



Upgrading of bio-oil and aqueous liquid product from pyrolysis of microalgae (*Nannochloropsis oculata*) by fractional distillation

Monet Concepcion Maguyon-Detras^{*1}, Sergio C. Capareda²

¹Department of Chemical Engineering, College of Engineering and Agro-Industrial Technology, University of the Philippines Los Baños, Los Baños, Laguna, Philippines

²Bio-Energy Testing and Analysis Laboratory (BETA Lab), Biological and Agricultural Engineering Department, Texas A & M University, College Station, Texas, USA

Key words: Bio-oil, Microalgae, *Nannochloropsis oculata*, Pyrolysis, Fractional distillation

<http://dx.doi.org/10.12692/ijb/10.1.218-231>

Article published on January 29, 2017

Abstract

The liquid product (bio-oil and aqueous) from pyrolysis of biomass such as microalgae contains a wide variety of compounds which makes it a potential source of biofuels and bio-chemicals. However, its complex composition limits its direct use as such. In this paper, fractional distillation at atmospheric conditions was explored as a technique to upgrade the quality of the pyrolytic bio-oil and aqueous liquid product (ALP) distillate fractions. By fractional distillation, the liquid product components were separated to relatively simpler distillate fractions which were then analyzed in terms of moisture, energy content, elemental and chemical compositions to assess their potential uses as fuel substitute or source of bio-chemicals. Fractional distillation of algal bio-oil resulted to 19% wt light fractions (BF1 and BF2), 7% wt middle fractions (BF3 and BF4), 51% wt heavy fraction (BF5) and 23% wt non-distillate residue. BF1 and BF2 were found to be comparable to diesel and BF6 to heavy fuel oil using van Krevelen diagram. For the ALP, the distillate fraction obtained at 150-180°C (AF5) was found to have a heating value (about 24 MJ/kg) which is higher than methanol and wood-derived bio-oils. It was also found to contain various components such as carboxylic acids and carboxylate esters which could be further processed to produce value-added chemicals. The separation factor, β , showed that complete separation can be achieved by fractional distillation for some compounds such as high molecular weight olefins and naphthenes.

* **Corresponding Author:** Monet Concepcion Maguyon-Detras ✉ mcmaguyon@up.edu.ph

Introduction

The rising demand for energy as a consequence of rapid population growth and economic progress is a continuing challenge that still needs to be addressed in the coming years. In a recent report by the US Energy Information Administration (2016), the total world energy consumption is expected to increase by 48% from 549 Quadrillion Btu in 2012 to 815 Quadrillion Btu in 2040, with liquid fuels remaining as the major energy being consumed. 62% of the increase in liquid fuel demand comes from the transportation industry, which at present, greatly depends on petroleum crude oil derivatives (US EIA, 2016). However, the intensified concern on the environmental effects of burning of fossil fuels (i.e. greenhouse gas emissions) has stimulated movements towards exploring energy sources that are less carbon-intensive and renewable.

Pyrolysis of CO₂-neutral and renewable biomass feedstock such as agricultural wastes, livestock manure, sewage sludge, and aquatic microorganisms such as microalgae is a potential alternative to produce liquid transport fuels (Wang, 2013). Pyrolysis is gaining more importance, nowadays, since it produces energy fuels with high fuel-to-feed ratio, and the process can be easily adjusted to favor solid, liquid, or gaseous fuel production (Bridgwater, 2006; Czernik and Bridgwater, 2004; Grierson *et al.*, 2009; Miao *et al.*, 2004). The pyrolytic bio-oil is comparable to crude oil, which can be easily stored and transported, and can be used for direct combustion or upgraded further to liquid transport fuels and bio-chemicals (Grierson *et al.*, 2009; Miao *et al.*, 2004).

In a previous study, microalgae (*N. oculata*) was used as a feedstock for the production of pyrolytic liquid (Maguyon and Capareda, 2013). Two immiscible liquid fractions were obtained, namely: (1) the dark-brown organic layer or bio-oil, and (2) the yellowish aqueous liquid product (ALP). Similar to bio-oils described elsewhere, the algal bio-oil contains a wide range of chemicals including alkanes, aromatics, esters and nitriles (Bridgwater, 2006; Czernik and Bridgwater, 2004; Marcilla *et al.*, 2009; Maguyon and Capareda, 2013).

This complex composition of the bio-oil restricts its direct use as liquid engine fuel. Hence, further upgrading of its quality to make it comparable to petroleum-derived fuels is necessary. According to Wang (2013), upgrading of the bio-oil can be done by separating its components to simpler fractions, which may have different uses depending on their characteristics. The ALP, on the other hand, is mostly composed of water but it may also contain sugars, organic acids, hydroxyacetone, hydroxyacetaldehyde, furfural, and small amounts of guiacols. Similar to bio-oil, these compounds when separated from water may be further processed into liquid fuel components such as alkanes, aromatics, or olefins (Cortright *et al.*, 2002; Huber *et al.*, 2004; Teella *et al.*, 2011).

The conventional techniques in the separation of bio-oil components include column chromatography, extraction, centrifugation, and distillation (Teella *et al.*, 2011). Column chromatography was used by Wang *et al.* (2011), Cao *et al.* (2010), and Zeng *et al.* (2011) to separate components of wood tar, and bio-oils from sewage sludge, and rice husk, respectively. Solvent extraction of valuable bio-oil components such as phenol can also be found elsewhere (Amen-Chen *et al.*, 1997; Lu *et al.*, 2011). Although these methods were found effective to a certain extent in separating specific compounds from the bio-oil, further processing is still needed to remove the solvent used in both processes. Centrifugation, on the other hand, is a simple pretreatment technique; however, the homogeneity of the bio-oil limits its applicability (Wang *et al.*, 2009). There are various distillation techniques which can be utilized for bio-oil separation including molecular distillation, flash distillation, steam distillation and fractional distillation (Wang *et al.*, 2009).

In this study, fractional distillation was explored as a technique to improve the quality of the distillate fractions of algal bio-oil and ALP. Fractional distillation or rectification is the traditional way of separating transport fuels such as gasoline and diesel from petroleum crude. Most of the researches conducted in bio-oil distillation used molecular distillation which is typically done under high vacuum (<0.01 torr) (Boucher *et al.*, 2000; Guo *et al.*, 2010a; Guo *et al.*, 2010b; Wang *et al.*, 2009).

Unlike molecular distillation, fractional distillation can be done at atmospheric conditions making the process less costly. The distillation characteristics of the algal bio-oil obtained in this study may be different from bio-oils derived from lignocellulosic feed stocks (i.e. rice husk, sawdust, softwood) used by other authors (Boucher *et al.*, 2000; Lu *et al.*, 2011; Xu and Lu, 1999). Also, to our knowledge, limited studies focused on the potential application of the aqueous liquid product (ALP) as liquid fuel and only specific compounds (i.e. alkanes and acetic acid) were separated from the ALP.

Hence, this study was primarily conducted to evaluate fractional distillation at atmospheric conditions as a technique to upgrade the quality of the distillate fractions of bio-oil and ALP obtained from pyrolysis of *N. oculata*. The specific objectives were as follows:

- a) determine the distillate yields at different vapor temperature ranges;
- b) compare the distillation characteristics of bio-oil and ALP using distillation curves;
- c) evaluate the characteristics of the distillate fractions in terms of moisture content, heating value, and elemental and chemical compositions;
- d) compare the bio-oil and ALP distillate fractions to petroleum-derived fuels and wood bio-oils using the van Krevelen diagram; and
- e) assess the degree of separation of the bio-oil components.

Materials and methods

Preparation of pyrolytic bio-oil and aqueous liquid product (ALP) from microalgae

The bio-oil and aqueous liquid product (ALP) used in this study was produced from pyrolysis of ground and dried *Nannochloropsis oculata* in a fixed-bed batch-type Parr pressure reactor as described in a previous work (Maguyon and Capareda, 2013) at 540°C and atmospheric pressure. Separation of the two liquid layers was done using a separatory funnel where the aqueous liquid layer at the bottom was first collected in a glass container followed by the organic dark-brown bio-oil layer.

