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Influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties

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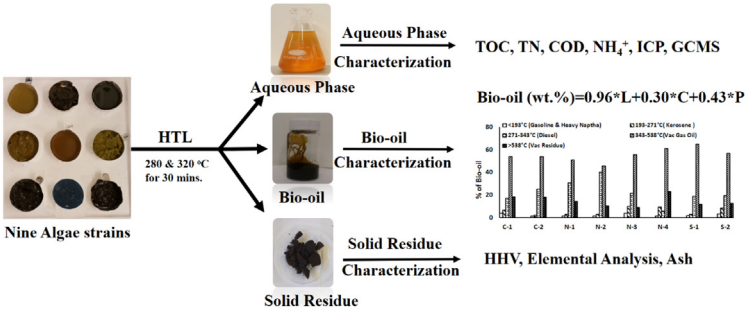
HIGHLIGHTS

- Hydrothermal liquefaction of nine algae species was performed.
- The regression model for bio-oil yield obtained was developed.
- The highest amounts of bio-oil yield was obtained from high lipid containing algae.
- Bio-oil yield showed the trend of lipid > protein > carbohydrate.
- Almost 50% of bio-oil was in vacuum gas oil range in most cases.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrothermal liquefaction (HTL) of nine algae species were performed at two reaction temperatures (280 and 320 °C) to compare the effect of their biomass composition on product yields and properties. Results obtained after HTL indicate large variations in terms of bio-oil yields and its properties. The maximum bio-oil yield (66 wt%) was obtained at 320 °C with a high lipid containing algae *Nannochloropsis*. The higher heating value of bio-oils ranged from 31 to 36 MJ/kg and around 50% of the bio-oils was in the vacuum gas oil range while high lipid containing algae *Nannochloropsis* contained a significant portion (33–42%) in the diesel range. A predictive relationship between bio-oil yields and biochemical compositions was developed and showed a broad agreement between predictive and experimental yields. The aqueous phases obtained had high amount of TOC (12–43 g/L), COD (35–160 g/L), TN (1–18 g/L), ammonium (0.34–12 g/L) and phosphate (0.7–12 g/L).

1. Introduction

Biomass is considered to be the only renewable source of energy that can be converted into liquid hydrocarbons and is abundant worldwide (Escobar et al., 2009). Among many sources of biomass, algae is a promising biomass feedstock because of its higher yield and faster growth rate (Rodolfi et al., 2009; Scott et al., 2010). Algae can be converted into biofuels by various processes such as pyrolysis (Nam et al., 2016; Thangalazhy-Gopakumar et al., 2012), gasification (Onwudili et al., 2013) and conventional extraction process (Scott et al., 2010). However, the major complication of those processes is that they require dried biomass. Algae, a wet feedstock, needs a huge amount of energy for drying, which

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makes the process uneconomical. An alternative process that is best suited for producing biofuels from wet biomass or algae is hydrothermal liquefaction (HTL). HTL uses water at a sub- and super-critical temperature (250–380 °C) and pressure (7–30 MPa) as reactant and reaction medium. This process not only eliminates energy-intensive drying process but also allows the utilization of whole algae for bio-oil production (Peterson et al., 2008).

Till date, numerous studies on HTL of algae have been conducted due to the synergistic relationship between algae and liquefaction process. Extensive studies were conducted to investigate the effect of different process variables such as temperature (Brown et al., 2010; Garcia Alba et al., 2012; Shakya et al., 2015; Jena et al., 2011a; Valdez et al., 2012), residence time (Anastasakis and Ross, 2011; Valdez et al., 2012; Jena et al., 2011a) and heating rate (Bach et al., 2014) on the bio-oil yield and its quality. Apart from these process variables, the biochemical composition of algae (which constitutes lipids, proteins, and carbohydrates) is an important factor found to influence the product yield and quality. Despite extensive studies on HTL of algae, only a handful of studies (Barreiro et al., 2013; Biller and Ross, 2011; Teri et al., 2014) on the effects of biochemical compositions of algae on the overall yields and product composition exist. Biller and Ross (2011) studied the effect of the biochemical composition in HTL using different model chemical compounds and microalgae species and reported that the conversion of lipids and proteins to bio-oil was more efficient compared to the conversion of carbohydrates. This finding was further confirmed by the work of Teri et al. (2014), who studied HTL of model chemical compounds of protein, carbohydrate, and lipid alone and in mixture. The authors reported that the conversion of model lipid showed the highest yield (>90 wt%) followed by protein and carbohydrate. They also performed binary and ternary mixtures of model compounds, and found that the bio-oil produced from the mixtures of polysaccharides and proteins exceeded the averaged bio-oil yield with an individual component. Barreiro et al. (2013) performed HTL of different microalgae species to examine the influence of strain-specific parameters like cell structure, biochemical composition and growth environment on product yields and properties. Less variation in the bio-crude yields (45.6–58.1 wt%) within species was obtained at a higher temperature of 375 °C, but significant differences in yields were observed at a lower temperature of 250 °C (17.6–44.8 wt%). The difference in yields was due to the cell wall structure of algae and severity of the reaction conditions. In an alternative approach, Sheehan and Savage (2017) incorporated algae HTL studies in their kinetics-based reaction model to study the effect of biochemical composition on product yields and found the activation energy required for biocrude formation was the lowest for proteins. To the best of our knowledge, only a limited number of studies were performed to evaluate the effect of algal biochemical compositions on bio-oil yields and its characteristics during HTL process. However, those studies were either focused on model compounds or limited number of algae species with narrow variation of biochemical composition. Hence, additional data on HTL using different type of algal species with varying biochemical composition will provide how the biochemical composition will affect HTL products yield and properties, which is very important in commercializing algae based biorefinery.

Hence, the main objective of this study was to perform HTL of nine types of algae species to investigate the influence of biochemical compositions on bio-oil yields and fuel characteristics. In addition, predictive models for predicting the bio-oil yields from HTL of algae at two temperatures of 280 and 320 °C were also obtained. Last, a comprehensive investigation of the aqueous phase obtained from HTL of different algae species was conducted for further aqueous phase utilization studies.

