Separation of chemical groups from bio-oil aqueous phase via sequential organic solvent extraction

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Abstract: The chemical complexity of bio-oil aqueous phase limits its efficient utilization. To improve the efficiency of the bio-oil biorefinery, this study focused on the separation of chemical groups from the bio-oil aqueous phase via sequential organic solvent extractions. Due to their high recoverability and low solubility in water, four solvents (hexane, petroleum ether, chloroform, and ethyl acetate) with different polarities were evaluated, and the optimum process conditions for chemical extraction were determined. Chloroform had high extraction efficiency for furans, phenolics, and ketones. In addition to these classes of chemical, ethyl acetate had a high extraction efficiency for organic acids. The sequential extraction using chloroform followed by ethyl acetate resulted in 62.2 wt.% of original furans, ketones, alcohols, and phenolics being extracted into chloroform, while 62 wt.% acetic acid was extracted into ethyl acetate, leaving behind a high concentration of levoglucosan (~53.0 wt.%) in the final aqueous phase. Chemicals separated via the sequential extraction could be used as feedstocks in a biorefinery using

processes such as catalytic upgrading of furans and phenolics to hydrocarbons, fermentation of levoglucosan to produce alcohols and diols, and hydrogen production from organic acids via microbial electrolysis.

Keywords: Bio-oil aqueous phase, Organic solvent, Solvent extraction, Chemical groups

1. Introduction

Bio-oil from biomass pyrolysis is a promising feedstock for the production of transportation fuels and value-added chemicals [1, 2]. However, some disadvantages make it difficult to directly use bio-oil as a transportation fuel. Bio-oil contains hundreds of chemicals that are classified according to their functional groups, including phenolics, furans, organic acids, ketones, aldehydes, esters, and anhydrosugars [3, 4]. Unlike petroleum oil, most chemicals in bio-oil are unstable oxygenated compounds, amounting to as high as 40 wt.% of oxygen [5]. Bio-oil also has a significant content of water generally ranging from 15−30 wt.%, depending on the different types of biomass and pyrolysis processes [5]. Furthermore, organic acids are the major contributors to the acidity of bio-oil, causing corrosion that requires special containers for the storage and transportation.

To recover and utilize the water-soluble chemicals, phase separation of bio-oil into an organic phase and an aqueous phase by adding water has been investigated [2, 6-9]. The extraction efficiency of chemicals in bio-oil by water is highly dependent on the polarity and solubility of the chemicals. Because of their high polarity and solubility in water, levoglucosan and organic acids, such as acetic acid, have high distribution coefficients in water, resulting in a high concentration in the aqueous phase. Furans, such as furfural and furanone, have a distribution coefficient similar to acetic acid [6]. Some phenolic compounds, such as syringol and guaiacol, have low polarity, but due to their low initial concentration in crude bio-oil, these

compounds also have a high distribution coefficient to water and a considerable fraction can also be extracted by water [6]. Thus, the bio-oil aqueous phase is a complex mixture requiring further separation to be efficiently utilized.

Due to the multitude of chemicals present in the aqueous phase, a variety of applications of bio-oil aqueous phase have been investigated, including aqueous phase reforming [10-12], extraction and recovery of value-added chemicals, such as acetic acid [13, 14], and ethanol production via hydrolysis and fermentation of levoglucosan [12, 15]. Recently, a novel application of the aqueous phase for hydrogen production has been reported via a microbial electrolysis (MEC) process [16]. Certain biological processes, such as microbial fermentation, are negatively affected by furans and phenolics [12, 16-18]. Therefore, the removal of non-desirable compounds from the bio-oil aqueous phase is required.

Several methods including distillation, solvent extraction, and column chromatography have been developed for separating and recovering the various components and to characterize bio-oil and the associated aqueous phase [7, 13, 19-22]. Among these methods, solvent extraction is considered cost-effective, as it can be operated at room temperature and atmospheric pressure [23]. Garcia-Perez *et al.* investigated different organic solvents to fractionate the chemicals for characterization [24]. Wei *et al.* found that chloroform had good performance in extracting phenolic compounds [7]. However, a considerable amount of furans, ketones, and alcohols were still detected by gas chromatography and mass spectrometry (GC/MS) in bio-oil aqueous phase after organic solvent extraction [7]. Moreover, organic acids and levoglucosan were not well separated by these organic solvents. Therefore, only using a single solvent might not sufficiently extract and separate these chemicals from the bio-oil aqueous phase.

Separating compounds in the bio-oil aqueous phase into different chemical groups is practically useful. It minimizes the effects of non-desirable chemicals on the application of specific chemical groups, develops strategies for the application of the bio-oil aqueous phase, and improves its application efficiency. Due to the very low concentration of individual compounds of furans, alcohols, ketones, and phenolics in the bio-oil aqueous phase, isolating these chemicals individually would be difficult and costly. Therefore, separating these chemicals as a group (Group 1) may be more practical. Due to the significant amount of organic acids (Group 2) and anhydrosugars (Group 3) in the bio-oil aqueous phase, separating these two chemical groups is feasible. Therefore, the purpose of this study was to develop a method to separate these three chemical groups from bio-oil aqueous phase using organic solvents. Four solvents including hexane, petroleum ether, chloroform, and ethyl acetate with different polarities were first evaluated individually for chemical extraction. Optimum conditions and extraction efficiency related to the different chemical groups were determined. According to these analyses, a sequential extraction using chloroform followed by ethyl acetate were further investigated for separating Group 1 (furans, alcohols, ketones, and phenolics), Group 2 (organic acids) and Group 3 (anhydrosugars).

2. Materials and Methods

2.1 Materials

Air-dried switchgrass (*Panicum virgatum L.*) obtained from a local producer in East 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 [25].

Hexane (a mixture of isomers, purity > 98.5%), petroleum ether (ACS certified grade), chloroform (purity > 99.0%), and ethyl acetate (purity > 99.5%) purchased from Thermo Fisher Scientific (Waltham, MA) were used as organic solvents for chemical extraction from bio-oil aqueous phase (BOAP). All these chemicals were used as received. Fifteen external standards, all purchased from Sigma-Aldrich (St. Louis, MO), were used for quantifying compounds in the BOAP.

2.2 Crude bio-oil production and bio-oil aqueous phase separation

A schematic diagram of the experiment for the separation of chemical groups by a sequential extraction is shown in Fig. 1. The process includes bio-oil production, aqueous phase separation, and organic solvent extractions. The separated chemical groups can be integrated into a biorefinery for the production of fuels and chemicals (Fig. 1).

A semi-pilot scale auger pyrolysis system (Proton Power, Inc., Lenoir City, TN) was used to pyrolyze switchgrass for bio-oil production. A detailed description of the pyrolysis system was provided elsewhere [25]. The pyrolysis was conducted at 500 ºC with a residence time of 72 seconds.

The separation of the bio-oil aqueous phase was achieved by simply adding water to crude bio-oil [26]. First, the crude bio-oil was mixed with four times by weight of distilled water. Then, the mixture was shaken vigorously using a mini vortexer (Model MS1 S7, Fisher Scientific) until a homogeneous mixture was formed. The mixture was stored at 4 °C overnight, followed by centrifugation using an IEC Model 120 clinical centrifuge (International Equipment Company) at a relative centrifugal force (RCF) of 2,400 g for 30 minutes to accelerate phase separation. After centrifugation, bio-oil aqueous phase (BOAP I) on top was collected and analyzed, as described below.

