories	Compounds	Crude oil	Extracted by ethyl lactate	Extracted by chloroform	Categories	Compounds	Crude oil	Extracted by ethyl lactate	Extracted by chloroform
Acids	Acetic acid	X			Ketones	1-Hydroxy-2-butanone	X	X	X
	propionic acid	X				2-Cyclopenten-1-one, 3-methyl-	X	X	X
	Succinic acid, methyl-	X				Cyclohexanone	X	X	X
	vanillic acid	X	X			2-Butanone, 3,3-dimethyl-	X	X	X
	Benzoic acid, 3-hydroxy-4-methyl-	X				2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-Cyclohexanone, 4-hydroxy-2,5-Cyclohexadiene-1,4-dione, 2-methyl-5-(1-methylethyl)-	X	X	X
Alcohols	1,3-Propanediol	X	X		PAHs	Anthracene	X		
	1,3-Propanediol, 2-(hydroxymethyl)-2-methyl-	X				Pyrene	X		
	3-Hexanol, 2,4-dimethyl-	X			Phenolics	Phenol	X	X	X
	Cyclodecanol	X	X	X		Phenol, 3-methyl-	X	X	
	3-Cyclobutene-1,2-dione, 3,4-dihydroxy-1,2-	X	X	X		Phenol, 2-methyl-	X	X	
	Cyclopentanedione, 3-methyl-	X	X	X		Guaiacol	X	X	X
	5-Isopropenyl-2-methylcyclohexanol	X				Phenol, 3-ethyl-	X	X	X
	Cyclohexanol, 2,3-dimethyl-	X	X	X		Phenol, 3-(1-methylethyl)-	X		
	4-Cyclopentene-1,3-diol, trans-	X				1,2-Benzenediol	X	X	X
	3-Nonyn-1-ol	X				Phenol, 2-methoxy-4-methyl-	X	X	
	2-Hexen-1-ol, 2-ethyl-	X	X	X		Phenol, 2,3,5,6-tetramethyl-	X		
	Cyclododecanol	X	X			Phenol, 4-ethyl-2-methoxy-	X	X	X
	Bicyclo [3.1.1] hept-3-en-2-ol, 4,6,6-trimethyl-	X	X			2(1H)-Naphthalenone, octahydro-, trans-	X		
	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	X	X	X		Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	X	X	X
	1-Cyclohexene-1-methanol, 4-(1-methylethenyl)-	X				Phenol, 2,6-dimethoxy-	X	X	X
Aldehyde	5-Methyl-2-hexanone	X	X			Benzaldehyde, 3-hydroxy-4-methoxy-	X	X	X
	1-Cyclohexene-1-acetaldehyde, 2,6,6-trimethyl-	X	X	X		1,2,3-Trimethoxybenzene	X	X	X
Esters	Butyric acid, 3-methyl-, allyl ester	X			Sugars	1,4-Benzenediol, 2-(1,1-dimethylethyl)-	X	X	X
	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	X	X	X		Benzene, 1,2,3-trimethoxy-5-methyl-	X	X	
	2-Octynoic acid, methyl ester	X	X	X		Phenol, 2,6-dimethoxy-4-(2-propenyl)-	X	X	X
	p-Menth-8-en-2-ol, acetate	X				Benzaldehyde, 2,4,5-trimethoxy-	X	X	X
	Pentanoic acid, 4-methyl-, ethyl ester	X				d-Mannitol, 1,4-anhydro-	X	X	
Furans	Furfural	X	X	X		1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)	X		
	2(5H)-Furanone	X	X	X					
	5-Hydroxymethyl-2-furaldehyde	X	X	X					
	2(5H)-Furanone, 5-methyl-	X	X						
	Benzofuran, 2,3-dihydro-	X	X	X					

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Compared to crude bio-oil, fewer chemicals were identified in BOAP. However, over 40 chemicals covering a wide range of chemical classes were identified in BOAP. The presence of a diverse range of compounds in BOAP makes purification of individual compounds difficult. Valorization of the BOAP may require conversion methods capable of accommodating a very broad substrate specificity.

3.6 Major compounds quantified by GC-FID and HPLC in crude bio-oil and BOAP

According to GC/MS analysis of crude bio-oil and BOAP, 16 main compounds were identified including three acids, three furans, two alcohols, six phenolics, one aldehyde and ketone, and one anhydrosugar in crude bio-oil. BOAP was further analyzed by GC-FID and HPLC-PDA and quantified using external standards.

Organic acids and sugars in crude bio-oil have been primarily determined by GC-FID or GC/MS in previous reports [30,32]. However, we found that the quantified mass content for these compounds in crude bio-oil by GC-FID is much less than those determined in BOAP by HPLC. The reason is that organic acids and sugars have poor volatility and are not easily evaporated in GC. Hence, the quantification for these compounds is not accurate when only using GC-FID. Therefore, in this study the contents of these compounds in both BOAP and BOOP were quantified by HPLC-RI and GC-FID, respectively. The reported contents for these compounds in crude bio-oil are the total contents quantified in BOAP and BOOP.