Distillation experiment

The distillation set-up used in this study consisted of a 50-ml distillation flask, Vigreux distilling column, distillation head, thermometer, condenser, cow head adapter and 15-ml receiving flasks as illustrated in Fig 1. For each distillation run, approximately 25 g of sample was placed inside the pre-weighed distillation flask with boiling chips to promote even boiling of the liquid. A heating mantle with a controller was used to heat the sample inside the distillation flask. The temperature inside the column was measured using a laboratory thermometer (maximum reading 400°C) with the bulb slightly below the arm of the distillation head. The distillation head was used to connect the distilling column to the condenser and thermometer. During distillation, the heater is first turned on and cooling water is allowed to flow through the annulus of the condenser. The temperature after the first drop is recorded then the distillate fractions were collected at various boiling point ranges as summarized in Table 1. The end of the condenser was attached to a cow head adapter, which directs the condensate from the condenser to a pre-weighed receiving flask. Each fraction was weighed using an analytical balance (Mettler Toledo, Model XP105DR, Switzerland). The yield for each fraction was calculated by dividing the weight of the distillate fraction obtained by the weight of the sample (aqueous, bio-oil) used. Distillation experiments for both the aqueous and bio-oil liquid products were done in triplicates and average values were reported.

Analysis of the distillate fractions

The distillates obtained from the fractional distillation experiments were collected in small glass vials and analyzed immediately for their chemical composition and properties. Heating values of the distillate samples was determined using PARR isoperibol bomb calorimeter (Model 6200, Parr Instrument Company, Moline, IL) following ASTM D2015. Ultimate analysis was determined using Vario MICRO Elemental Analyzer (Elementar Analyse systeme GmbH, Germany) in accordance with ASTM D 3176. Moisture content was analyzed by following ASTM E203 using KF Titrino 701 (Metrohm, USA, Inc).

The chemical composition of the distillate fractions was also determined by GC-MS analysis using Shimadzu QP2010 Plus, with the following parameters: bio-oil dissolved in dichloromethane (10 % vol); column – DB-5ms (25m length, 0.25 μ m thickness and 0.25mm diameter); column temperature program: 40°C (held for 5 minutes) then ramped to 320°C at 5°C/min, then held for 5 minutes at 320°C; ion source temperature at 300°C. The functional groups present in each distillate fraction were also analyzed using Shimadzu IRAffinity-1 FTIR (Fourier Transform Infrared) Spectrophotometer

(Shimadzu, Inc). Results of the FTIR analysis were interpreted using IR Solutions (version 1.5) software.

Results and discussion

*Characteristics of the liquid products from pyrolysis of *N. oculata**

The pyrolytic dark-brown bio-oil from *N. oculata* was found to contain about 72% carbon, 10% hydrogen, 8% nitrogen, 0.15% sulfur and 10% oxygen by weight. Based on its O:C and H:C ratios, this product could be a potential alternative to crude oil after further processing to improve its quality.

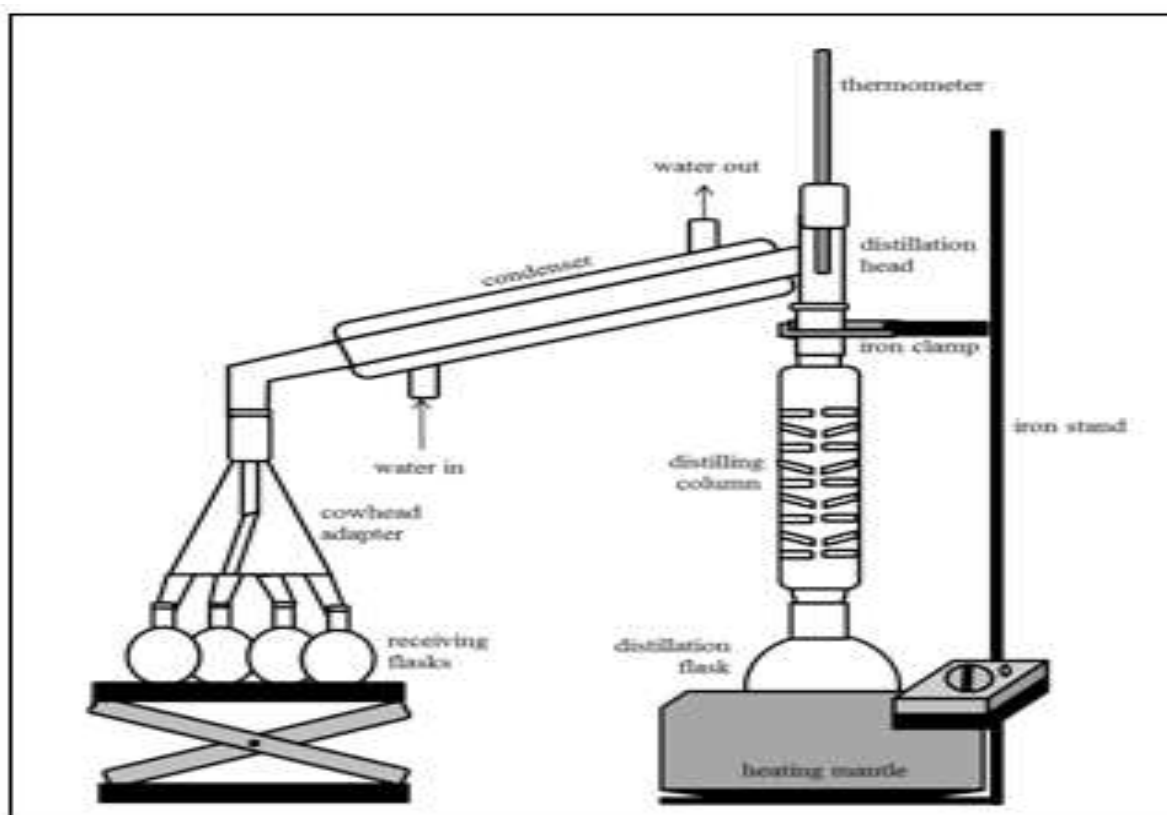


Fig. 1. Distillation set-up.

Removal of its moisture content (6% wt) by distillation could further improve its heating value (36 MJ/kg) which is still lower than heavy fuel oil (40 MJ/kg) (Czernik & Bridgwater, 2004). Moreover, heteroatoms such as nitrogen and oxygen may also be removed during the distillation process and improve the bio-oil quality. On the other hand, the yellowish aqueous liquid product (ALP), which settles at the bottom layer of the liquid product, consists of about 65% wt moisture.

The remaining 35% wt may consist of water-soluble organic compounds as indicated by the FTIR results shown in a previous work (Maguyon and Capareda, 2013).

Distillate yields

Fig. 2 shows the distribution of the distillate fractions of algal bio-oil at different vapor temperature ranges after fractional distillation at atmospheric conditions.