123 Physical properties of crude bio-oil and BOAP I, including density, pH, viscosity, water
124 content, solid content, ash content, and total acid number, were measured in triplicate. Density
125 was measured according to the ASTM D1217 (2012) standard [27], and pH was measured with
126 an Extech pH meter. A Schott TitroLine Karl Fischer volumetric titrator was used to measure
127 water content according to ASTM D4377 (2011) [28]. Viscosity was measured at 40 °C with
128 serialized Schott Ubbelohde capillary viscometers according to ASTM D445 (2012) [29]. Ash
129 content was measured according to ASTM D482 (2013) at 575 °C [30]. The solid content was
130 determined according to Boucher *et al*. [31]. Total acid number (TAN) was measured by
131 titrating bio-oil (0.1 g) or BOAP I (0.2 g) in a solvent of water, isopropyl alcohol and toluene
132 (volume ratio of water: isopropyl alcohol: toluene = 1: 99:100) with 0.1M KOH isopropyl
133 alcohol solution to an end point of pH 11 according to ASTM D664 (2011) [32].

134 **2.3 Chemical extraction from bio-oil aqueous phase**

135 The separated BOAP I was used for extraction experiments. Four organic solvents, hexane,
136 petroleum ether, chloroform, and ethyl acetate, were first individually investigated to separate
137 chemicals from BOAP I. Four different volumetric ratios of solvent to BOAP I (0.5:1, 1:1, 2:1,
138 and 3:1) were employed to determine the effect of solvent-to-feed ratio (S/F ratio) on the
139 chemical extraction. A fixed volume of BOAP I at 20 mL was weighted and measured into a
140 100 mL beaker. Then a predesignated volume of organic solvent was added into the beaker. The
141 mixture of BOAP I and the organic solvent was magnetically stirred for 30 min. After stirring,
142 the mixture was transferred to a separatory funnel and left undisturbed for 24 hours to allow
143 phase separation. During the stirring and separation, the beaker and funnel were sealed to
144 minimize solvent evaporation. After separation, the solvent phase and BOAP II were collected

and weighed. Chemicals extracted to the organic solvent were recovered by evaporating the organic solvent via a rotary evaporator at 40 ^{o}C.

First, distribution coefficient and extraction efficiency [33] were used to evaluate the four solvents in extracting BOAP I. The distribution coefficient (D_i) is defined as the ratio of equilibrium mass concentration (g/L) of compounds in the solvent (*i* indicates six groups based on their functional groups: acids, furans, alcohols, ketones, phenolics, and levoglucosan, that is the only anhydrosugar detected) to their equilibrium mass concentration in BOAP I, according to Equation 1.

$$D_i = \frac{mass\ concentration\ of\ i\ in\ solvent}{mass\ concentration\ of\ i\ in\ BOAP\ I} \quad \text{(Equation 1)}$$

Extraction efficiency (X_j) is defined as the mass percentage (wt.%) of 15 individual chemical compounds (*j*) transferred from BOAP I to the organic solvent after the extraction process according to Equation 2.

$$X_j = \frac{mass\ of\ j\ in\ BOAP\ I - mass\ of\ j\ in\ BOAP\ II}{mass\ of\ j\ in\ BOAP\ I} \times 100\% \quad \text{(Equation 2)}$$

According to the results evaluating the four solvents, chloroform and ethyl acetate were chosen for sequential extraction to separate the chemical groups from BOAP I, which was first extracted by chloroform to recover furans, ketones, and phenolics. After chloroform extraction, BOAP II and the solvent phase were collected and weighed. BOAP II was further extracted using ethyl acetate. The organics extracted to chloroform and ethyl acetate were recovered by

evaporating the organic solvents via a rotary evaporator at 40 °C. BOAP II and III were collected and analyzed. The experiment was performed in triplicate.

2.4 Chemical identification and quantification by GC/MS, GC-FID and HPLC

Chemical compounds in BOAP I were identified using gas chromatography/mass spectrometry (GC/MS). A Shimadzu GC/MS (QP2010S) with a Restek Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The column temperature was programmed at 45 °C for 3 min and increased to 150 °C at 5 °C/min; then, it was further increased to 260 °C at 10 °C/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.

The acids, levoglucosan, hydroxymethylfurfural, furfural, phenol, and 1,2-benzenediol in BOAP I-III were quantified using a high pressure liquid chromatography system (HPLC, Jasco 2000Plus, Jasco Analytical Instruments, Easton, MD) equipped with a MD-2018 plus photodiode array detector (PAD), a RI-2031 Plus intelligent RI detector, and an AS-2055 plus auto sampler [26]. The liquid chromatography was conducted at 50 °C using a Bio-Rad column HPX-87H (300 × 8 mm) The injected sample volume was 20 μL. The mobile phase was 5 mM H_2SO_4 in deionized water with a flow rate of 0.6 mL/min.

The following compounds in BOAP I-III, which have been reported in switchgrass bio-oil analysis [34-37], were quantified using a gas chromatography-flame ionization detector (GC-FID) with a HP-5 column (30 m × 0.32 mm × 0.25 μm): 2(5H)-furanone, 1-hydroxy-2-butanone, 1,3-propanediol, 3-methyl-1,2-cyclopentanedione, guaiacol, creosol, 2,6-dimethoxyphenol, 3-ethylphenol . The same temperature program as that with GC/MS was used in the GC-FID. The

compounds extracted to organic solvent phase were also quantified by the GC-FID. Compounds were quantified using external standards in both the HPLC and GC-FID analysis.

3. Results and Discussion

3.1 Characterization of bio-oil and BOAP I

The physical properties of crude bio-oil and BOAP I are shown in Table 1. The crude bio-oil obtained from the pyrolysis was an even mixture containing about 41.1 wt.% water. After aqueous phase separation, BOAP I showed a light yellow color containing about 90.8 wt.% water. After the separation, most polar compounds were extracted by water to BOAP I. The oligomers derived from lignin remained in the black and viscous organic phase. Due to the dilution by water, the pH in BOAP I slightly increased compared with the crude bio-oil, while the density, ash content, viscosity, and TAN decreased.

GC/MS analysis showed that more than 50 compounds were detected in BOAP I. The relative peak areas in GC/MS chromatogram were considered a useful indication of the relative abundance of chemicals, which has been used in bio-oil analysis [38-41]. Among these compounds, levoglucosan, acetic acid, furfural, and phenol were the most abundant as detected by GC/MS spectrometry. According to their functional groups, the detected compounds were classified into anhydrosugars, acids, furans, alcohols, phenolics, aldehydes, ketones, esters, and others (nitrogen-containing compounds). The distribution of these chemicals in BOAP I according to their relative GC/MS peak area is shown in Fig. 2.

Previous study has pointed out that levoglucosan, organic acids, alcohols, furans such as furfural, and acetol have high distribution coefficient in water [6]. After the bio-oil aqueous phase separation, the majority of these compounds can be extracted to the aqueous phase. Although phenolic compounds have a lower polarity than acids and furans, their low initial

concentration in the crude bio-oil affords them a high distribution coefficient in water [6]. GC/MS analysis detected a number of phenolic compounds with small peaks in BOAP I, but these small peaks added up to a total relative area of about 28 area%. A number of aldehydes, ketones, and esters with relatively small area percentage were also found in BOAP I.