Table 7 shows the details of mass content of the 16 major chemical compounds in crude bio-oil. Acetic acid and levoglucosan were the two largest amounts of chemicals in the crude bio-oil and the mass content of each was over 7 wt.% of total crude bio-oil. Besides these two compounds, propionic acid, 1-hydroxy-2-butanone, furfural, and 5-hydroxymethyl-2-

338 furaldehyde (HMF) had considerable mass content (over 1.2 wt.%) in the crude bio-oil. The
 339 mass content for individual phenolic compounds was low (< 0.5 wt.%). However, the total mass
 340 content of the six quantified phenolic compounds in crude bio-oil was significant (about 1.44
 341 wt.%). The total quantified mass content of the 16 compounds was about 24.18 wt.% which
 342 accounts for about 41.9 wt.% of organics in the crude bio-oil.

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344 Table 7 Mass content of 16 major compounds in crude bio-oil and BOAP

Classifications	Major compounds	Content in crude bio-oil (wt.%)	Concentration (g/L) based on BOAP	Percentage of the original chemicals extracted to BOAP (wt.%)	Methods used for BOAP analysis	Wavelength used in HPLC-PDA analysis (nm)
Carboxylic acid	Acetic acid	7.12±0.24	15.88±0.01	94.38	HPLC-RI	
	Propionic acid	2.81±0.12	6.61±0.03	99.54	HPLC-RI	
	Vanillic acid	0.10±0.02	0.22±0.01	93.09	HPLC-PDA	220
Sugars	Levogluconan	7.77±0.05	18.05±0.14	98.17	HPLC-RI	
Furans	HMF	1.70±0.05	1.32±0.01	32.86	HPLC-PDA	280
	Furfural	1.22±0.03	0.99±0.06	34.34	HPLC-PDA	277
	2(5H)-Furanone	0.46±0.01	0.79±0.01	72.67	GC-FID	
Alcohols	1-Hydroxy-2-butanone	1.20±0.04	2.30±0.07	81.1	GC-FID	
	1,3-propanediol	0.17±0.07	0.20±0.03	49.78	GC-FID	
Aldehydes and ketones	3-methyl-1,2-cyclopentanedione	0.19±0.04	0.36±0.01	80.18	GC-FID	
Phenolics	1,2-benzendiol	0.47±0.01	0.70±0.01	63.02	HPLC-PDA	195
	3-ethylphenol	0.27±0.01	0.23±0.01	36.05	GC-FID	
	Phenol	0.22±0.01	0.24±0.02	46.16	HPLC-PDA	193
	Guaiacol	0.21±0.01	0.25±0.06	50.38	GC-FID	
	2,6-Dimethoxyphenol	0.18±0.01	0.22±0.01	51.72	GC-FID	
	2-methyl-4-methylphenol	0.09±0.01	0.12±0.01	56.42	GC-FID	
Total		24.18	48.48			

Quantification results for these 16 major chemical compounds in BOAP are also shown in Table 7. Levoglucosan, acetic acid, and propionic acid were detected in large amounts in BOAP at 18.05 g/L, 15.88 g/L, and 6.61 g/L, respectively. Over 90 wt.% of these compounds in crude bio-oil were extracted to BOAP. Furans and alcohols were also observed in relatively high concentrations in BOAP; about 61 wt.% of the original furans and 77 wt.% of the original alcohols in crude bio-oil were extracted to BOAP. Other compounds representing aldehydes, ketones, and phenolics were detected at low concentrations in BOAP, generally less than 0.4 g/L. However, total concentration of quantified phenolics was about 1.76 g/L which accounts for about 51.7 wt.% of original phenolics in crude bio-oil, indicating that considerable amounts of phenolic compounds were extracted into BOAP. Furans, alcohols, and phenolics are valuable chemicals for hydrocarbon fuel production in a biorefinery [33-35]. However, a large portion of these compounds can be extracted to BOAP in the aqueous phase separation by simply adding DI water. Furthermore, the presence of a considerable amount of phenolics in BOAP could be harmful to microbial growth thereby reducing the efficiency of hydrogen production via biological routes such as the microbial electrolysis process [21,36]. Therefore, better separation methods should be further investigated for reducing the content of furans, alcohols, and phenolics partitioned into BOAP.

4. Conclusions

Physicochemical properties and chemical constituents of crude bio-oil and associated aqueous phase partitioned by simply adding water were investigated in this study. The partitioning of bio-oil by this aqueous phase separation removed most of organic acids, leading to the improvement of bio-oil stability. A large portion of water, oxygen, and hydrogen in crude bio-oil were partitioned into BOAP, making it a potential feedstock for hydrogen production via

processes such as microbial electrolysis. However, the separation of bio-oil by simply adding water (at water:bio-oil weight ratio of 4:1) resulted in ~61 wt.% organics in crude bio-oil being partitioned into BOAP, including a significant portion of chemicals such as furans, alcohols, and phenolics that are valuable chemicals to be upgraded to biofuels. Further, the presence of a diverse range of compounds in BOAP could bring out challenges for its utilization by microbes, because some of these chemicals such as furfural and phenolics may inhibit the microbial activity. A better separation strategy is needed to selectively remove acidic and polar components from crude bio-oil to improve the overall economics of biorefinery operations.

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