2. Materials and methodology

2.1. Hydrothermal liquefaction of algae

Nine algae species were used for HTL experiments. Algae species were obtained from Arizona Center for Algae Technology and Innovation (AzCATI), Mesa, Arizona and Reed Mariculture, Inc. (California). HTL of algae species were performed in a high-pressure experimental unit as described in the previous paper (Shakya et al., 2015). The reactor was loaded with approximately 10 g of algae (dry weight basis) at a solid loading of 15 wt%, and purged with helium gas (>99% purity, Airgas Inc., Charlotte, NC) to remove residual air and create an inert headspace. After purging, the reactor was pressurized at 100 psig with helium gas at room temperature. The reactor was then heated to a desired temperature (280 °C and 320 °C) at heating rate of 30 °C min^{-1}. After holding the reactor at the reaction temperatures for 30 min, it was cooled down to room temperature using cold water. Then the residual pressure created by gas formation was vented off and was not analyzed in this study. Product separation procedure and yield equations were adopted from the previous paper (Shakya et al., 2015). In this study, bio-oil, solid residue and gaseous yields were gravimetrically measured, and the remaining balance was considered as WSP (water soluble phase).

2.2. Biomass and product analysis

Algae biomass were analyzed for their moisture content, ash and elemental composition using the methods described in the previous paper (Shakya et al., 2015) whereas biochemical composition was provided by the suppliers. Fatty Acid Methyl Esters [FAME] analysis was performed according to the National Renewable Energy Laboratory's laboratory analytical procedure (Van Wychen and Laurens, 2013).

Bio-oils were characterized for water content, ash content, higher heating value (HHV), total acid number (TAN), elemental analysis and chemical composition according to the methods described by Shakya et al. (2015). Fourier Transform Infrared (FTIR) spectroscopic analysis of bio-oils was performed by using Thermo Nicolet iS10 (Thermo Scientific, Waltham, MA). The samples were analyzed for 34 scans over a range of 400–4000 cm^{-1} wavenumbers. Simulated distillation was performed as according to the method described by Wang et al. (2016). Energy recovery for the bio-oil obtained from different algae species was calculated using Eq. (1).

$$Energy\ recovery = \frac{HHV_{BIO\text{-}OIL} \times Mass_{BIO\text{-}OIL}}{HHV_{ALGAE\ BIOMASS} \times Mass_{ALGAE\ BIOMASS}} \times 100\% \quad (1)$$

The aqueous phase produced from HTL of different algae species was analyzed for its pH, total organic carbon (TOC), chemical oxygen demand (COD), total nitrogen (TN), phosphate (PO_4^{3-}), ammonium (NH_4^+), chemical composition and metal content. TOC and TN were determined using a TOC-L analyzer attached with TNM-L unit (Shimadzu Corp., Japan). For the measurement, aqueous phase samples were filtered using 0.2 μm filter paper to remove any suspended particles. The filtrate samples of 50 dilution factor were prepared using ultra high purity water and kept in auto-sampler for measurement. The COD of the aqueous phase was measured according to the standard method (Eaton et al., 1998). Ammonium, phosphate, and magnesium were determined using YSI reagent kits according to the supplier's protocol. The aqueous phase was also characterized for its chemical composition using GC–MS. 10 mL of dichloromethane was mixed with 5 mL of the aqueous phase and the dichloromethane extracted/soluble part was used for GC–MS analysis using the same DB 17901 capillary column

and heating program as described by Shakya et al. (2015). The trace metal concentration in the aqueous phase was measured using an Optima 5300 DV inductively coupled plasma spectrometer (ICP) with optical emission spectrometry (Perkin Elmer, Cambridge, UK). Approximately 0.05 g of the aqueous phase was digested by 25 mL of nitric acid overnight and then adjusted to 100 mL with ultrahigh purity (Type 1) water (Synergy Ultrapure Water Systems, EMD Millipore) and then filtered using 0.45 μm filter before the analysis.

The char from the HTL of algae was measured for its HHV, ash content and elemental composition by using the methods as described by Shakya et al. (2015). The char was also measured for BET (Brunauer-Emmett-Teller) surface area using a Quantachrome Autosorb- iQ with nitrogen absorption. Before measurement, all the samples were outgassed to 10^{-3} Torr at 300 °C for 10 h.

2.3. Predictive modeling

Multiple linear regression of biomass composition parameters (lipids, carbohydrates, and proteins) against the corresponding bio-oil was performed using SAS to obtain a linear model for predicting bio-oil yield from HTL of algae. The confidence level of the regression was 95%, and the intercept was set at zero. Model validation was also performed comparing prediction with experimental results reported in other microalgae HTL literature.

3. Results and discussion

3.1. Algal characterization

Algae selected for this study had diverse biochemical composition; lipids ranging from 13 to 55 wt%, carbohydrates ranging from 9 to 54 wt% and proteins ranging from 7 to 63 wt% as shown in Table 1. In the case of lipids, FAMEs analysis of the algae showed fatty acids (FAs) to range from C6 to C20. Based on the area percentage, palmitic acid (C16:0), palmitoleic acid (C16:1), linoleic acid (C18:2) and linolenic acid (C18:3) were the predominant FAs in all algae strains.

Table 2 illustrates proximate and elemental analyses of different algae strains. Ultimate analysis showed that the algae having higher lipids (N-1 and N-2) had higher C and H% resulting in higher heating values. Algae having higher protein (N-4) had maximum N % as expected. The sulfur content was not measured in this study since previous studies (Brown et al., 2010; Shakya et al., 2015) have reported their values to be less than 1%. Relatively low oxygen contents were observed with *Nannochloropsis* species (except N-3) compared to others.

Table 1
Biochemical composition of different algae strains.

Sample[¥] (Nomenclature)	Biochemical composition (wt%)[a]		
	Lipids	Carbohydrates	Proteins
Chlorella (C-1)	15.78	16.10	46.80
Chlorella (C-2)	30.28	49.70	14.63
Nannochloropsis (N-1)	55.36	12.39	12.92
Nannochloropsis (N-2)	49.26	15.94	18.15
Nannochloropsis (N-3)	20.09	9.21	46.62
Nannochloropsis (N-4)	18.12	8.92	62.78
Pavlova (P-1)	13.88	28.00	46.94
Scenedesmus (S-1)	35.66	50.40	7.15
Scenedesmus (S-2)	17.83	54.17	30.06

¥ All the algae, except for N-4 and P-1 were provided by AzCATI whereas; N-4 and P-1 were purchased from Reed Mariculture Inc.

[a] Values reported by AzCATI and Reed Mariculture on a dry basis. AzCATI used ATP3 protocol for determination of the biochemical composition, and experiments were performed in duplicates (n = 2) whereas Reed Mariculture obtained their values from Medallion laboratory (MN, USA).