Taking their high peak area percentages into account, 15 compounds were further quantified using GC-FID and HPLC, including two acids, three furans, two alcohols, six phenolics, one ketone, and one anhydrosugar (levoglucosan). These quantified chemicals accounted for about 53.9 wt.% of total chemicals in BOAP I. The concentrations of these 15 compounds in BOAP I are presented in Table 2. Acids and anhydrosugars are the two major chemical groups extracted to BOAP I by water. Among the acids, acetic acid was detected at the highest concentration, which was about 16.54 g/L. The concentration of levoglucosan was about 18.99 g/L in BOAP I. These two compounds were the most abundant chemicals observed in BOAP I. The quantification of other chemicals in BOAP I showed that 1-hydroxy-2-butanone and hydroxymethylfurfural with concentration of 2.25 g/L and 1.37 g/L, respectively, are the major chemicals following levoglucosan, acetic acid, and propionic acid. The total quantified concentration of furans and alcohols were about 3.17 g/L and 2.6 g/L, respectively. While the individual phenolic compound had a low concentration in BOAP I, the total concentration of quantified phenolic compounds added up to 1.5 g/L. These 15 quantified compounds (6 groups) were used in the next step to evaluate the extraction efficiency of organic solvents from BOAP I.

3.2 Effects of organic solvents on the extraction of total chemicals

Fig. 3 shows the total mass percentages of the 15 chemicals extracted by the four different organic solvents. Among the four, hexane has the lowest affinity for chemicals in BOAP I, extracting less than 30 wt.%. Petroleum ether has slightly higher affinity for chemicals than

hexane, with the highest amount of chemicals extracted at 33.2 wt.% from BOAP I. Chloroform has a higher affinity for chemicals than hexane and petroleum ether, in agreement with a previous report [7]. Ethyl acetate has the highest affinity for chemicals in BOAP I, followed by chloroform. These two can extract over 50 wt.% of total chemicals in BOAP I, due to their high polarity (polarity of chloroform at 4.1 and polarity of ethyl acetate at 4.4). In BOAP I , most chemicals present are oxygenated compounds, having high polarity and solubility in water [6]. Therefore, hexane and petroleum ether with very low polarity and solubility in water limit the dispersion of the oxygenated compounds. These results suggest that to recover more chemicals from BOAP I, solvents with high polarity, such as ethyl acetate and chloroform would be more preferable than hexane and petroleum ether with low polarity.

The effect of different volumetric ratios of organic solvent to BOAP I (S/F ratio) was also investigated (Fig. 3). With a S/F ratio of 0.5, all the four solvents showed poor extraction performance for chemicals, with the maximum extracted amount being less than 22 wt.%. Increasing the S/F ratio to 1, the amount of chemicals extracted using hexane and petroleum ether did not change significantly. However, the amount of extracted chemicals was significantly increased using chloroform and ethyl acetate from 14.7 wt.% to 47.8 wt.% and 21.1 wt.% to 54.1 wt.%, respectively. When the S/F ratio further increased to 2, the amount extracted by hexane, petroleum ether and ethyl acetate, except for chloroform (increased only 2 wt.%), significantly increased as compared to that at S/F ratio of 1.This result indicates that the S/F ratio of chloroform to BOAP I at 1 is desirable for chemical extraction from an economic viewpoint, consistent with a previous study [7]. When the S/F ratio further increased to 3, there were no significant changes in extracted chemicals for all the tested solvents. Therefore, the optimum S/F ratio were 2 for hexane, petroleum ether, or ethyl acetate and 1 for chloroform.

3.3 Effects of solvents on the separation of 15 individual chemicals

After organic solvent extraction of BOAP I, 15 chemicals in BOAP II were quantified using GC-FID and HPLC to determine the effects of different organic solvents and S/F ratios on the extraction of these chemicals. The extraction efficiency of individual chemical was calculated according to Equation 2. The results are presented in Fig. 4 for Group 2 (two organic acids) and Group 3 (one anhydrosugar) chemicals, and Figs. 5 and 6 for Group 1 (twelve chemicals of furans, alcohols, ketones, and phenolics).

As Fig. 4 shows, the extraction efficiencies for anhydrosugar (levoglucosan), and organic acids (acetic acid and propionic acid) in BOAP I by hexane and petroleum ether were less than 10 wt.%. Increasing loading of the two solvents did not significantly increase the extraction efficiency. Chloroform had low extraction efficiencies for anhydrosugar (less than 8.8 wt.%) and organic acids (less than 14 wt.% for acetic acid and less than 3.9 wt.% for propionic acid) in BOAP I at low S/F ratios (volume ratio at 0.5 and 1); when the S/F ratio increased to 3, the extraction efficiencies for levoglucosan, acetic acid, and propionic acid increased to 11.1 wt.%, 23.8 wt.%, and 34 wt.%, respectively. Ethyl acetate showed a performance similar to chloroform for the extraction of levoglucosan. The extraction efficiency for acetic acid was low using ethyl acetate when the S/F was at 0.5. However, when the S/F ratios were increased to 2 and 3, the extraction efficiency of acetic acid was significantly increased to 55 wt.% and 61.2 wt.%, respectively. This result suggests that the S/F ratio has great effect on acetic acid extraction. Because ethyl acetate can be used as a hydrogen bond acceptor in extraction [42], the increase in its loading provides more receptors for hydrogen bond, thus breaks the hydrogen bond between acetic acid and water.

Fig. 5 illustrates the extraction efficiencies for two alcohols (1-hydroxy-2-butanone, 1,3-Propanediol), one ketone (3-methyl-1,2-cyclopentanedione), and three furans (hydroxymethylfurfural, 2(5H)-furanone, furfural) in BOAP I using the four organic solvents. Both hexane and petroleum ether had poor performance, even with increased loading, for the extraction of alcohols and the ketone. The extraction efficiencies using these two solvents varied greatly for the different compounds of furans. Extraction efficiencies for hydroxymethylfurfural were observed at less than 8 wt.%. Hexane and petroleum ether had good extraction performance for 2(5H)-furanone with extraction efficiencies at about 58 wt.% and 59 wt.%, respectively. Extraction efficiencies for furfural using hexane and petroleum ether were 13.3 wt.% and 19.9 wt.% at the S/F ratio of 0.5, respectively; when the S/F ratio was increased to 2, a significant increase in extraction efficiency for furfural was observed using both solvents. Further increasing the S/F ratio to 3 using hexane and petroleum ether increased the extraction efficiencies for furfural by about 7 and 10 wt.%, respectively. However, the highest extraction efficiencies using hexane and petroleum ether for furfural were achieved at 54.5 and 53.6 wt.%, respectively. Compared to hexane and petroleum ether, chloroform and ethyl acetate had a better extraction performance for these chemical groups (two alcohols, one ketone, and three furans). The extraction efficiencies using chloroform reached to about 29 wt.% for 1-hydroxy-2-butanone, 10 wt.% for 1,3-Propanediol, 68 wt.% for 3-methyl-1,2-cyclopentanedione, 54 wt.% for hydroxymethylfurfural, 63 wt.% for 2(5H)-furanone, and 95 wt.% for furfural at the low S/F ratio of 0.5. When the S/F ratio increased to 1, the extraction efficiencies for 1-hydroxy-2-butanone, 1,3-Propanediol, 3-methyl-1,2-cyclopentanedione, and hydroxymethylfurfural greatly increased to 48.1 wt.%, 19 wt.%, 81 wt.%, and 69.6 wt.%, respectively. When the S/F ratio increased to 2, the extraction efficiencies for these four compounds further increased to 57 wt.%,