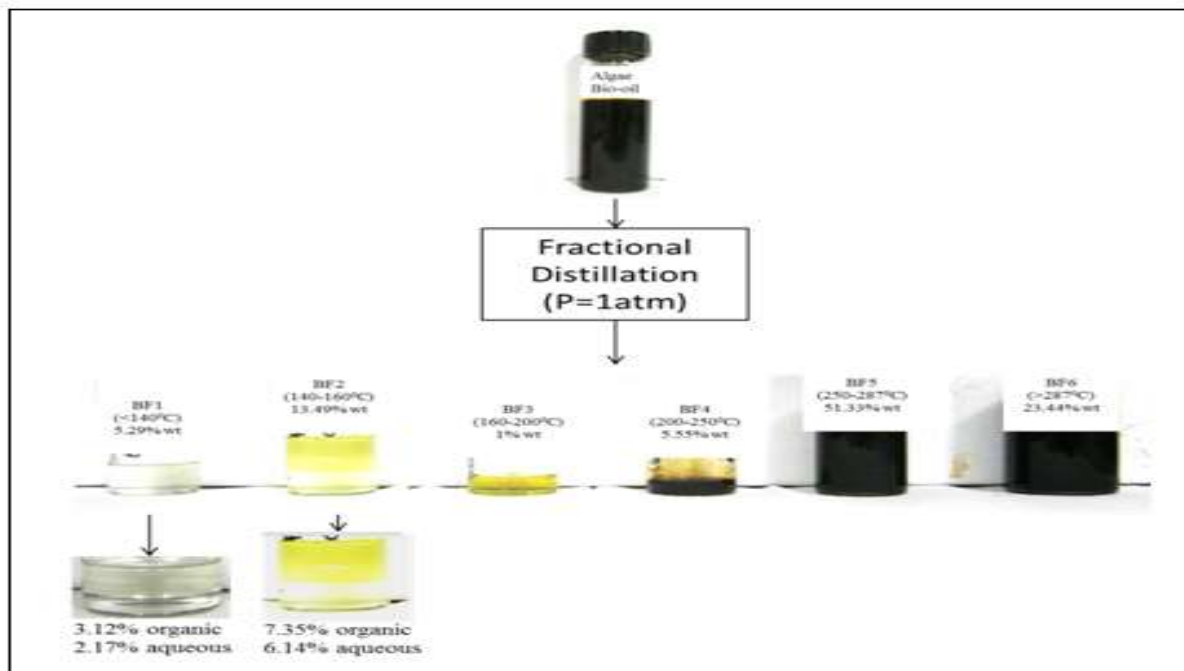


Fig. 2. Distribution of distillate fractions of algal bio-oil.

The six (6) distillate fractions of the bio-oil were referred in this paper as BF1 to BF6. The first two fractions (BF1 and BF2) consist of two immiscible aqueous and organic layers. BF1 has about 59% wt organic layer (3% wt of the bio-oil) and 41% wt aqueous layer (2% wt of the bio-oil) while BF2 contains about 54% wt (or 7% wt of the bio-oil) organic and 46% wt (or 6% of the bio-oil) aqueous

fractions. The presence of aqueous layers in BF1 and BF2 may be attributed to the water present in the bio-oil, which typically condenses in the first fractions. In the separation of sawdust bio-oil components using molecular distillation, Fraction I also contains about 50-70% wt water content while the middle fraction has only about 1-2% wt (Wang *et al.*, 2009).

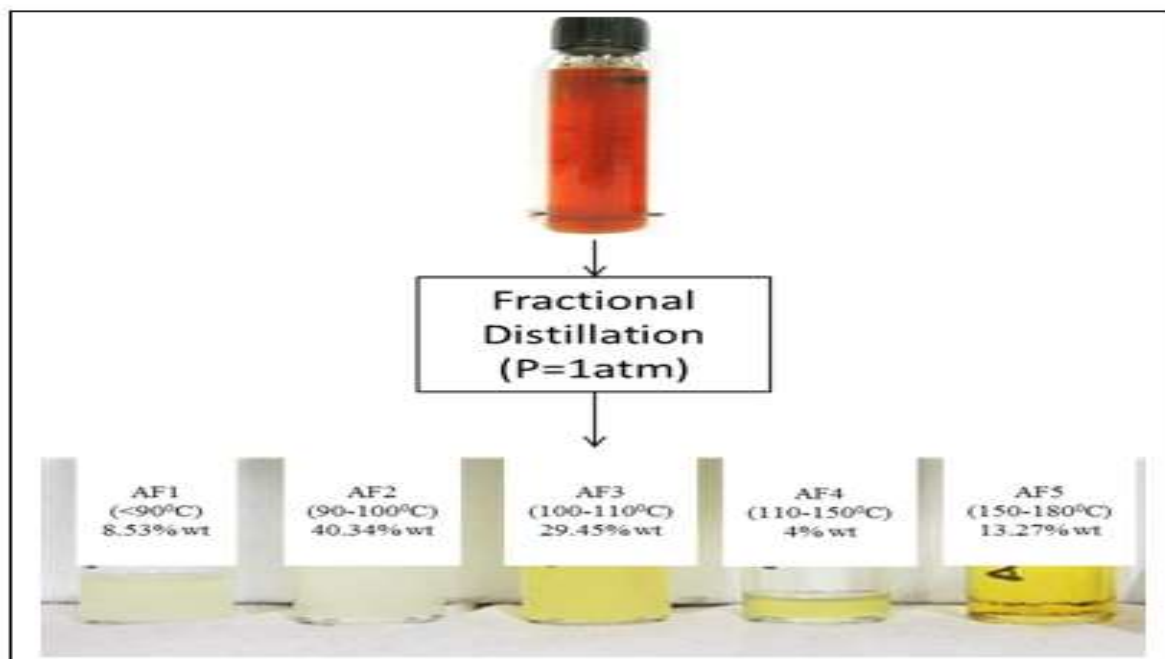


Fig. 3. Distribution of distillate fractions of the aqueous liquid product (ALP).

Based on the yields, BF5 (51% wt) was the major product of the fractional distillation of bio-oil followed by the non-distillate residue, BF6 (23% wt). BF5 has almost the same color as

the algal bio-oil while BF6 is darker in color and more viscous than BF5. On the other hand, lower yields were observed from the middle fractions, BF3 (1% wt) and BF4 (6% wt).

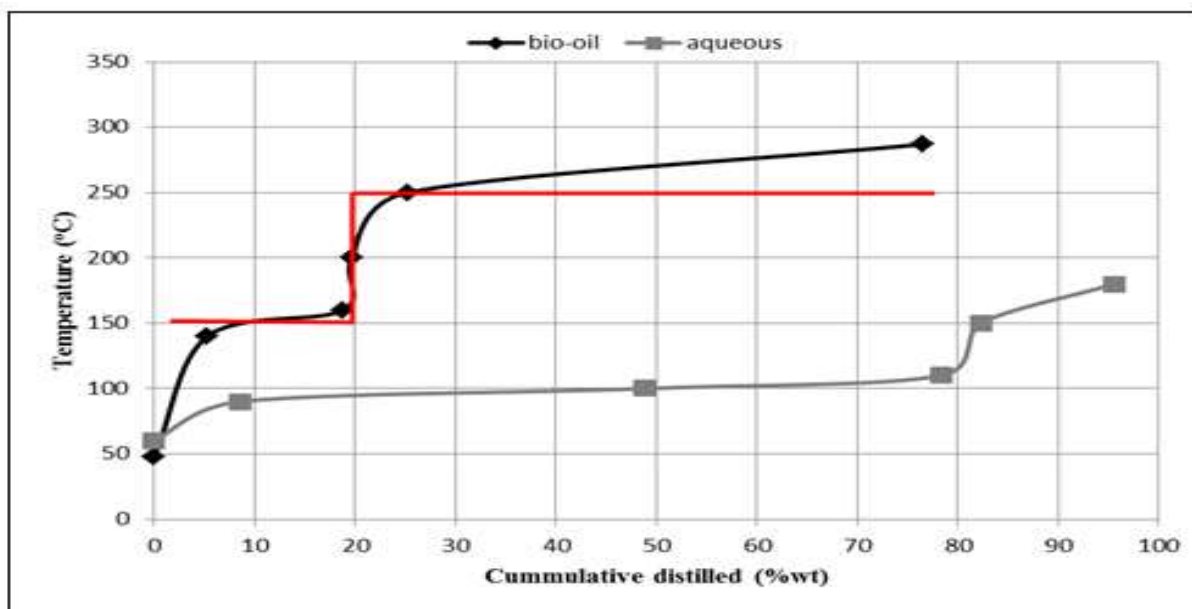


Fig. 4. Distillation curves for algal bio-oil and aqueous liquid product.