3.2. Liquefaction yields

The product yields (on dry weight basis) from HTL of nine algae strains at two reaction temperatures (280 °C and 320 °C) are presented in Fig. 1. Duplicate experiments were conducted with selected species (S-1, C-1, N-3, P-1, and N-4) to demonstrate reproducibility of data. The variation in bio-oil yields was 1–5 wt%, which was consistent with our previous work (Shakya et al., 2015).

During HTL, different concentrations of lipids, proteins, and carbohydrates in the biomass undergo complex reactions processes of hydrolysis, depolymerization, condensation, re-polymerization and thermal cracking, etc. to form bio-oil, char, WSP and gaseous product. In this study, a higher bio-oil and gas yields and lower WSP yield were observed at 320 °C compared to that obtained at 280 °C. Relatively, less difference in char yield were observed at two temperatures. An increase in bio-oil yields at 320 °C can be due to the attainment of activation energy required for bond cessation, thus resulting in extensive biomass decomposition and depolymerization to bio-oil (Brown et al., 2010). *Nannochloropsis* species (N-1 and N-2) having high lipid contents yielded relatively large amount of bio-oil at 320 °C (56.47% for N-1 and 65.96% for N-2), which supports the hypothesis that more than 90% of lipids in the biomass is converted into products during HTL process (Elliott, 2016). In the biological system, triacylglycerides (TAGs) are the most common form of lipids (Peterson et al., 2008), which can be hydrolyzed to fatty acids and glycerols in hydrothermal media. Generally, fatty acids contribute to bio-oil formation whereas water soluble glycerols contribute to the aqueous phase. According to Tavakoli and Yoshida (2006), maximum glycerol concentration (4–6 wt%) was observed in the aqueous phase which was produced at 260 °C HTL temperature. The amount of glycerol decreased with an increase in operating temperature which may support the reduced WSP of high lipid containing algae at a higher temperature. In the case of high protein containing algae (N-4), bio-oil yields increased from 32.92 wt% at 280 °C to 46.41 wt% at 320 °C. Proteins in hydrothermal media undergo hydrolysis and form amino acids and peptides (Ross et al., 2010). An increase in WSP and a decrease in bio-oil at a lower temperature (280 °C) may be due to the presence of maximum amounts of hydrolyzed proteins and amino acids in the aqueous phase (Peterson et al., 2008). However, as the temperature and residence time increases, proteins in aqueous phase decomposes into bio-oil phase which results in an increase in the bio-oil and a decrease in WSP at higher temperatures (Garcia-Moscoso et al., 2015). Carbohydrates also get hydrolyzed during HTL to water soluble products. The maximum glucose in the aqueous phase was obtained at 260 °C and reduced drastically over 300 °C (Minowa et al., 1998). Thus, this explains the high amount of WSP at a lower temperature for high carbohydrate containing algae. The glucose in the aqueous phase can convert into bio-oil under the harsher environment and it can further go secondary decomposition to form char and gaseous product, resulting in an increase in their yields.

Algae strains investigated in this study constitute all the biochemical components (lipid, protein, and carbohydrate) in varying proportions. Therefore, the overall bio-oil yields observed may not be only due to the sole effect of individual biochemical components, but also due to interaction effect between these components (Teri et al., 2014). This cross-interaction between biochemical compounds may be the reason for high amounts of bio-oil yields obtained for algae S-1, which had both high carbohydrates and ample amounts of lipids. Although a detailed study on the interaction effects were not performed in this study, the formation of melanoidin compounds (formed by the interactions between proteins

Table 2
Proximate and ultimate analyses of algae strains.[¥]

Sample (Nomenclature)	Ash content (wt%)	HHV (MJ/kg)	Water Content (wt%)	Elemental analysis (wt% on dry basis)[γ]			
				C	H	N	O[*]
Chlorella (C-1)	7.42	22.18	78.97	51.41	7.59	9.10	24.47
Chlorella (C-2)	2.99	23.82	72.36	54.34	8.04	2.50	32.12
Nannochloropsis (N-1)	6.65	29.44	64.05	62.51	9.24	1.76	19.85
Nannochloropsis (N-2)	7.37	28.92	76.50	62.12	9.36	2.64	18.52
Nannochloropsis (N-3)	8.28	22.72	74.14	50.76	7.35	7.37	26.24
Nannochloropsis (N-4)	3.42	24.02	68.88	56.83	9.32	10.13	20.30
Pavlova (P-1)	3.47	22.69	75.8	54.34	8.69	8.67	24.83
Scenedesmus (S-1)	2.02	25.45	75.11	56.31	8.32	2.01	31.35
Scenedesmus (S-2)	2.10	22.34	70.30	50.82	7.46	6.87	32.74

[¥] The reported values for ash, HHV and water content are from average of two (n = 2).
[γ] The reported values are single data point (n = 1).
[*] By balance.

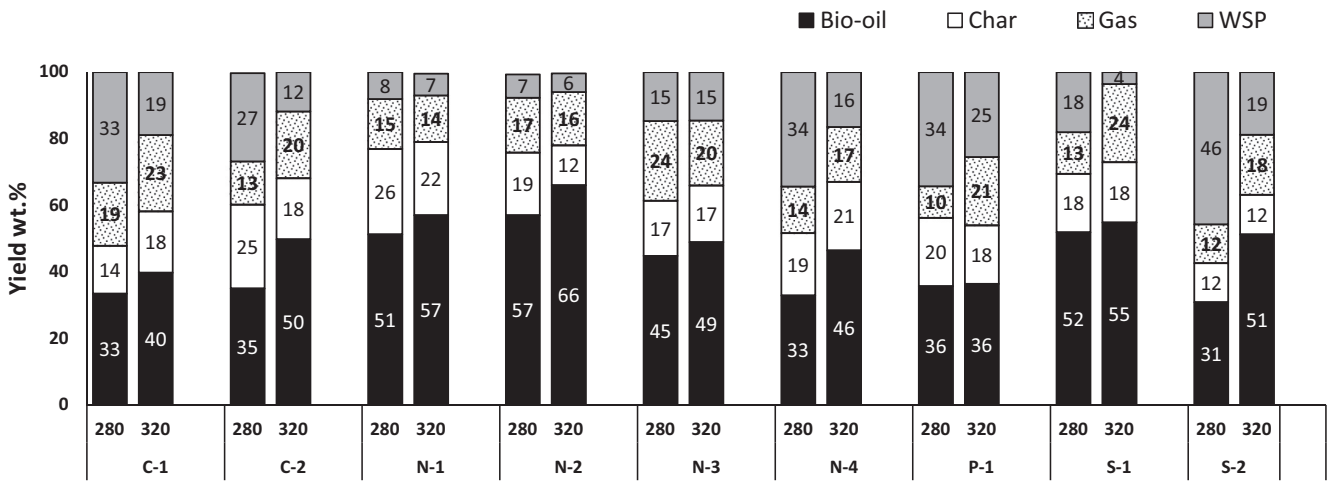

Fig. 1. Product distribution from hydrothermal liquefaction of nine algae strains.

and carbohydrates) (Peterson et al., 2010), and fatty acid amides (formed by the interaction between lipids and proteins) (Albrecht et al., 2016), in the bio-oil indicates the presence of interactions between different biochemical components.