304 36 wt.%, 89 wt.% and 82 wt.%, respectively. Further increasing the S/F ratio to 3, the extraction
305 efficiency for 1,3-Propanediol increased to 56 wt.% while only a slight increase was observed for
306 the other three compounds. No significant change in the extraction efficiency was observed for
307 2(5H)-furanone and furfural when the S/F ratio was increased from 0.5 to 3. The extraction
308 efficiency for 2(5H)-furanone by ethyl acetate was higher than that by chloroform. However, the
309 extraction efficiency for 1-hydroxy-2-butanone, 3-methyl-1,2-cyclopentanedione,
310 hydroxymethylfurfural, and furfural by ethyl acetate was lower than that by chloroform,
311 especially at low solvent loading (S/F ratio at 0.5 and 1).
312 Hexane and petroleum ether had good performance for the extraction of phenolic
313 compounds (3-ethylphenol, guaiacol, 2,6-dimethoxyphenol, and creosol) as shown in Fig. 6.
314 When the S/F ratio was increased to 2, all creosol in BOAP I was extracted to solvents. At this
315 S/F ratio, extraction efficiencies for 3-ethylphenol, guaiacol, and 2,6-dimethoxyphenol were
316 about 73.5 wt.%, 80.1 wt.%, and 36.8 wt.%, respectively, by hexane, and 68.5 wt.%, 74.4 wt.%
317 and 51.2 wt.%, respectively, by petroleum ether. When the S/F ratio increased to 3, the changes
318 of 3-ethylphenol, guaiacol, and 2,6-dimethoxyphenol were insignificant. Hexane showed a poor
319 extraction performance for 1,2-benzenediol and phenol; the highest extraction efficiencies for
320 these two chemicals were 7 wt.% and 20 wt.%, respectively, when the S/F ratio was increased to
321 3. Petroleum ether had a slightly better performance for 1,2-benzenediol and phenol than hexane.
322 But the highest extraction efficiency was observed at 12.6 wt.% for 1,2-benzenediol and 35.5 wt.%
323 for phenol. Compared to hexane and petroleum ether, chloroform and ethyl acetate showed
324 superior extraction efficiency for phenolic compounds (Fig. 6). All guaiacol, 2,6-
325 dimethoxyphenol, and creosol were extracted to solvents even at the low S/F ratio of 0.5,
326 suggesting that the two had very high affinity for the three compounds. Extraction efficiency for

phenol and 3-ethylphenol using chloroform was over 94 wt.% and 87 wt.% respectively, while the extraction efficiency for these two chemicals using ethyl acetate was at 100 wt.% and 90 wt.%, respectively. Chloroform had low affinity for 1,2-benzenediol as indicated by the extraction efficiency of only about 36 wt.%. However, ethyl acetate showed a high affinity for 1,2-benzenediol resulting in an extraction efficiency of over 96 wt.% at a S/F ratio of 2.

According to the above analyses, the extraction efficiency by the organic solvents for chemicals in BOAP I was not only affected by the different solvents and S/F ratios, but also subjective to the different chemical groups. Generally, chloroform had high extraction efficiency for furans, alcohols, and phenolics, while ethyl acetate had high extraction efficiency for organic acids in addition to furans and phenolics, indicating non-selective extraction by ethyl acetate. The optimum S/F ratio and extraction efficiency by the four solvents for the 15 quantified chemicals are summarized in Table 3.

To further understand the effects of solvent on the chemical extraction, the distribution coefficients of six chemical groups were summarized in Fig. 7. The distribution coefficient of furans, alcohols, or ketones in hexane and petroleum ether was less than 0.6, much lower than that in chloroform or ethyl acetate. Due to the large difference in polarity between the solvent and the chemicals to be extracted, the distribution coefficients of levoglucosan and acids in hexane and petroleum ether were very low, generally less than 0.1 (Fig. 7). These results indicate that hexane and petroleum ether cannot be efficiently used to extract furans, alcohols, ketones, anhydrosugars, and organic acids. The highest distribution coefficient of alcohols and furans in chloroform was observed at 0.79 and 4.49, respectively. Compared to those in chloroform, the distribution coefficients of alcohols and furans in ethyl acetate were lower. Therefore, chloroform would be the preferred choice for the extraction of alcohols and furans from the bio-

oil aqueous phase. Levoglucosan and organic acids such as acetic acid can form hydrogen-bonding with water in the bio-oil aqueous phase [6, 43]. Although chloroform has a high polarity, it barely breaks the hydrogen bond in extraction. However, in the case of ethyl acetate with a slightly higher polarity, when the S/F ratio was increased from 0.5 to 2, the hydrogen bond between acids and water could be broken, thereby increased the distribution coefficient of acids in ethyl acetate from 0.01 to 0.67 (Fig. 7). Therefore, ethyl acetate at an S/F ratio of 2 would be suitable for extracting acetic acid from BOAP I. The distribution coefficients of phenolics in chloroform were greater than 1.5 at the S/F ratios of 0.5 and 1, and they were greater than 10 at all the investigated S/F ratios in ethyl acetate (Fig. 7), much higher than those in hexane and petroleum ether, indicating that both chloroform and ethyl acetate are suitable for extracting phenolics from the bio-oil aqueous phase.

Based on the above analysis, we further developed a sequential extraction method to separate chemical groups (Fig. 1). First by using chloroform, we can separate furans, alcohols, ketones, and phenolics together (Group 1). Then by using ethyl acetate, we can separate organic acids (Groups 2). Finally, we can recover anhydrosugars (Group 3) from the final aqueous phase of BOAP III. This sequential extraction was investigated and the results are reported in the following section.

3.4 Sequential extraction by chloroform and ethyl acetate

After chloroform extraction, the number of chromatography peaks of BOAP II was reduced and major peaks observed were levoglucosan, acetic acid, propionic acid, and 1,2-benzenediol. After ethyl acetate extraction of BOAP II, the major peak in BOAP III was only levoglucosan. The chemical compositions of aqueous phase before and after extraction, and the extracted organics are presented in Table 2 and Fig. 8. In this study, about 53.9 wt.% of total chemicals in

BOAP I was quantified. A number of chemicals (46.1 wt.% of total chemicals) with very low concentration listed in alcohol, ketones, aldehydes, etc., in BOAP I were not quantified. About 62.2 wt.% of the total of furans, ketones, alcohols, and phenolics and 85 wt.% unquantified chemicals in BOAP I were extracted to chloroform. After chloroform extraction, BOAP II was mainly composed of organic acids and levoglucosan, which accounted for about 43.4 wt.% and 36.1 wt.%, respectively (Fig. 8). Other components were about 2.0 wt.% furans, 3.0 wt.% alcohols, 1.1 wt.% phenolics, 0.56 wt.% ketones, and 13.8 wt.% unquantified chemicals. The sequential extraction by chloroform followed by ethyl acetate well recovered furans, ketones, and phenolics from aqueous phase, leaving behind no ketones and only about 0.7 wt.% furans, and 0.04 wt.% phenolics in BOAP III after extraction (Fig. 8). The sequential extraction also concentrated levoglucosan resulting in about 53 wt.% levoglucosan in BOAP III. However, we also observed that BOAP III contained about 32.5 wt.% organic acids. In this study, BOAP II was extracted only once by ethyl acetate. Multiple extractions using ethyl acetate may be implemented to extract larger fraction of acids from BOAP II. The organics extracted by chloroform were mainly furans, ketones, alcohols, phenolics. Due to the very low concentration of these compounds, it will be difficult to further purify them individually. The preferred application of these compounds could be as a feedstock for the biorefinery process to generate hydrocarbons fuels. The organics extracted by ethyl acetate contains over 57.6 wt.% organic acids, in which acetic acid accounts for about 75 wt.%. It will be costly to produce glacial acetic acid from this stream. Therefore, these organics could serve as a source for hydrogen production using microbial electrolysis [16].