The vapor temperature ranges and distillate yields for ALP, on the other hand, are shown in Fig. 3. Lower boiling points close to the boiling point of water were employed for ALP since it consists mostly of water (65% wt). The five (5) ALP distillate fractions were referred in this paper as AF1 to AF5. Based on Fig. 3, about 49% wt were distilled below 100°C (AF1 and AF2) and an additional 30% wt (AF3) was condensed just above the boiling point of water (100-110°C). Based on the total amount of distillates (79% wt), which is greater than the moisture content of ALP (65% wt), and the color of the distillates (AF1, AF2 and AF3), there may be other organic components with similar or lower boiling points than water that were distilled from ALP at temperatures lower than 110°C. A light yellow distillate fraction, AF4 (4% wt), was distilled at around 110-150°C while a darker yellow transparent distillate fraction, AF5 (13% wt), was obtained at about 150-180°C. A black solid residue amounting to about 4% wt adhered to the surface of the distillation flask after fractional distillation at 180°C. This residue was not considered in the later characterization of distillation products.

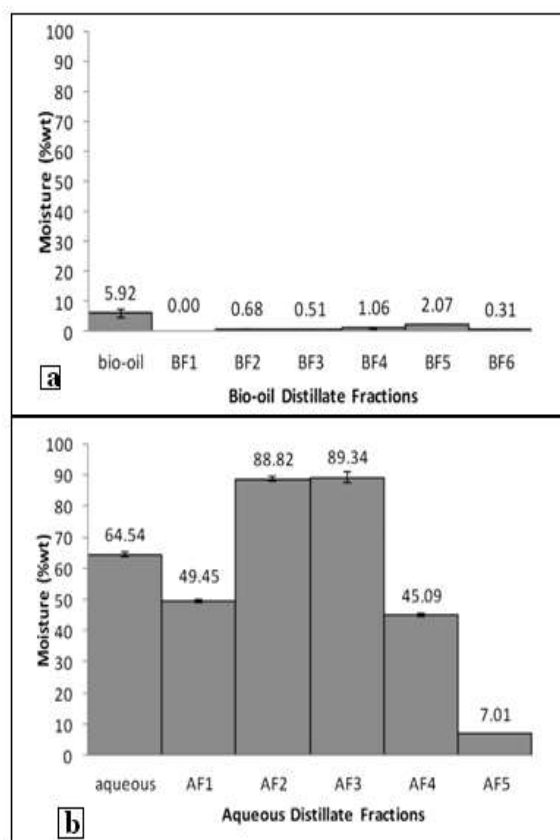


Fig. 5. Moisture contents of algal bio-oil and aqueous liquid product distillate fractions.

Distillation curves

Fig. 4 shows the distillation curves of the algal bio-oil and ALP. A distillation curve represents the temperature of the distilling vapor as a function of cumulative mass percentage of the distillate (Aglevor *et al.*, 2012; Boucher *et al.*, 2000).

The figure (Fig. 4) clearly shows that bio-oil fractions boil at higher temperatures than ALP distillates. Also, both bio-oil and ALP distillation curves slightly varied from an ideal distillation plot since both plots are empirical and a

wide variety of compounds are present in the bio-oil and ALP. Nonetheless, both plots show distinct boiling point ranges for the lower and higher boiling components. For bio-oil, lower boiling components condensed at temperatures ranging from 50 to 160°C while higher boiling components were obtained from 250°C to about 290°C. The lower boiling components for ALP, on the other hand, were collected at temperatures below 100°C while the higher boiling components were obtained at temperatures starting from 150 °C to 180 °C.

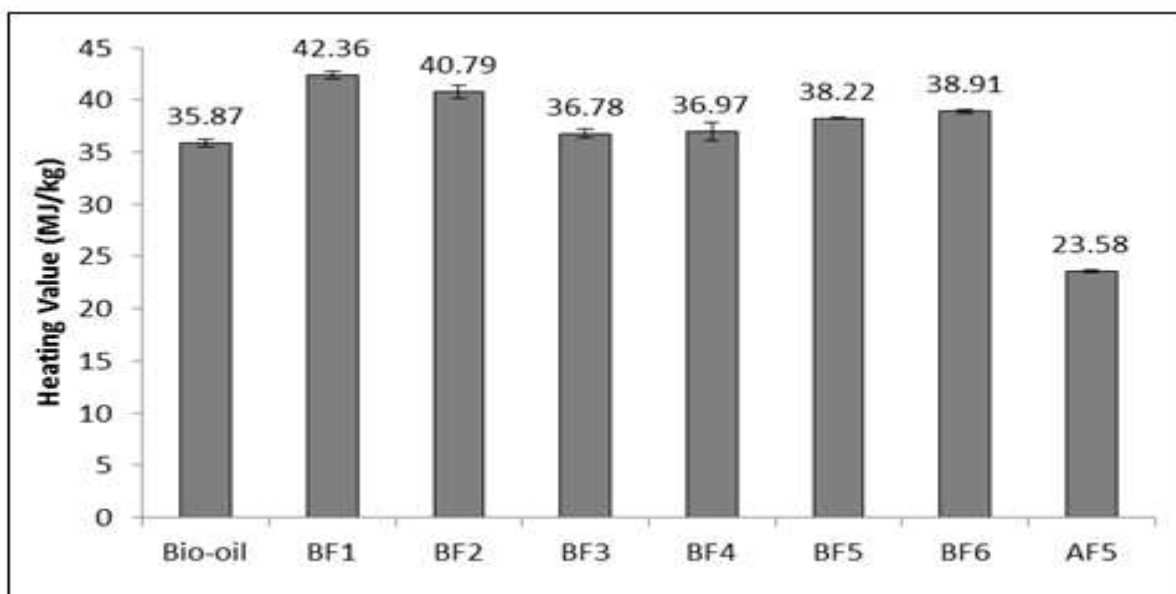


Fig. 6. Comparison of the heating values of algal bio-oil, bio-oil distillates and AF5.

Characteristics of distillate fractions

One of the main goals in further processing bio-oil either by chemical or physical means is to improve the quality of the bio-oil or its components. This section shows the variation of the bio-oil and ALP distillate fractions from their corresponding original material (i.e. bio-oil, ALP).

Moisture content

The original bio-oil contains about 6% moisture as shown in Fig. 5a. Water present in the bio-oil obtained from pyrolysis is typically generated by the dehydration reactions occurring during pyrolysis (Czernik and Bridgwater, 2004; Marcilla *et al.*, 2009; Peng *et al.*, 2001). It has positive effect on the flow characteristics of the bio-oil by reducing its viscosity and NO_x emission during combustion. However,

water also tends to lower the heating value and decreases the combustion rate of the bio-oil (Czernik and Bridgwater, 2004). Due to this, the removal of water from the bio-oil may be necessary to improve its heating value and combustion characteristics. ALP, on the other hand, is expected to be mainly composed of water. However, as mentioned earlier, 35% wt of ALP could be water-soluble organics which may have potential uses.

Fig. 5 shows the moisture contents of the bio-oil and ALP distillate fractions. For BF1 and BF2, only the top organic layers were analyzed for moisture content. As can be seen from Fig. 5a, there is a considerable decrease in the moisture contents of the bio-oil fractions as compared to the original bio-oil.

BF5 has the highest moisture content among other bio-oil fractions while the top organic layer of BF1 was found to be moisture-free. Fig. 5b, on the other hand, shows that most of the water from ALP was present in the distillates that were condensed near the boiling point of water (AF2 and AF3). Lower moisture contents were detected for AF1 (49% wt) and

AF4 (45% wt), which were condensed at $>90^{\circ}\text{C}$ and $110\text{--}150^{\circ}\text{C}$, respectively. AF5, on the other hand, has only about 7% wt moisture, which indicates that most of the organic water-soluble compounds in ALP were condensed in this fraction. Hence, AF5 was further characterized and compared with bio-oil fractions in the subsequent sections.