3.3. Prediction of Bio-oil yield

Eqs. (2) and (3) show the predictive models of the bio-oil yields obtained from the linear regression of the data obtained from fourteen HTL experiments performed at each temperature of 280 and 320 °C, respectively.

$$\textit{Bio-oil Yield}\ (wt\%)_{280} = 0.90 \times L + 0.22 \times C + 0.32 \times P \quad (2)$$

$$\textit{Bio-oil Yield}\ (wt\%)_{320} = 0.96 \times L + 0.30 \times C + 0.43 \times P \quad (3)$$

where L, C and P are fractions of lipids, carbohydrates and proteins in the algae biomass. The predicted models explained about 97–98% variation in the observed data as shown by the adjusted R-square values. The coefficient of the respective component showed that the yield followed the trend of lipids > proteins > carbohydrates. The overall coefficients of lipids and proteins in this study were similar to other studies obtained by using model compounds (Biller and Ross, 2011; Teri et al., 2014). In contrast, the coefficient of carbohydrates were 5–7 times higher than the value reported. Higher conversion of carbohydrates may be due to its cross-interactions with other biochemical compounds, such as proteins, in the algae (Peterson et al., 2010) or due to the effect of inorganics present in the algae, which might have catalyzed the carbohydrates to give higher yields (Biller and Ross, 2011; Abdullah and Gerhauser, 2008). Also, algae have cell walls that are made up of bio-polymers like algaenans, which can be converted into bio-oil. Apart from the model chemical compound studies, the carbohydrate conversion in this study was 2–2.5 times greater than that obtained by Leow et al. (2015), who performed HTL of 10 batches of *Nannochloropsis*, having different biochemical compositions. This increase in carbohydrate conversion may be due to the use of different algae species in this study as compared to *Nannochloropsis* only in the previous work (Leow et al., 2015). Different algae species have different cell wall thickness and have different proportion of cell matrices which could have altered yields (Barreiro et al., 2013; Graham et al., 2016). Although cross-interactions among various biochemicals play a vital role in the bio-oil yield, the predictive model considering cross-interaction effect showed poorer accuracy compared to the model considering the effect of a single model compounds (Leow et al., 2015). Therefore, the predictive models of Eqs. (2) and (3) better predict HTL bio-oil yields from algae as it considered diverse algae species and different operating temperatures. Model validation was performed for the model obtained at 320 °C. As only few studies have been conducted at the same reaction conditions used in this study, other literature with varied process conditions were used for the validation. The model obtained at 320 °C showed the difference in the predicted and experimental data to be ±8%.

3.4. Bio-oil properties

Table 3 illustrates the properties of bio-oils obtained from different algae strains. The bio-oils obtained were thick, black viscous

Table 3
Properties of bio-oil obtained from HTL of different algae strains.[¥]

Sample	Temp. (°C)	Water content (wt%)	TAN (mg KOH/g)	Ash content (wt%)	HHV (MJ/kg)	Elemental analysis (wt% in daf)				Atomic ratio (mole/mole)		Energy recovery (ER)
						C	H	N	Balance[*]	H/C	N/C	
C-1	280	8.55	45.38	0.89	31.10	72.89	12.09	5.74	9.27	1.99	0.07	46.89
	320	9.88	27.44	0.25	33.11	77.67	12.72	7.34	2.27	1.97	0.08	59.31
C-2	280	9.10	101.61	0.22	32.92	77.80	13.64	2.50	6.05	2.10	0.03	48.79
	320	5.98	97.22	0.19	34.23	79.53	13.07	4.76	2.64	1.97	0.05	71.50
N-1	280	5.07	114.21	0.93	35.14	76.75	13.23	2.48	7.55	2.07	0.03	61.12
	320	6.32	96.60	0.30	35.83	79.11	12.90	5.37	2.63	1.96	0.06	68.72
N-2	280	6.63	118.71	0.67	36.44	77.34	14.19	3.74	4.74	2.20	0.04	72.25
	320	5.25	94.48	0.31	36.30	78.72	14.44	4.64	2.19	2.20	0.05	82.80
N-3	280	6.28	37.39	0.65	34.63	76.36	12.15	4.43	7.06	1.91	0.05	68.25
	320	7.41	30.11	0.22	35.46	78.53	12.98	6.66	1.84	1.98	0.07	76.37
N-4	280	7.75	28.91	0.69	34.82	76.24	10.01	5.24	7.83	1.57	0.06	47.72
	320	5.74	25.51	0.18	36.97	79.56	10.85	5.37	4.04	1.64	0.06	71.42
P-1	280	4.87	36.15	0.33	33.42	69.32	10.46	4.07	15.83	1.81	0.05	52.58
	320	4.22	30.42	0.19	35.29	76.81	10.50	3.81	8.68	1.64	0.04	56.54
S-1	280	5.96	118.29	0.59	34.34	77.95	12.28	2.61	7.17	1.89	0.03	69.97
	320	5.39	107.06	0.31	36.28	78.58	13.76	3.91	3.76	2.10	0.04	78.11
S-2	280	5.92	47.61	0.62	33.93	72.73	11.80	4.99	10.48	1.95	0.06	46.87
	320	9.96	29.70	0.29	34.37	76.49	12.53	5.76	5.21	1.97	0.06	78.79

[¥] The reported values for ash content, TAN, HHV, water content and elemental analysis are from average of two (n = 2), daf: dry and ash free basis.
[*] Balance is the sum of % O and % S.

layer. Bio-oils from all the algae strains were hydrophobic in nature. The water content of bio-oils was in the range of 4–9 wt%. TAN in bio-oil is directly influenced by the amount of carboxylic acid (Wang et al., 2016) and also by phenolic present in the samples. Therefore, bio-oils obtained from high lipid (>30 wt%) containing algae were observed to have maximum TAN (101–118 mg KOH/g). Bio-oils from high protein (>45 wt%) containing algae strains had the lowest TAN (25–48 mg KOH/g). The decrease in TAN was observed for all the bio-oils obtained at 320 °C due to further degradation or decomposition of fatty acids at the severe environment.