4. Conclusions

Four organic solvents were evaluated for chemical extraction from bio-oil aqueous phase. The amount of chemicals extracted by these four solvents from bio-oil aqueous phase was in the order of hexane < petroleum < chloroform < ether ethyl acetate at the same S/F ratio. Further, chloroform had similar extraction efficiency with ethyl acetate for furans, alcohols, ketones and phenolics. Ethyl acetate also had high extraction efficiency for acetic acid when the S/F ratio was at 2. According to the distribution coefficients and extraction efficiencies obtained for the different chemical groups, a sequential extraction using chloroform followed by ethyl acetate is recommended. The first step of extraction using chloroform separated most of the furans, ketones, alcohols, and phenolics from bio-oil aqueous phase. The second step of extraction employing ethyl acetate concentrated organic acids (over 62 wt.% acetic acid in BOAP I). In the final aqueous phase, levoglucosan was concentrated at 53 wt.%. These results suggest that sequential extraction with different solvents can be used to separate chemical groups in bio-oil aqueous phase and these extracted chemical groups could serve as feedstocks for the specific production of chemicals, hydrogen, or hydrocarbons.

Acknowledgements

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525

526 **Figure captions**

527 **Figure 1.** Experimental procedures (dotted box) with separation of chemical groups from bio-oil
528 aqueous phase (BOAP I-III) by sequential extraction and their applications for the production of
529 fuels and chemicals.

Figure 2. Chemical distribution (relative peak area percentage analyzed by GC/MS) in BOAP I.

Figure 3. The mass percentage of chemicals extracted by different organic solvents and S/F ratios.

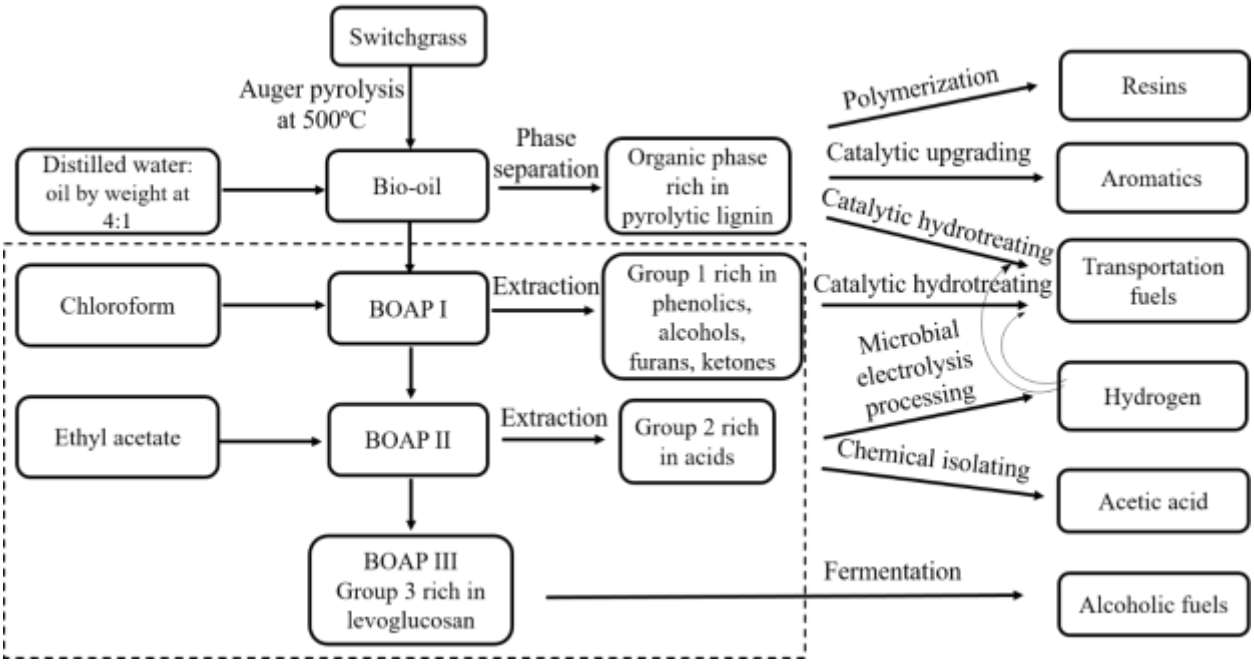
Figure 4. Extraction efficiencies for levoglucosan, acetic acid, and propionic acid in BOAP I by different solvents and S/F ratios.

Figure 5. Extraction efficiencies for alcohols, ketones, and furans in BOAP I by different solvents and S/F ratios.

Figure 6. Extraction efficiencies for phenolics in BOAP I by different solvents and S/F ratios.

Figure 7. Distribution coefficients for chemical groups from BOAP I in different solvents. Distribution coefficients reported are average numbers based on three replicates.

Figure 8. Chemical compositions of aqueous phase before and after sequential extraction and the extracted organics in solvents.



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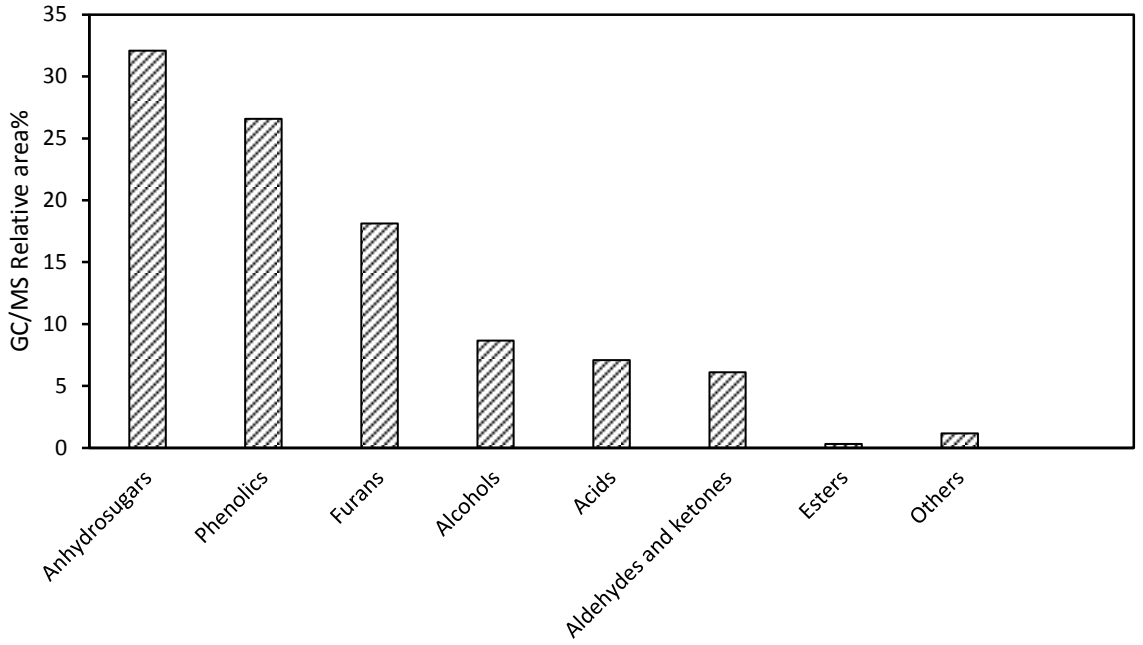