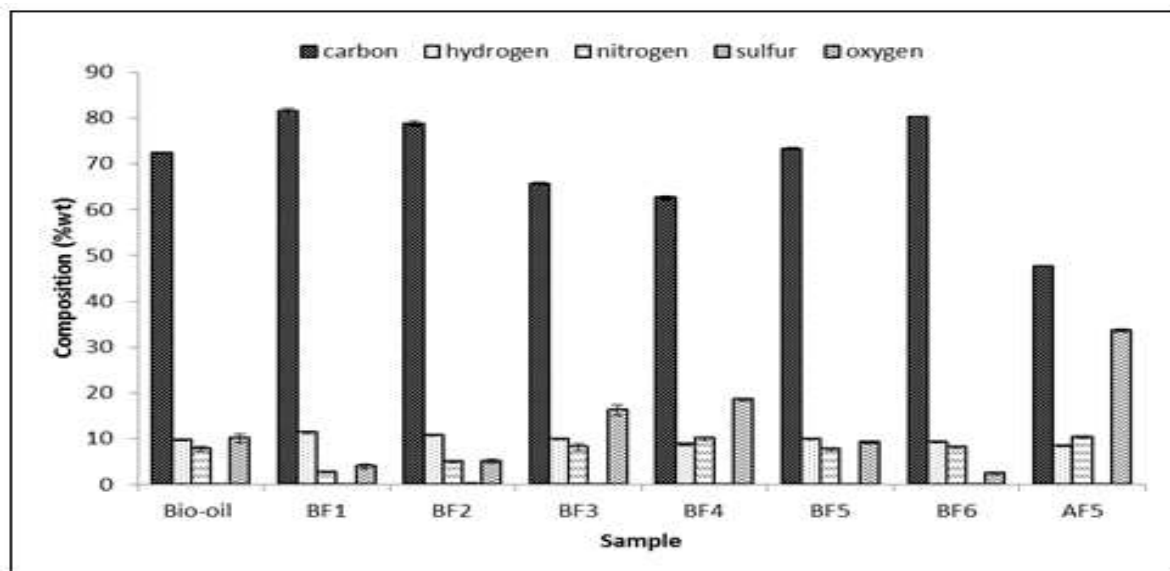


Fig. 7. Comparison of the elemental composition of algal bio-oil, bio-oil distillates and AF5.

Heating value

Fig. 6 compares the heating values of the original bio-oil, bio-oil distillate fractions (BF1 and BF6) and AF5. The original bio-oil has a heating value of about 36 MJ/kg, which is still lower than that of heavy fuel oil (40 MJ/kg), FAME (40.5 MJ/kg), diesel (45.7 MJ/kg) and gasoline (46.4 MJ/kg) (API, 2001). Based on the figure, the heating value of the distillate fractions increased significantly as compared to the original bio-oil. BF1 has a heating value of about 42 MJ/kg, which is higher than heavy fuel oil and FAME. Whereas, the heating value of BF2 (40 MJ/kg) is almost equal to that of heavy fuel oil and FAME. The four (4) remaining bio-oil fractions (BF3, BF4, BF5 and BF6) have heating values that are still slightly lower than petroleum-derived fuels but slightly higher than the original bio-oil. AF5, which is an ALP distillate, has a heating value of about 24 MJ/kg. Although lower in value as compared to the bio-oil fractions, the heating value of AF5 is higher than that of a typical wood-derived bio-oil (16–19 MJ/kg) (Czernik and Bridgwater, 2004) and methanol (22.8 MJ/kg) (API, 2001).

Elemental composition

The elemental composition in terms of C, H, O, N and S % wt of the bio-oil distillate fractions and AF5 were analyzed to further assess their suitability as alternative energy sources. The results were also compared with the elemental composition of the original bio-oil as shown in Fig. 7.

Fig. 7 shows that BF1 has the highest carbon (82% wt) and hydrogen (11% wt) contents compared to the original bio-oil and other distillate fractions followed by BF6 and BF2. This result agrees well with the heating values of the distillate fractions as discussed previously. Higher amounts of combustible carbon and hydrogen components in the distillate fractions increase their energy content. Hence, the increase in the heating values of BF1, BF2 and BF6 compared to the original bio-oil may be attributed to the enrichment of carbon and hydrogen in these distillates. Aside from that, reduction in oxygen content was also observed in BF1 (4% wt), BF2 (5% wt) and BF6 (2% wt).

Low oxygen content is desirable since oxygen causes several disadvantages including low energy density, immiscibility with hydrocarbons, and instability (Czernik and Bridgwater, 2004). BF₅, on the other

hand, has almost similar elemental composition as the original bio-oil as can be seen in Fig. 7. However, the heating value of BF₅ (38 MJ/kg) is higher than the bio-oil (36 MJ/kg) as discussed previously.

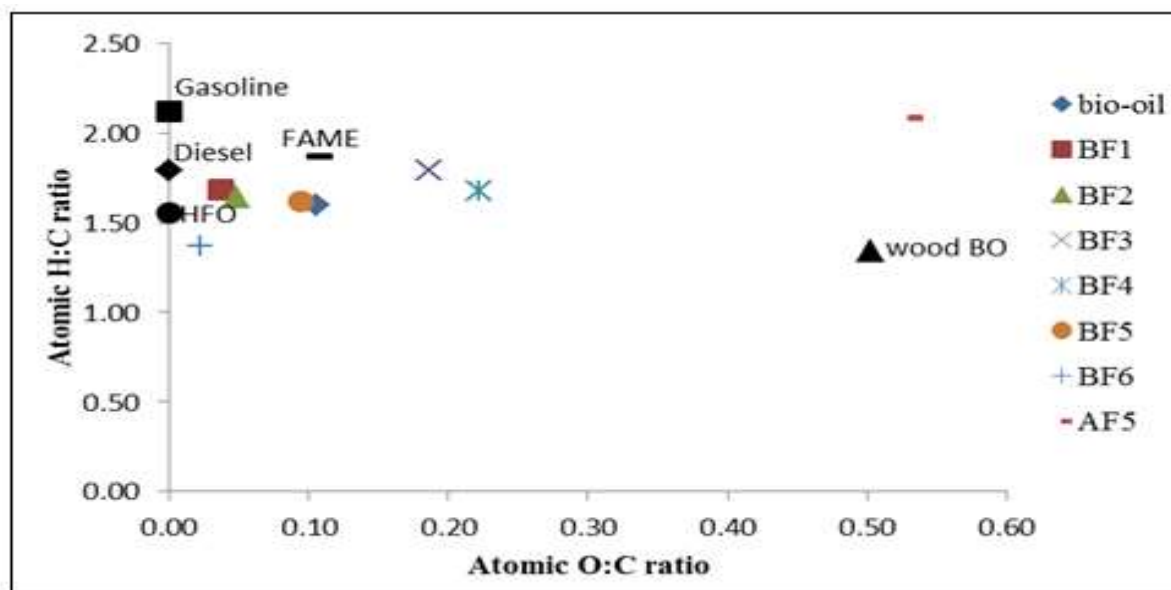


Fig. 8. Van Krevelen diagram.

This may be attributed to the higher moisture content of the bio-oil as compared to BF₅ (see Fig. 5a). In general, the heating value of a substance is negatively correlated to moisture content (Zhang *et al.*, 2007). The middle fractions (BF₃ and BF₄) have lower % carbon and hydrogen and higher % oxygen compared to the bio-oil. However, these distillate fractions (BF₃ and BF₄) have higher heating values than the bio-oil, which may also be due to their lower moisture contents. AF₅, on the other hand, contains about 48% carbon, 8% hydrogen, 10% nitrogen, 0.05% sulfur and 34% oxygen, which is very much different from the bio-oil distillates. Its carbon content is lower than lingo cellulosic bio-oils (54-58% wt). However, AF₅ has higher hydrogen and lower oxygen contents than wood-derived bio-oils (5.5-7% hydrogen, 35-40% oxygen).

Based on the properties discussed, bio-oil distillates and AF₅ could be potentially used as fuel. Further investigation on their application as fuel was done by comparing them to traditional petroleum-derived fuels and wood derived-bio-oils using the van Krevelen diagram (Fig. 8).