The carbon and hydrogen contents were in the range of 69–79 wt% and 10–14 wt%, respectively which were higher than that of the original algae. An increase in carbon and hydrogen concentration in the bio-oil was observed with higher HTL temperature. The H/C ratio of the bio-oils was in the range of 1.8–2.2, which compared favorably with that of the petroleum crude. Higher carbon and hydrogen content and lower oxygen content resulted in a increase in higher heating value (HHV) of the bio-oils. The HHV of bio-oils were in the range of 32–37 MJ/kg, which showed no trend with respect to biochemical compositions. As expected, the nitrogen content of the bio-oils from HTL of high protein containing algae was comparatively higher than others. The presence of relatively high amounts of nitrogen and oxygen makes the bio-oil unfavorable for blending with petroleum crude. Therefore, further upgrading of the bio-oil is required. Energy recovery in the bio-oil from algae samples were evaluated and higher energy (56–82%) was recovered at higher temperature compared to that (47–72%) at lower temperature. A maximum recovery of 83% was observed for a high lipid containing algae (N-2).

The distribution of major compounds in bio-oil produced from different algae strains are shown in Fig. 2. Organic acids in the bio-oils obtained at both 280 and 320 °C were not only high for higher lipid containing algae (N-1 and N-2) but were also high for algae having relatively large amount of both, lipids and carbohydrates (C-2 and S-1). This suggests that not only lipids but carbohydrates also contribute to the formation of organic acids. The bio-oils produced from all the algae species, except C-2 and S-1, had higher organic acids at 280 °C than at 320 °C. The high amount of organic acids at 280 °C may be due to the higher conversion of triglycerides into fatty acids at a lower temperature, whereas its reduction at 320 °C may be due to the decarboxylation of fatty acids to form hydrocarbons at a higher temperature (Teri et al., 2014). In the case of S-1 and C-2, a slight increase in organic acid was observed at 320 °C, which may be due to the degradation and decomposition of carbohydrate in the algae to form formic acid, lactic acid and acetic acid (Srokol et al., 2004). These changes in organic acid are correlated with the TAN of the bio-oil. Hydrocarbons in the bio-oils were mostly aliphatic hydrocarbons formed due to decarboxylation of organic acids and decarbonylation of aldehydes and ketones. No specific trend was found with an increase in temperature for hydrocarbons. In the case of nitrogenated compounds, both amides and nitrogen heterocycles were observed in the bio-oils. The amino acids present in proteins undergoes decarboxylation and deamination reaction in hydrothermal media and produce amines, ammonia, carbonic acids and other organics (Sato et al., 2004). As expected, algae having high initial protein content (C-1, N-3, P-1 and N-4) had a significantly high amount (42–48% of total peak area) of nitrogenated compounds present in bio-oil. Oxygenates and phenols were also present in the bio-oils, but no trend was observed with an increase in temperature. Oxygenates and phenols were formed mainly due to decomposition of carbohydrates. Overall, the production of each chemical composition in bio-oil did not follow a single reaction pathway but multiple reaction pathways in this complex mixture.

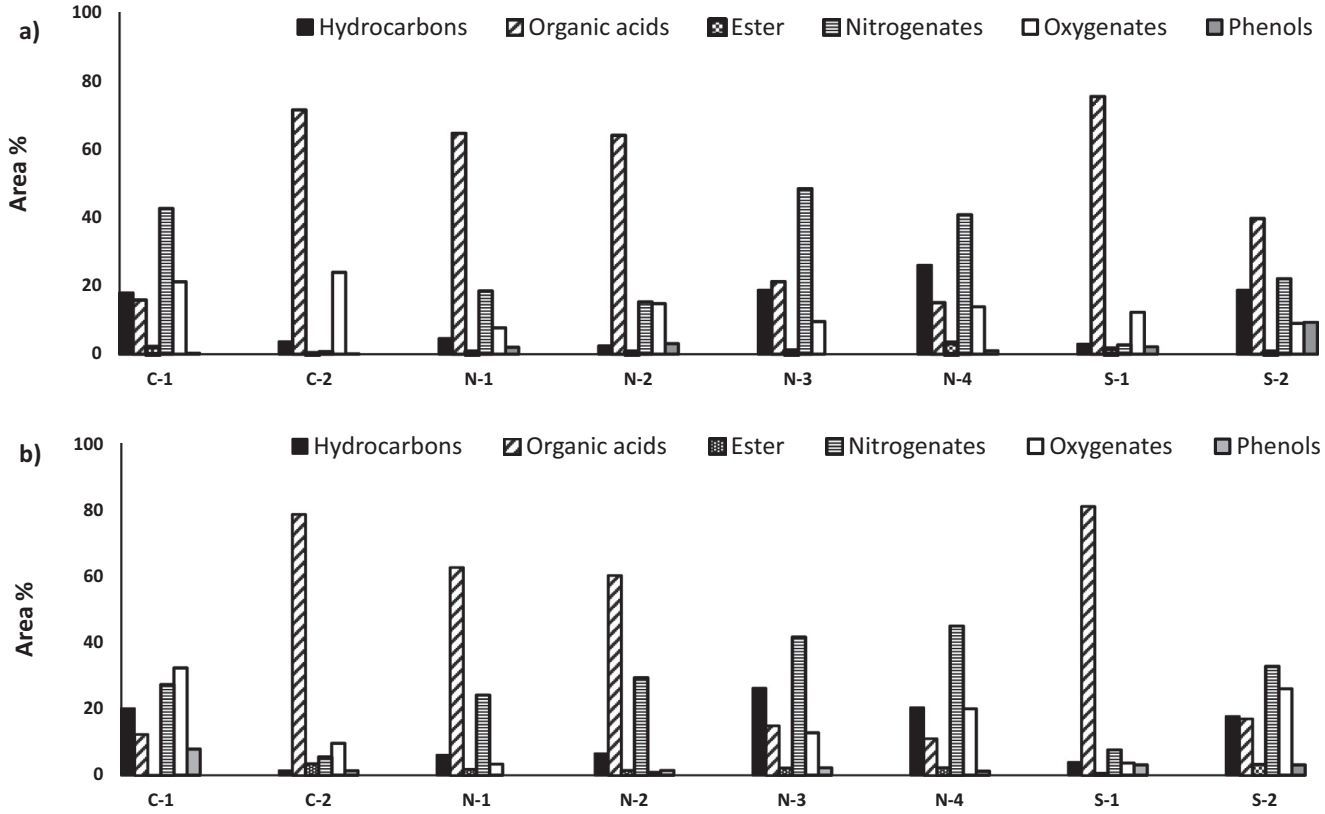

Fig. 2. Distribution of chemical compounds in bio-oils obtained from HTL of different algae strains at a) 280 °C and b) 320 °C.