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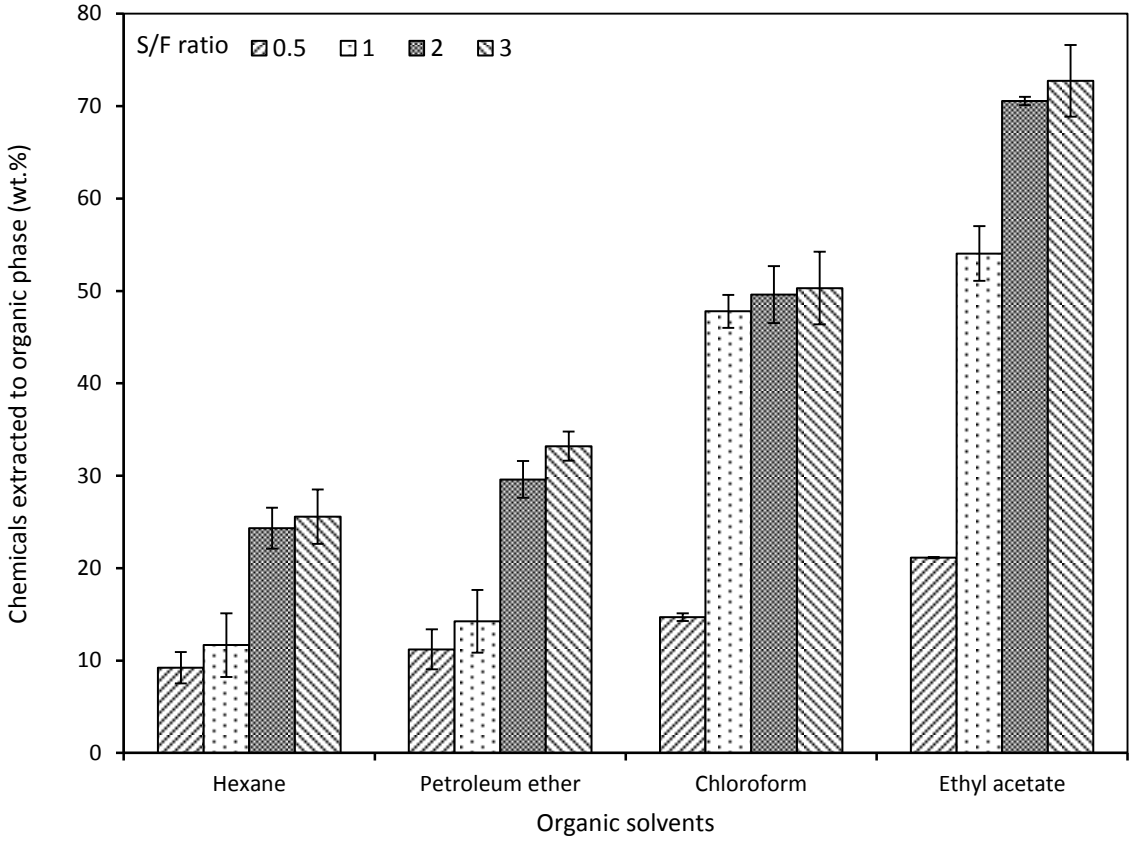

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583 **Figure 3.** The mass percentage of total chemicals extracted by different organic solvents and S/F
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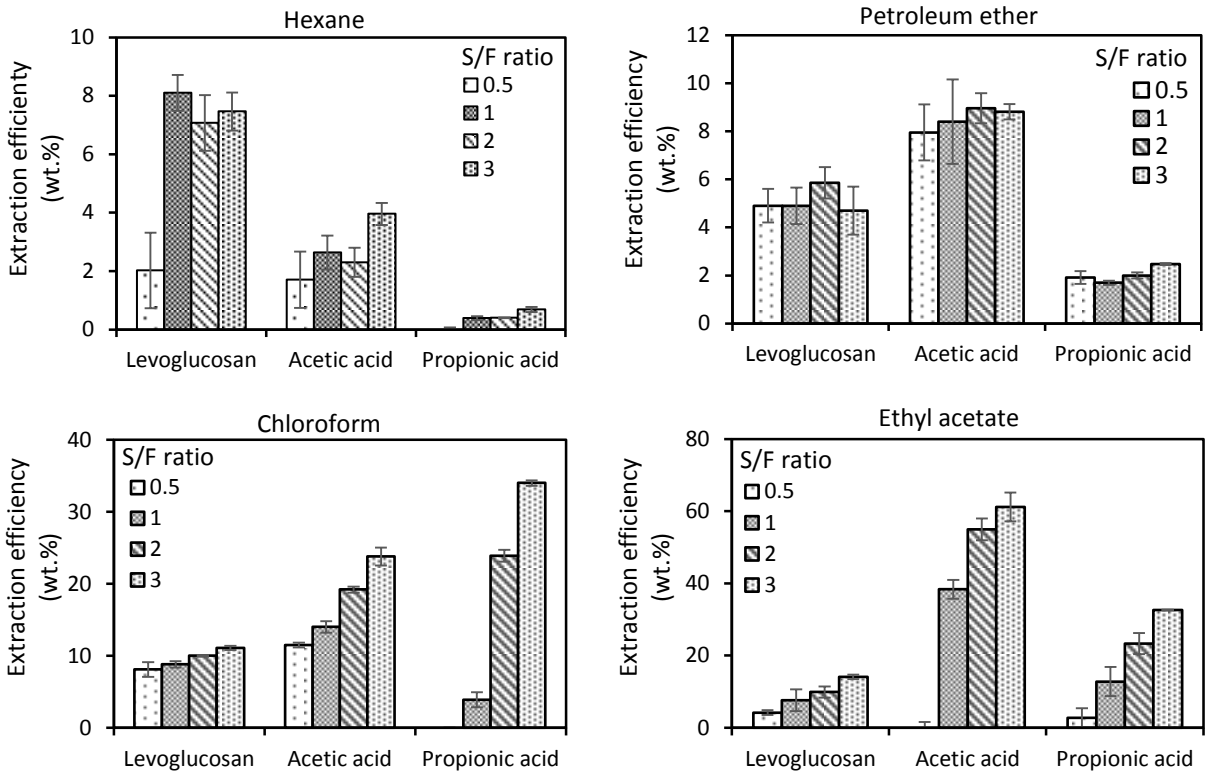
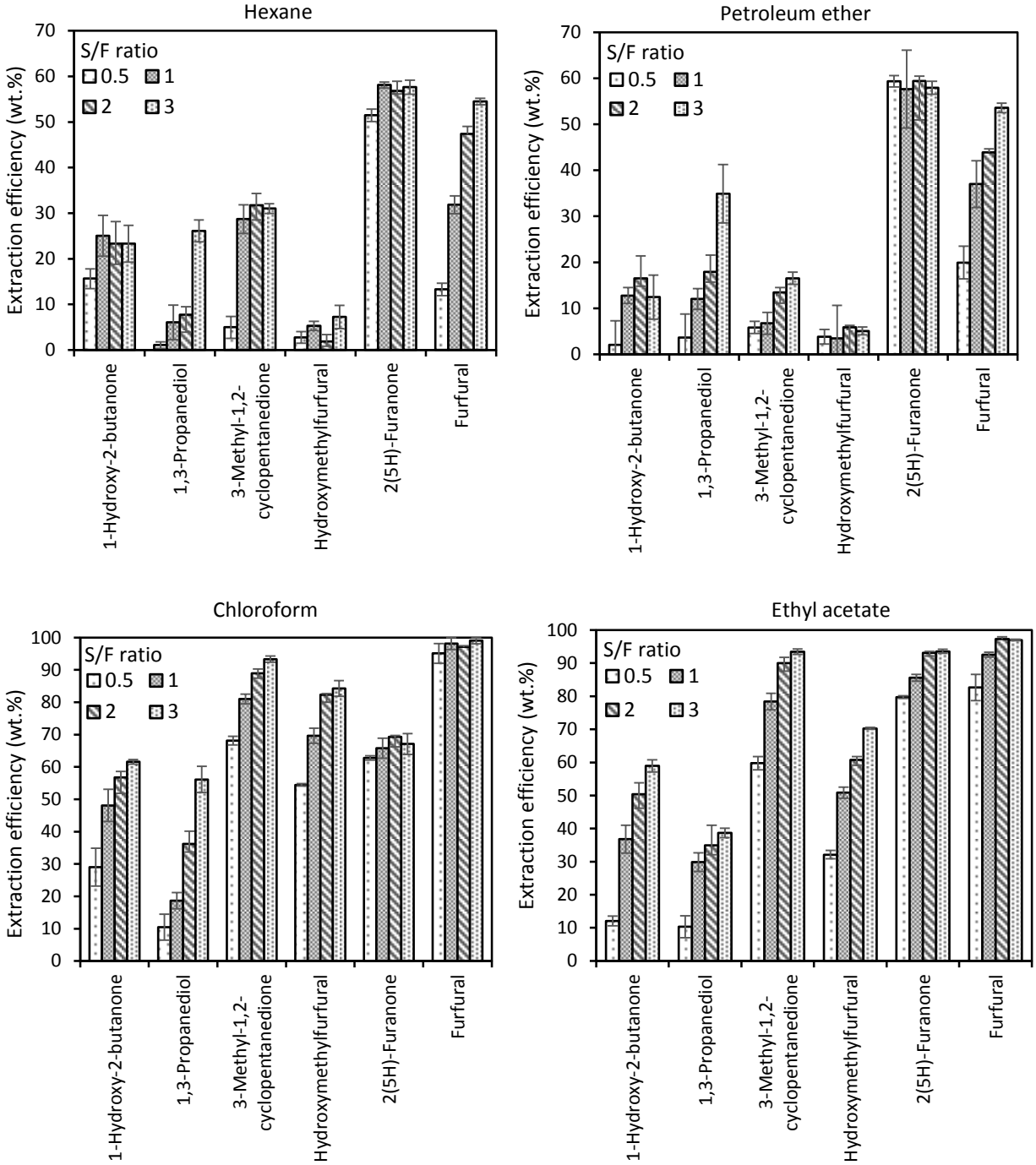