According to Mc Kendry (2002), biofuels can be compared to fossil fuels using the plot of atomic H:C and O:C ratios, known as the van Krevelen diagram. Based on Fig. 8, the original bio-oil, which is comparable to BF₅, has similar O:C ratio but lower H:C ratio compared to FAME. BF₁ and BF₂ are approaching the diesel region while BF₆ is quite near the heavy fuel oil (HFO) region. BF₃ and BF₄ plots, on the other hand, were positioned farther from the liquid fossil fuels region than the bio-oil due to their higher O:C ratios, which also indicates the enrichment of oxygenated compounds in these fractions. Fig. 8 also illustrates the large difference between AF₅ and the bio-oil distillates. Based on the van Krevelen diagram, AF₅ is near the wood-derived bio-oil region while the bio-oil distillates are near the petroleum-derived fuels.

Functional groups

Functional group compositional analysis of the bio-oil and ALP distillates was done using FTIR spectrometry. This nondestructive technique was also used to determine the groups of compounds present in pyrolytic oils (Apaydin-Varol *et al.*, 2007; Du *et al.*,

2011; Ertaş and Hakkı-Alma, 2010; Jiang *et al.*, 2011; Singh and Shadangi, 2011; Zhou *et al.*, 2009). Fig. 9 shows the FTIR spectra for the bio-oil and ALP distillate fractions while Table 2 summarizes the functional groups and their corresponding band position (in cm^{-1}) in the FTIR spectra. Based on Fig. 9a, AF1 and AF4 follow a similar pattern while AF2 and AF3 are also the same. This result coincides with the moisture content analysis in the previous section. Also, ALP distillates except AF5 have distinct peaks in the $3600\text{--}3200\text{ cm}^{-1}$ frequency range, which indicates the presence of water. Based on Fig. 9a and Table 2, other organic compounds such as alkanes, ketones, aldehydes, carboxylic acids,

esters and aromatic compounds may still be present in AF2 and AF3 although the major component is water. For AF1 and AF4, however, alkanes, ketones, aldehydes, alcohols, carboxylic acids, esters, phenols and aromatic compounds may be present aside from water based on the band position of the peaks detected. AF5, on the other hand, mainly consists of organic compounds which may include carboxylic acids, esters, ketones, aldehydes ($1750\text{--}1650\text{ cm}^{-1}$), alkenes or amides ($1660\text{--}1630\text{ cm}^{-1}$), alkanes ($1470\text{--}1350\text{ cm}^{-1}$), alcohols and phenols ($1300\text{--}950\text{ cm}^{-1}$), and aromatic compounds ($975\text{--}525\text{ cm}^{-1}$) with minimal amount of water consistent with the results presented in the previous section.

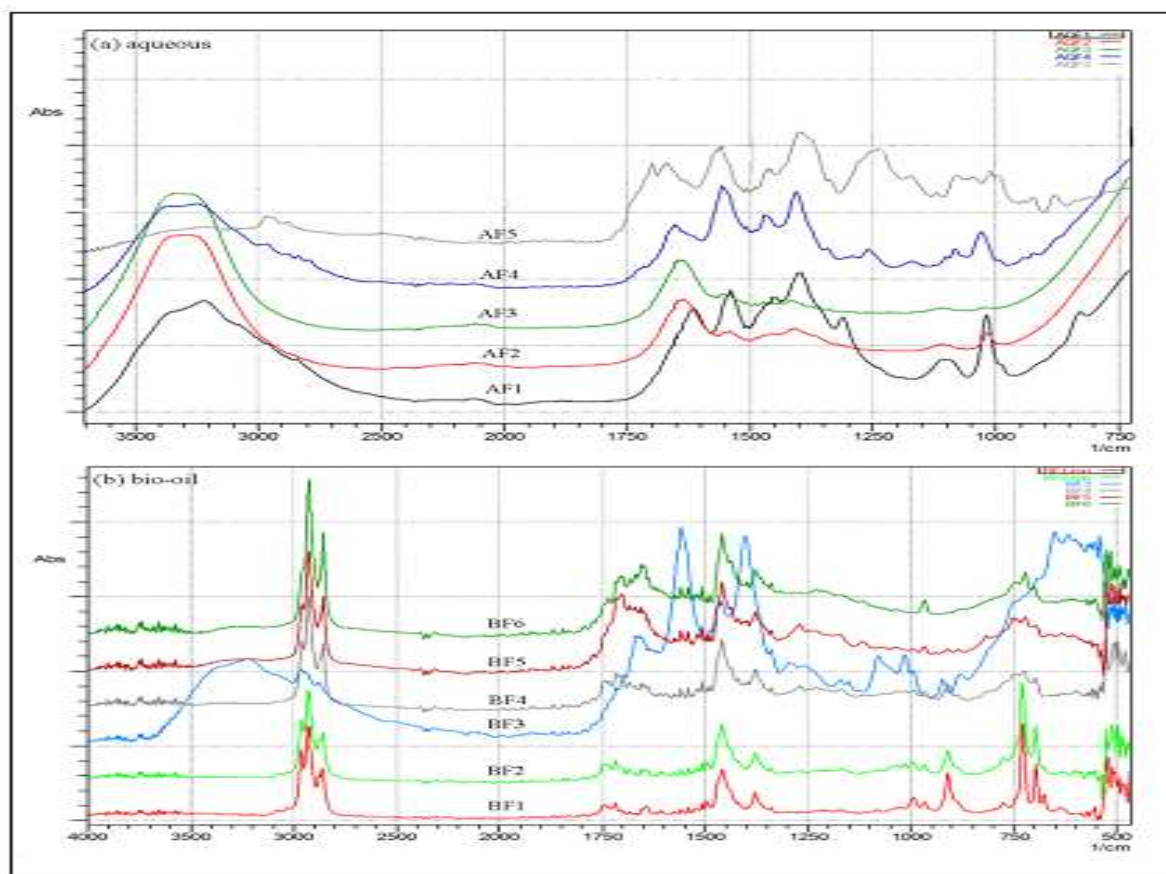


Fig. 9. FTIR spectra of aqueous and bio-oil distillate fractions.

Bio-oil distillate fractions, on the other hand, have almost similar functional groups (except BF3) based on the band positions of the peaks detected as shown in Fig. 9b. BF3 may contain more oxygenated compounds such as alcohols and phenols based on the absorbance peaks. The peak detected at $3200\text{--}2800\text{ cm}^{-1}$ and $1475\text{--}1350\text{ cm}^{-1}$ for C-H stretching and

C-H deformation, respectively, indicate the presence of alkanes. C=C stretching vibrations at $1660\text{--}1630\text{ cm}^{-1}$ suggests the presence of alkenes or amides. Esters may also be present based on the peaks observed at $1310\text{--}1250\text{ cm}^{-1}$. Lastly, the peak absorbances at $975\text{--}525\text{ cm}^{-1}$ could indicate the presence of aromatic compounds in the bio-oil distillates.

Chemical composition

The chemical compositions of the bio-oil distillates (BF1 to BF6) and AF5 were identified using gas chromatography coupled with mass spectrometry (GC-MS).

The quantity of each component was expressed in terms of relative percentage area of the chromatographic peaks. This kind of method was also used by Wang *et al.* (2009) to directly assess the separation level of bio-oil components by molecular distillation.