Fig. 3 illustrates the bio-oil fraction from all the algae strains in different boiling point ranges. Boiling points were grouped as described by Vardon et al. (2011). The boiling points of bio-oils obtained from all the algae strains were predominantly (40–68%) in vacuum gas oil (VGO) range, except for N-1 and N-2, which had a maximum portion in diesel range (33–42%). An increase in diesel range for N-1 and N-2 may be due to the large amount of organic acids, mostly myristic acid and palmitic acid (55–75% of total lipids) present in them. The bio-oils from S-1 and C-2 also had high organic acids whereas they contained mostly stearic and oleic acid (50–75% of total lipids), which have higher boiling points than myristic and palmitic acids. All the bio-oils had a minimal amount of compounds in gasoline (0.5–5%) and kerosene (1–13%) ranges. About 9–30% of the bio-oils had a boiling point higher than 538 °C and N-4 (high protein containing algae) showed the maximum of 30%. An increase in the reaction temperature at 320 °C slightly increased the fraction of bio-oil in lower boiling points ranges and consequently decreased the fraction of bio-oil in vacuum residue range for all the bio-oils. However, the simulated distillation showed that the bio-oil fraction in lower boiling point range was still very low, which requires a further upgrading of the bio-oil to be used as a transportation fuel substitutes or to blend it with conventional fuels.

FTIR analysis of all the bio-oils was performed to study their functional group characteristics. The spectral band assignments and interpretation were based on the literature (Gai et al., 2014; Mahadevan et al., 2016). No significant difference was observed between the spectral band observed at 280 °C and 320 °C. All the bio-oil samples showed a broad-band in 3100–3650 cm^{-1}, which is possibly from O—H and N—H stretching vibrations indicating the presence of alcohols and carboxylic acids for O—H stretching and the presence of amine and amides (N—H stretching). This is supported by the presence of prominent N—H (amines and amides) bending peaks at 1600–1680 cm^{-1} and 1525–1575 cm^{-1}. Bio-oils obtained from high protein containing algae (N-4) had more absorption in the 3100–3500 cm^{-1} spectral region. The prominent C—H stretching (2800–3000 cm^{-1}) and —CH_3 bending (1360–1380 cm^{-1}) were observed in all the bio-oils. Bio-oils obtained from algae (N-1) having higher lipid content had prominent stretching in these regions, which may be due to the C—H stretching of fatty acids present in the bio-oils. This was supported by the prominent stretching observed for the bio-oils in the C=O stretching (1709 cm^{-1}) region. Apart from carboxylic acids, the presence of other compounds having C=O bonding such as ketones and aldehydes in the bio-oil also causes the stretching in 1709 cm^{-1} region. Some adsorption peaks were also observed at 500–900 cm^{-1}, which may be attributed to the C—H bending from aromatics such as naphthalene and phenols.

3.5. Solid residue analysis

Proximate and ultimate analyses of the solid residue obtained from HTL of different algae strains are summarized in Table 4. Ash content was observed to be in the range of 8–40 wt% and followed the trend of original algae ash content. The HHV of solid residue were in the range of 15–32 MJ/kg which were in agreement with the previous studies (Garcia Alba et al., 2012; Shakya et al., 2015). Ultimate analysis of the solid residue showed that it still had a significant portion of unconverted C, H and N. No significant trend in elemental values were observed at 280 °C and 320 °C. However, high C and H values of solid residue related to a higher HHV. The surface area of the solid residue obtained from HTL at 280 °C for S-1 and C-2 were found to be 15.27 and 25.22 m^2/g, respectively whereas that obtained at 320 °C were observed to be 13.33 and 13.69 m^2/g, respectively.