Figure 4. Extraction efficiencies for levoglucosan, acetic acid, and propionic acid in BOAP I by different solvents and S/F ratios



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608 **Figure 5**. Extraction efficiencies for alcohols, ketones, and furans in BOAP I by different

609 solvents and S/F ratios

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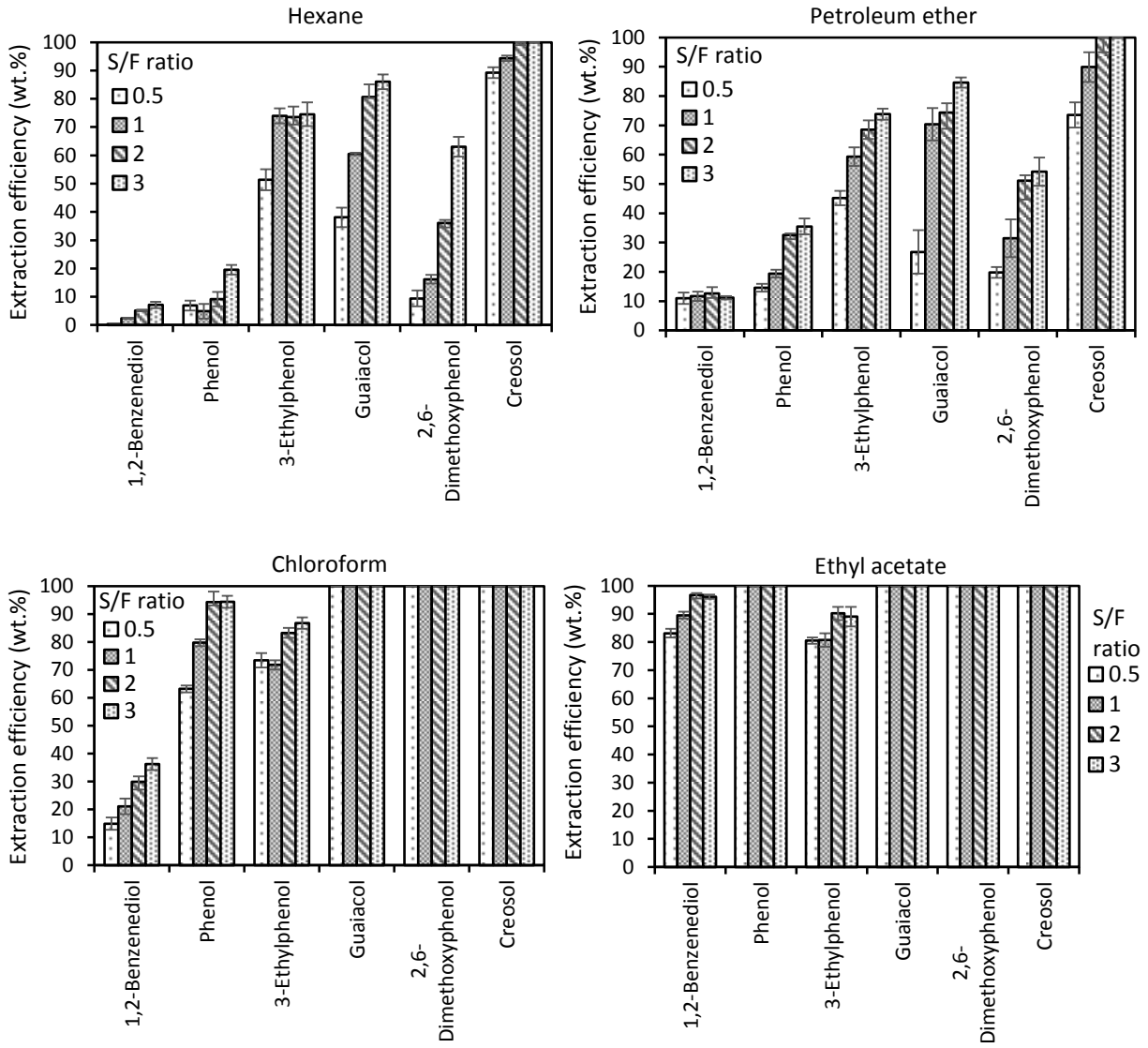


Figure 6. Extraction efficiencies for phenolics in BOAP I by different solvents and S/F ratios