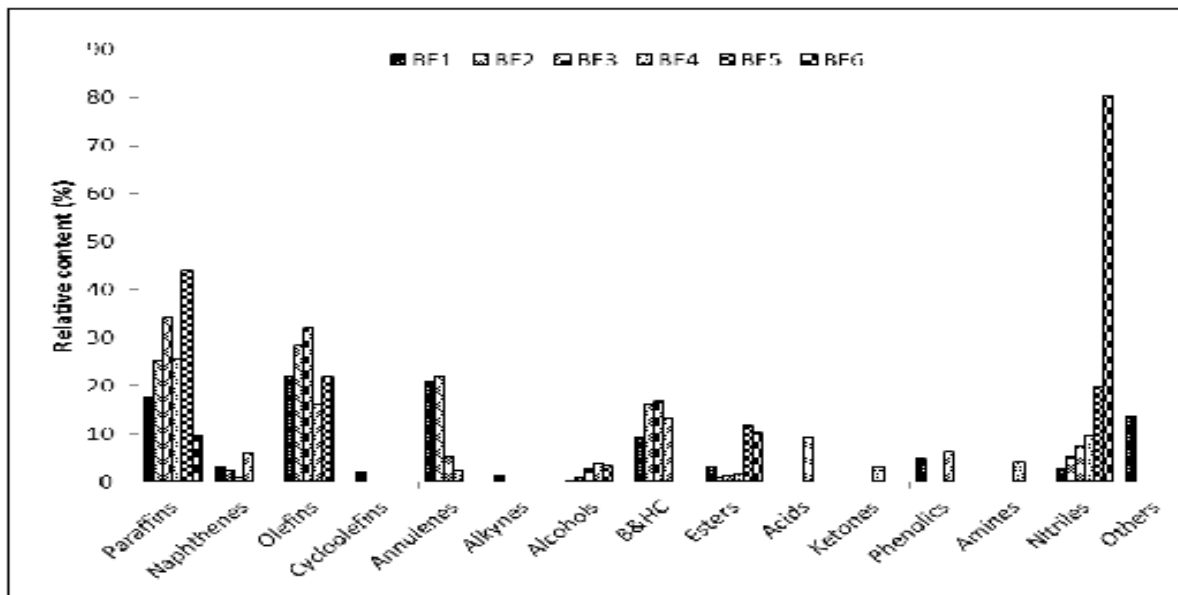


Fig. 10. The chemical distribution of algal bio-oil distillates.

Table 3 summarizes the compounds detected from the ALP distillate, AF5. Based on the table, AF5 consists mostly of oxygenated compounds such as carboxylic acids (21.06%) and carboxylate esters (53.85%), N-containing amides (9.89%), lactams (11.59%) and some acetates (3.61%). Typically, smaller carboxylic acids with 1 to 5 carbon atoms are water-soluble but have higher boiling points than water; hence, can be separated from water by distillation. The carboxylate esters and nitrogen containing compounds present in AF5, on the other hand, could be derived from the lipid and protein contents of microalgae, respectively. According to Schlten and Leinweber (1993) as cited by Cao *et al.* (2010), acetamide usually comes from pyrolysis of microbial cells walls and chitin, and from other precursors such as proteins and amino sugars. On the other hand, the decarboxylation and cyclization of γ - and δ -amino acids during pyrolysis typically produces five- and six-membered cyclic lactams, respectively (Cao *et al.*, 2010).

Further processing to remove or reduce the nitrogen-containing species may be necessary for AF5 to be a suitable fuel replacement since these nitrogen species can be converted to NO_x and N₂O during combustion causing adverse environmental effects (i.e. acid rain, photochemical smog).

The chemical compositions of bio-oil distillates, on the other hand, are shown in Fig. 10. The identified compounds were grouped into the following categories: paraffins, naphthenes, olefins, cycloolefins, alkynes, alcohols, benzene and heterocyclic compounds (B&HC), esters, acids, ketones, phenolics, amines, nitriles and others. The groups of compounds identified for algal bio-oil distillates were very much different from that of bio-oil derived from lignocellulosic material such as sawdust, which mostly contains oxygenated compounds (i.e. phenols, acids) (Du *et al.*, 2011; Guo *et al.*, 2010a). Based on Fig. 10, all bio-oil distillate fractions contain considerable amounts of saturated paraffins and unsaturated olefins.

BF4 has the highest amount of paraffins (43.92%) while BF3 contains the highest amount of olefins (31.96%). Most of the annulenes in the form of cycloheptatriene and cyclooctatetraene are present in BF1 (20.86%) and BF2 (21.96%). BF1, BF2, BF3 and BF4 contain benzene and heterocyclic compounds while BF5 and BF6 contain most of the esters. Most of the oxygen-containing compounds such as carboxylic acids, ketones and phenols are present in BF4, which is consistent with the elemental analysis presented in Fig. 7. Nitriles concentration was enriched in BF6, which contains about 80.40% nitriles. The other compound detected in BF1 was levoglucosan, which is typically used as a chemical tracer in thermal treatment of biomass at atmospheric pressure. Wang *et al.* (2009) also detected levoglucosan in the heavy fraction produced from molecular distillation of sawdust bio-oil.

The degree of separation of bio-oil components was assessed in terms of a separation factor, β . The separation factor was first defined by Guo *et al.* (2010b) to define the separation characteristics of typical sawdust bio-oil compounds. In his study, β was calculated as follows:

$$\beta_{m-n} = M_{m-n,DF} / (M_{m-n,DF} + M_{m-n,RF}) \quad (1)$$

where m represents the two distillation processes, n represents the compound in the bio-oil, DF is the distillate fraction, RF is the residual fraction and $M_{m-n,DF}$ is the weight of the compound in DF calculated using the formula:

$$M_{m-n,DF} = Y_{m,DF} \times R_n \quad (2)$$

Where $Y_{m,DF}$ is the yield of DF and R_n is the proportion of the compound n corresponding to the relative content (%). There were only two fractions obtained by Guo *et al.* (2010b) from molecular distillation of sawdust bio-oil. Hence, in this study, Equation 1 was modified to include all six (6) fractions obtained from the algal bio-oil. The modified equation for separation factor, β , of algal bio-oil distillate fractions is shown in Equation 3.

$$\beta = M_{DF} / \sum M_{BFi} \quad (3)$$

where M_{DF} is the amount of the component in the boiling point range to which it should be collected, and M_{BFi} is the amount of the component in each distillate fraction where i ranges from 1 to 6.

Based on Guo *et al.* (2010b), a separation factor of 1 ($\beta=1$) means that the compound has been completely separated in the boiling range where it should be. Additionally, since there are six (6) bio-oil distillates in this study, $\beta > 0.16$ means that the compound is enriched in the distillate fraction where it should belong. $\beta=0$, on the other hand, indicate that the compound did not condense in the distillate fraction where it should be. Based on the calculated separation factor (β), paraffins such as heptane, decane, undecane and dodecane, most of the higher molecular weight olefins (i.e. tetradecene, hexadecane) and naphthenes were completely separated ($\beta = 1$) while phenolic compounds were not condensed in the boiling point range where it should belong ($\beta = 0$). According to Guo *et al.* (2010b), phenols and aldehydes are hard to distill. Moreover, most alcohols, nitriles and annulenes were enriched ($\beta > 0.16$) in the distillate fraction where each compound should be.

Although it appears that acids, amides and ketones were completely separated based on Fig.10, the distillate fraction where they were condensed does not correspond to the boiling point range where they should be grouped; hence, their separation factors were either low or zero. This may be attributed to the complexity of the bio-oil. Also, other factors such as heating rate may have a potential effect on the separation of components (Guo *et al.*, 2011).

Acknowledgements

The authors would like to acknowledge funding of this work by the US Department of Energy administered by the National Alliance for Advanced Biofuels and Bio-Products (NAABB).

References

American Petroleum Institute (API). 2001. Alcohols and ethers - A technical assessment of their application as fuels and fuel components. In: P. N. 4261, 3rd ed. API, Washington, DC.