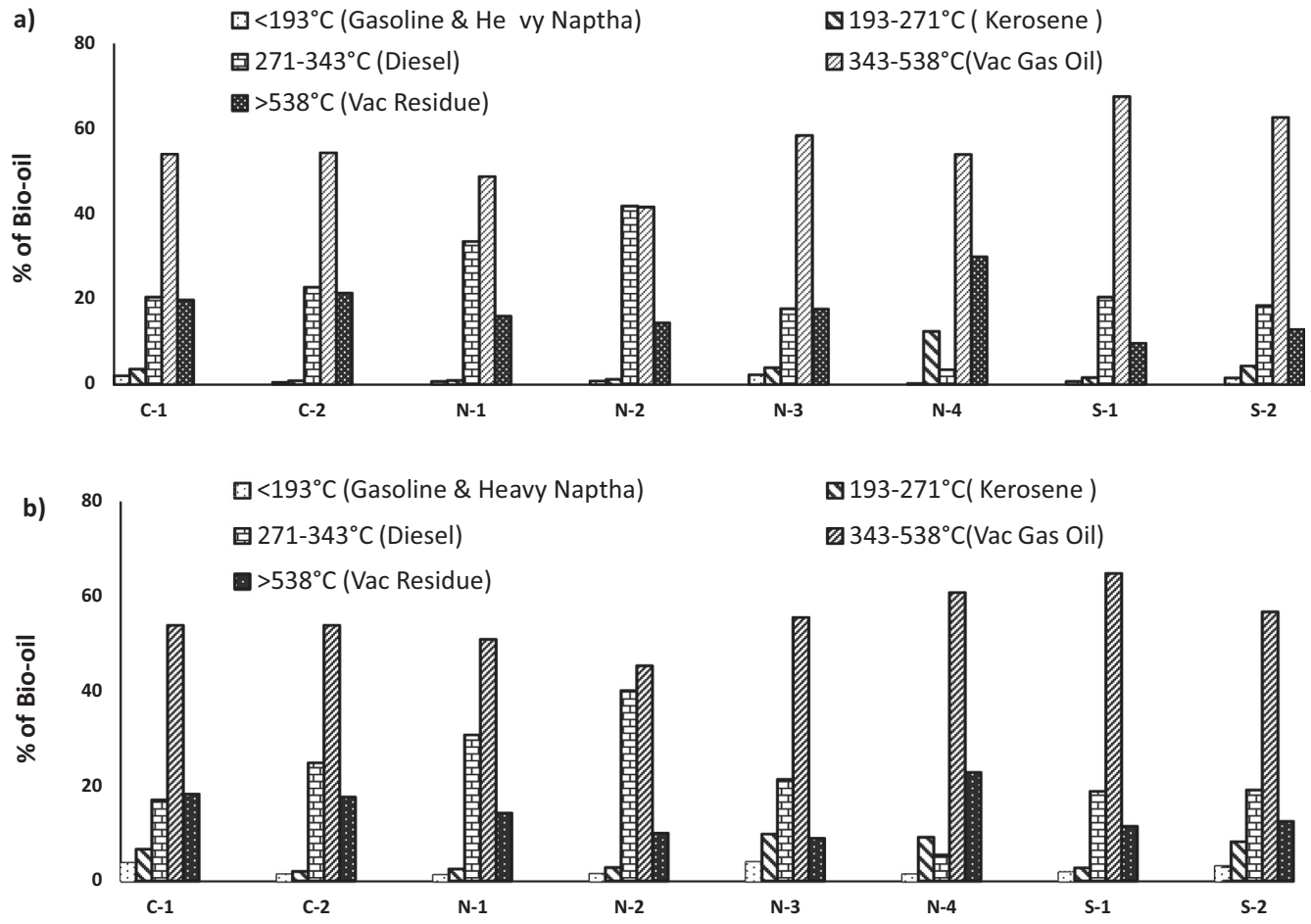

Fig. 3. Boiling point distribution of bio-oil derived from HTL of different algae strains at a) 280 °C and b) 320 °C.

Table 4
Proximate and ultimate analyses of solid residue of seven different algae.[¥]

Sample	Temp. (°C)	Ash Content (wt%)	HHV (MJ/kg)	Ultimate analysis (wt% on dry basis)			
				C	H	N	Balance[*]
C-2	280	12.32	25.97	61.59	5.97	3.92	28.52
	320	13.48	27.53	66.32	4.85	4.60	24.23
N-1	280	19.05	29.86	57.97	8.96	0.43	32.64
	320	21.51	31.87	64.32	10.02	0.03	25.63
N-2	280	40.27	21.89	39.95	5.58	1.24	53.23
	320	40.54	20.19	39.76	6.15	0.52	53.57
N-3	280	33.66	20.72	36.43	4.90	2.25	56.42
	320	36.15	22.24	31.54	4.57	1.51	62.38
N-4	280	15.79	20.15	27.67	4.53	5.34	62.46
	320	21.71	22.87	35.79	5.27	2.10	56.84
P-1	280	24.88	18.61	40.05	4.77	4.29	50.89
	320	30.26	19.25	37.62	4.88	3.01	54.49
S-1	280	10.26	28.77	67.09	5.84	4.32	22.75
	320	8.89	31.49	70.72	6.32	4.17	18.79

Note: data for algae S-2 and C-1 were not shown due to less amount of char.
[¥] The reported values for ash content, HHV, and ultimate analysis are single point data (n = 1).
[*] Balance is the sum of % O and % S.

3.6. Aqueous phase analysis

The aqueous phase is the major byproduct from HTL of algae, which is rich in nutrients and chemicals. Understanding the composition of the aqueous phase is critical to the overall economics of the HTL process. Table 5 illustrates the total organic carbon, total nitrogen, phosphate, ammonium, magnesium and chemical oxygen demand of the aqueous product. The amount of total organic carbon (TOC) and chemical oxygen demand (COD) ranged between 12–43 g/L and 35–160 g/L, respectively, indicating a significant proportion of organic carbon distributed in the aqueous phase as dissolved organics. A slight decrease in organic carbon was observed with the increase in reaction temperature from 280 °C to 320 °C, which further supports the theory that at higher temper-

Table 5
TOC, TN, COD and phosphate measurement of the aqueous phase at both temperature.[¥]

Samples	Temperature (°C)	TOC (g/L)	TN (g/L)	COD (g/L)	PO_4^{3-} (g/L)	NH_4^+ (g/L)	Mg (g/L)
C-1	280	29.75 ± 6.90	10.36 ± 1.38	93.50 ± 12.02	9.65 ± 1.77	8.95 ± 0.35	2.00 ± 0.71
	320	23.62 ± 1.57	9.77 ± 2.37	70.50 ± 47.38	5.95 ± 1.12	9.30 ± 0.71	0.90 ± 0.07
C-2	280	12.62 ± 0.82	1.49 ± 0.01	52.00 ± 24.04	9.04 ± 1.46	0.13 ± 0.01	1.45 ± 0.07
	320	13.34 ± 0.43	2.11 ± 0.03	61.50 ± 13.44	1.96 ± 0.19	0.50 ± 0.28	0.88 ± 0.04
N-1	280	19.78 ± 1.24	3.13 ± 0.02	52.00 ± 00	1.33 ± 0.07	0.65 ± 0.07	2.68 ± 0.18
	320	12.71 ± 0.85	3.07 ± 0.07	52.00 ± 00	0.94 ± 0.21	0.90 ± 0.42	1.85 ± 0.14
N-2	280	16.75 ± 0.32	2.51 ± 1.98	35.50 ± 23.33	2.06 ± 0.16	0.65 ± 0.07	1.48 ± 0.46
	320	11.60 ± 0.15	2.48 ± 1.77	61.00 ± 33.94	1.15 ± 0.57	1.70 ± 0.57	0.73 ± 0.11
N-3	280	30.42 ± 2.03	11.12 ± 2.79	102.00 ± 00	11.95 ± 0.64	7.55 ± 0.92	1.45 ± 0.07
	320	24.14 ± 1.44	10.48 ± 3.17	71.00 ± 00	8.87 ± 1.51	9.75 ± 0.35	1.22 ± 0.11
N-4	280	43.10 ± 4.15	15.01 ± 0.18	160.50 ± 12.02	11.70 ± 0.57	9.00 ± 1.41	1.25 ± 0.21
	320	29.26 ± 0.20	18.52 ± 1.67	87.50 ± 23.33	8.12 ± 1.86	12.05 ± 0.49	1.55 ± 0.07
P-1	280	28.88 ± 1.52	9.48 ± 0.91	35.00 ± 00	2.12 ± 0.50	8.70 ± 0.28	0.78 ± 0.11
	320	26.01 ± 2.79	9.50 ± 2.03	54.00 ± 24.04	6.26 ± 0.62	9.20 ± 0.14	1.02 ± 0.04
S-1	280	14.62 ± 0.94	0.98 ± 0.90	35.00 ± 00	0.72 ± 0.04	0.08 ± 0.03	1.98 ± 0.39
	320	12.89 ± 0.01	1.26 ± 1.06	35.00 ± 00	0.84 ± 0.05	0.34 ± 0.06	1.28 ± 0.04
S-2	280	26.05 ± 0.86	8.54 ± 0.29	69.00 ± 00	4.12 ± 0.04	7.40 ± 0.85	0.93 ± 0.11
	320	20.64 ± 0.01	8.48 ± 0.07	37.00 ± 00	5.02 ± 1.32	4.00 ± 0.14	0.32 ± 0.11