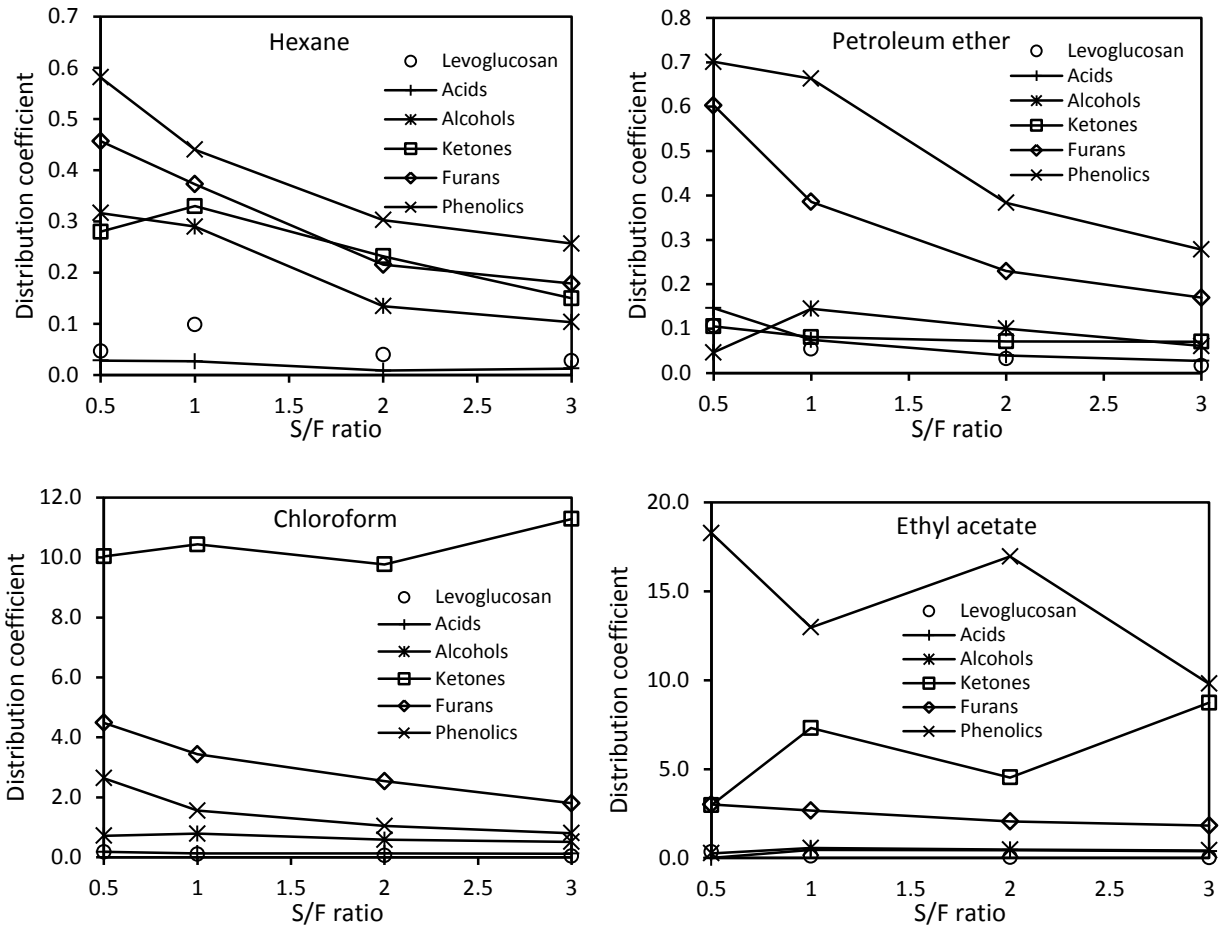
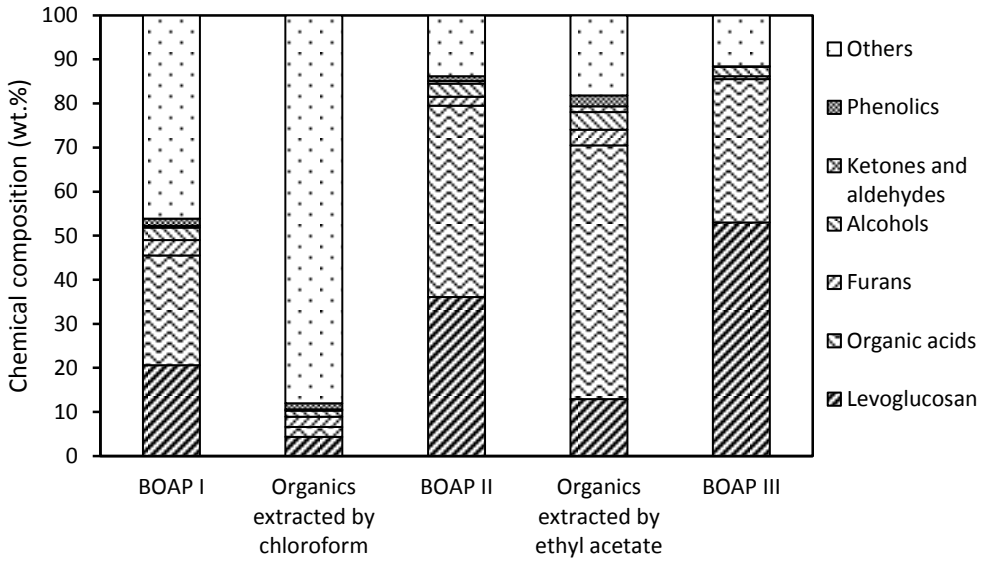


Figure 7. Distribution coefficients for chemical groups from BOAP I in different solvents. Distribution coefficients reported are average numbers based on three replicates.



634

635 **Figure 8**. Chemical compositions of aqueous phase before and after sequential extraction and the

636 extracted organics in solvents

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Table 1. Properties of crude bio-oil and BOAP I (Deionized water added at 4:1 by weight for BOAP I separation)

Properties	Crude bio-oil	BOAP I
Water content (wt%)	41.1±0.5	90.8±1.4
Total solid (wt%)	0.77±0.05	Not detected
pH value	2.4±0.1	2.6±0.1
Density (g/mL)	1.14±0.01	1.02±0.00
Ash (wt%)	0.09±0.01	0.02±0.01
Viscosity at 40 °C centistokes (cSt)	3.23±0.03	0.71±0.01
TAN, mg KOH/g	130.3±1.9	25.3±0.1

Table 2. Concentrations of 15 compounds quantified in BOAP I, BOAP II (aqueous fraction produced post chloroform extraction), and BOAP III (aqueous fraction remaining after ethyl acetate extraction). Data reported are average numbers based on three replicates.

Classifications	Major compounds	Concentration in BOAP I (g/L)	Concentration in BOAP II (g/L)	Concentration in BOAP III (g/L)
Acids	Acetic acid	16.54	14.64	6.26
	Propionic acid	6.37	6.35	3.93
Anhydrosugars	Levoglucosan	18.99	17.46	16.62
Furans	Furfural	0.98	0.05	BDL
	2(5H)-Furanone	0.82	0.31	0.04
	Hydroxymethylfurfural	1.37	0.62	0.17
Alcohols	1-Hydroxy-2-butanone	2.25	1.17	0.51
	1,3-Propanediol	0.35	0.29	0.18
Ketones	3-Methyl-1,2-cyclopentanedione	0.40	0.27	BDL
Phenolics	1,2-Benzenediol	0.63	0.54	0.01
	Phenol	0.15	BDL	BDL
	Guaiacol	0.20	BDL	BDL
	Creosol	0.15	BDL	BDL
	2,6-Dimethoxyphenol	0.17	BDL	BDL
	3-Ethylphenol	0.20	BDL	BDL

BDL: The concentrations are below the detection limit.

Table 3. Extraction efficiency of different solvents for 15 quantified chemicals at the optimum conditions. Data reported are average numbers based on three replicates.

		Major compounds	Extraction efficiency (wt.%)			
			Hexane (2:1)[a]	Petroleum ether (2:1)	Chloroform (1:1)	Ethyl acetate (2:1)
Group 1	Furans	Furfural	56.8	43.9	98.1	97.3
		2(5H)-Furanone	47.3	59.4	65.8	93.1
		Hydroxymethylfurfural	1.8	5.9	69.6	60.8
	Alcohols	1-Hydroxy-2-butanone	23.3	16.5	48.1	50.4
		1,3-Propanediol	7.7	17.9	18.6	35.0
	Ketones	3-Methyl-1,2-cyclopentanedione	31.7	13.5	81.0	90.1
	Phenolics	1,2-Benzenediol	5.1	12.6	21.1	96.7
		Phenol	9.1	32.6	79.8	100.0
		3-Ethylphenol	73.5	68.5	71.8	90.3
		Guaiacol	80.7	74.4	100.0	100.0
		2,6-Dimethoxyphenol	36.1	51.2	100.0	100.0
		Creosol	100.0	100.0	100.0	100.0
Group 2	Acids	Acetic acid	1.3	9.0	14.0	55.0
		Propionic acid	0.4	1.2	3.9	23.3
Group 3	Anhydro-sugars	Levoglucosan	7.1	5.9	8.8	7.6

[a] : The optimum volume ratio of solvent-to-feed