- Agblevor FA, Mante O, McClung R, Oyama ST.** 2012. Co-processing of standard gas oil and biocrude oil to hydrocarbon fuels. *Biomass & Bioenergy* **45**, 130-137.
<http://dx.doi.org/10.1016/j.biombioe.2012.05.024>
- Amen-Chen C, Pakdel H, Roy C.** 1997. Separation of phenols from eucalyptus wood tar. *Biomass & Bioenergy* **13**, 25-37.
[http://dx.doi.org/10.1016/S0961-9534\(97\)00021-4](http://dx.doi.org/10.1016/S0961-9534(97)00021-4)
- Apaydin-Varol E, Pütün E, Pütün AE.** 2007. Slow pyrolysis of pistachio shell. *Fuel* **86(12-13)**, 1892-1899.
<http://dx.doi.org/10.1016/j.fuel.2006.11.041>
- Boucher ME, Chaala A, Roy C.** 2000. Bio-oils obtained by vacuum pyrolysis of softwood bark as a liquid fuel for gas turbines. Part I: Properties of bio-oil and its blends with methanol and a pyrolytic aqueous phase. *Biomass & Bioenergy* **19**, 337-350.
[http://dx.doi.org/10.1016/S0961-9534\(00\)00043-X](http://dx.doi.org/10.1016/S0961-9534(00)00043-X)
- Bridgwater T.** 2006. Review: Biomass for energy. *Journal of Science and Food Agriculture* **86**, 1755-1768.
<http://dx.doi.org/10.1002/jsfa.2605>
- Cao JP, Zhao XY, Morishita K, Wei XY, Takarada T.** 2010. Fractionation and identification of organic nitrogen species from bio-oil produced by *Technology* **101**, 7648-7652.
<http://dx.doi.org/10.1016/j.biortech.2010.04.073>
- Cortright RD, Davda RR, Dumesic JA.** 2002. Hydrogen from catalytic reforming of biomass-derived hydrocarbons in liquid water. *Nature* **418**, 964-967.
<http://dx.doi.org/10.1038/nature01009>
- Czernik S, Bridgwater AV.** 2004. Overview of Applications of Biomass Fast Pyrolysis Oil. *Energy Fuels* **18**, 590-598.
<http://dx.doi.org/10.1021/ef034067u>
- Du Z, Li Y, Wang X, Wan Y, Chen Q, Wang C, Ruan R.** 2011. Microwave-assisted pyrolysis of microalgae for biofuel production. *Bioresource Technology* **102(7)**, 4890-4896.
<http://dx.doi.org/10.1016/j.biortech.2011.01.055>
- Ertas M, Hakkı AM.** 2010. Pyrolysis of laurel (*Laurus nobilis* L.) extraction residues in a fixed-bed reactor: Characterization of bio-oil and bio-char. *Journal of Analytical and Applied Pyrolysis* **88(1)**, 22-29.
<http://dx.doi.org/10.1016/j.jaap.2010.02.006>
- Grierson S, Strezov V, Ellem G, Mcgregor R, Herbertson J.** 2009. Thermal characterisation of microalgae under slow pyrolysis conditions. *Journal of Analytical and Applied Pyrolysis* **85**, 118-123.
- Guo X, Wang S, Guo Z, Liu Q, Luo Z, Cen K.** 2010a. Pyrolysis characteristics of bio-oil fractions separated by molecular distillation. *Applied Energy* **87**, 2892-2898.
<http://dx.doi.org/10.1016/j.apenergy.2009.10.004>
- Guo X, Wang S, Wang Q, Guo Z, Luo Z.** 2011. Properties of bio-oil from fast pyrolysis of rice husk. *Biotechnology & Bioengineering* **19**, 116-121.
- Guo Z, Wang S, Gu Y, Xu G, Xin L, Luo Z.** 2010b. Separation characteristics of biomass pyrolysis oil in molecular distillation. *Separation and Purification Technology* **76**, 52-57.
<http://dx.doi.org/10.1016/j.seppur.2010.09.019>
- Huber GW, Cortright RD, Dumesic JA.** 2004. Renewable alkanes by aqueous-phase reforming of biomass-derived oxygenates. *Angewandte Chemie International Edition* **116**, 1575-1577.
<http://dx.doi.org/10.1002/anie.200353050>
- Jiang X, Naoko E, Zhong ZP.** 2011. Structure properties of pyrolytic lignin extracted from aged bio-oil. *Chinese Science Bulletin* **5 (14)**, 1417-1421.
<http://dx.doi.org/10.1007/s11434-011-4465-4>

- Lu R, Sheng G-P, Hu Y-Y, Zheng P, Jiang H, Tang Y, Yu H-Q.** 2011. Fractional characterization of a bio-oil derived from rice husk. *Biomass & Bioenergy* **35**, 671-678.
<http://dx.doi.org/10.1016/j.biombioe.2010.10.017>
- Maguyon, MCC, Capareda, SC.** 2013. Evaluating the effects of temperature on pressurized pyrolysis of *Nannochloropsis oculata* based on products yields and characteristics. *Energy Conversion and Management* **76**, 764-773.
<http://dx.doi.org/10.1016/j.enconman.2013.08.033>
- Marcilla A, Gomez-Siurana A, Gomis C, Chapuli E, Catala MC, Valdes FJ.** 2009. Characterization of microalgal species through TGA/FTIR analysis: Application to *nannochloropsis* sp. . *Thermochimica Acta* **484**, 41-47.
<http://dx.doi.org/10.1016/j.tca.2008.12.005>
- McKendry P.** 2002. Energy production from biomass (part 1): overview of biomass. *Bioresource Technology* **83** (1), 37-46.
[http://dx.doi.org/10.1016/S0960-8524\(01\)00118-3](http://dx.doi.org/10.1016/S0960-8524(01)00118-3)
- Miao X, Wu Q, Yang C.** 2004. Fast pyrolysis of microalgae to produce renewable fuels. *Journal of Analytical and Applied Pyrolysis* **71**, 855-863.
- Peng W, Wu Q, Tu P, Zhao N.** 2001. Pyrolytic characteristics of microalgae as renewable energy source determined by thermogravimetric analysis. *Bioresource Technology* **80**, 1-7.
- Schlten HR, Leinweber P.** 1993. Pyrolysis-field ionization mass spectrometry of agricultural soils and humic substances: effect of cropping systems and influence of the mineral matrix. *Plant soil* **151**, 77-90.
<http://dx.doi.org/10.1051/ebr:2006012>
- Singh RK, Shadangi KP.** 2011. Liquid fuel from castor seeds by pyrolysis. *Fuel* **90**(7), 2538-2544.
<http://dx.doi.org/10.1016/j.fuel.2011.03.015>
- Teella A, Huber GW, Ford DM.** 2011. Separation of acetic acid from the aqueous fraction of fast pyrolysis bio-oils using nanofiltration and reverse osmosis membranes. *Journal of Membrane Science* **378**, 495-502.
<http://dx.doi.org/10.1016/j.memsci.2011.05.036>
- US Energy Information Administration.** 2016. International energy outlook 2016 with projections to 2040. U.S. Department of Energy, Washington, DC, USA.
[http://www.eia.gov/outlooks/ieo/pdf/0484\(2016\).pdf](http://www.eia.gov/outlooks/ieo/pdf/0484(2016).pdf)
- Wang S.** 2013. Chapter 16: High-Efficiency Separation of Bio-Oil. *Biomass Now - Sustainable Growth and Use*. Rijeka: In Tech.
<http://dx.doi.org/10.5772/51423>
- Wang S, Gu Y, Liu Q, Yao Y, Guo Z, Luo Z, Cen K.** 2009. Separation of bio-oil by molecular distillation. *Fuel Processing Technology* **90**, 738-745.
<http://dx.doi.org/10.1016/j.fuproc.2009.02.005>
- Wang Z, Lin W, Song WL, Du L, Li ZJ, Yao JZ.** 2011. Component fractionation of wood-tar by column chromatography with the packing material of silica gel. *Chinese Science Bulletin* **56**(14), 1434-1441.
- Xu BJ, Lu N.** 1999. Experimental research on the bio oil derived from biomass pyrolysis liquefaction. *Transactions of Chinese Society of Agricultural Engineers* **15**, 177-181.
- Zeng F, Liu W, Jiang H, Yu H-Q, Zeng RJ, Guo Q.** 2011. Separation of phthalate esters from bio-oil derived from rice husk by a basification-acidification process and column chromatography. *Bioresource Technology* **102**, 1982-1987.
<http://dx.doi.org/10.1016/j.biortech.2010.09.024>
- Zhang Q, Chang J, Wang TJ, Xu Y.** 2007. Review of biomass pyrolysis oil properties and upgrading research. *Energy Conversion and Management* **48**, 87-92.
<http://dx.doi.org/10.1016/j.enconman.2006.05.010>
- Zhou L, Zong ZM, Tang SR, Zong Y, Xie RL, Ding MJ, Wei XY.** 2009. FTIR and Mass Spectral Analyses of an Upgraded Bio-oil. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* **32**(4), 370-375.
<http://dx.doi.org/10.1080/15567030802467340>