[¥] The reported values are the mean of duplicates (n = 2).

ature the organics in the aqueous phase is converted to bio-oil or gaseous products. Shakya et al. (2015) observed a similar trend in TOC for the aqueous phase from hydrothermal liquefaction of *Nannochloropsis*. On comparing with other algal feedstock, the HTL of high proteinaceous algae resulted in a high TOC (N-4: 43 g/L). As the COD values of the aqueous phase are higher than the permissible wastewater discharge parameter (120 mg/L) set by the EPA (2009), further recovery of the chemicals from the aqueous phase is required before dumping it into the wastewater streams. Due to the high amount of organic carbon (20–35 wt%) in the aqueous phase, there is the potential of converting it into high-value chemicals and fuels. Elliott et al. (2013) and Shanmugam et al. (2017a) demonstrated the production of methane from aqueous phase via catalytic gasification and anaerobic digestion, respectively.

Total nitrogen (TN) content in the aqueous phase ranged from 1 to 15 g/L and was similar to that obtained by Gai et al. (2015). The variation in TN in the aqueous byproduct is mostly due to the type of algal strains. Algae containing the highest proteins (N-4) gave the maximum TN (18 g/L) whereas that with the lowest protein content (S-1) resulted in the minimum TN (0.98 g/L). It is also known from the literature (Gai et al., 2015; Valdez et al., 2012) that around half to two-third of nitrogen in the algae is retained in the aqueous phase. No significant trend was observed in total nitrogen content with the increase in temperature in this study. Ross et al. (2010) reported a decrease in nitrogen concentration with an increase in temperature for *Spirulina* but reported no change for *Chlorella* which shows that change in nitrogen concentration with temperature depends on the algae strains. The ammonium in the aqueous phase ranged between 0.34 and 12.05 g/L, which were observed to be higher than that observed by Ross et al. (2010), this might be due to the use of catalysts in their work or the variation in the reaction temperatures (300 & 350 °C). From the observation of TN and NH_4^+ content, this study shows that most of the nitrogen in the aqueous phase are in NH_4^+ form than in NO_3^-. A higher level of ammonium in the aqueous phase demonstrates its potential for nitrogen recovery by algal growth as ammonium can be directly used to synthesize cellular N-compounds than the nitrate form in the algae (Graham et al., 2016). The aqueous phase obtained were mainly alkaline (pH of 7.0–8.5) in nature due to the presence of a high amount of ammonium and other forms of basic nitrogenous compounds. However, the aqueous phase obtained from the algae having lower protein content (S-1 and C-2) were acidic (pH of 3.5–5) in nature, this was in agreement with the work of Maddi et al. (2016), who observed low pH in the aqueous phase obtained from high lipids and low nitrogen containing algae.

The chemical composition of the aqueous phase from HTL contained diverse chemical compounds like cyclopentenones, phenolics, carboxylic acids and nitrogen heterocycles. The chemical compounds present in the aqueous phase depended on the biochemical composition of the algae. The aqueous phase obtained from high protein containing algae had the maximum amount of nitrogenous compounds whereas maximum oxygenates were obtained from high carbohydrates containing algae. The high amount of nitrogenous and phenolics compounds present have a wide variety of applications. High-value chemicals and drugs can be produced from pyridine, pyrazine, and their alkyl derivatives (Maddi et al., 2016).

Phosphorus is one of the primary nutrients needed for algal growth (Graham et al., 2016; Shanmugam et al., 2017b). During HTL, the phosphorus in the algae also gets partitioned into different products. Phosphorus in the aqueous phase is mostly found in the form of phosphate (Garcia Alba et al., 2012; Valdez et al., 2012). In this study, phosphate in the aqueous phase ranged from 0.7 to 11.95 g/L, which were in agreement with the values obtained by Maddi et al. (2016). The phosphate concentration slightly decreased with the increase in temperature for all the algae species except for P-1, S-1, and S-2, which increased slightly. Magnesium content in the aqueous phase was in the range between 0.7 and 2.6 g/L. Phosphate, ammonium, and magnesium in the aqueous phase can be recovered by precipitating it in the form of struvite (Magnesium ammonium phosphate), a slow releasing fertilizer, as shown by Shanmugam et al. (2017b). According to Shanmugam et al. (2017b), around 0.10–0.12 g of struvite can be produced from 10 mL of aqueous phase, which had phosphate concentration of 3.9 g/L. The study also showed that more than 99% of phosphate and around 44–100% ammonia were recovered as struvite.

Apart from phosphorus and magnesium, the range of the other major trace elements in the aqueous phase was 24–758, 807–4151 and 209–1841 mg/L for Ca, K and Na, respectively. These data were consistent with the previous studies (Jena et al., 2011b). These variations in the metal elements are mainly attributed to their take up by biomass during its growth. Other trace elements present in the aqueous phase were copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), etc. The presence of high amount of nutrients and chemicals in the aqueous phase can not only be recovered as fuels or chemicals but can also be used for algal growth (Jena et al., 2011b).

4. Conclusions

The effect of biochemical composition of nine algae species on the HTL bio-oil yields and properties at 280 and 320 °C were investigated. The bio-oil yields were higher at the higher temperature of 320 °C. The predictive yield model for bio-oil yield followed the trend lipid > protein > carbohydrate. A large concentration (40–68%) of algae bio-oils were in VGO range. The aqueous phase had high amount of organic chemicals which could be recovered for fuels and chemicals. The high amount of phosphate, ammonia, and magnesium in the aqueous phase showed a potential of struvite production.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.07.046.

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