



# Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil



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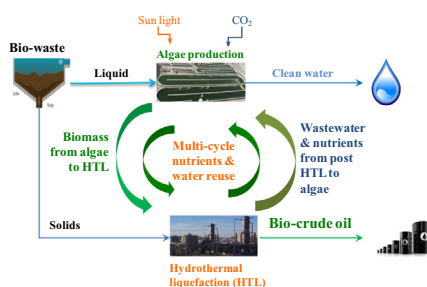
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## HIGHLIGHTS

- The first work using mixed-culture algae directly from wastewater as HTL feedstock.
- Improved bio-crude oil yield using mixed-culture algal biomass.
- Lower nitrogen recovery in the bio-crude oils compared to studies using pure algae.

## GRAPHICAL ABSTRACT

A schematic Environmental-Enhancing Energy road map.



## ARTICLE INFO

### Article history:

Received 4 September 2013  
 Received in revised form 27 October 2013  
 Accepted 30 October 2013  
 Available online 7 November 2013

### Keywords:

Bio-crude oil  
 Hydrothermal liquefaction (HTL)  
 Algae  
 Wastewater treatment  
 Nitrogen recovery

## ABSTRACT

In this study, a mixed-culture algal biomass harvested from a functioning wastewater treatment system (AW) was hydrothermally converted into bio-crude oils. The highest bio-crude oil yield (**49% of volatile matter**) and the highest energy recovery were obtained at 300 °C with 1 h retention time. The highest heating value of the bio-crude oil was **33.3 MJ/kg**, produced at 320 °C and 1 h retention time. Thermogravimetric analysis showed approximately 60% of the bio-crude oils were distilled in the range of 200–550 °C; and the solid residue might be suitable for use in asphalt. GC–MS results indicated that the bio-crude oil contained hydrocarbons and fatty acids, while the aqueous product was rich in organic acids and cyclic amines. The nitrogen recovery (NR) in the bio-crude oil ranged from 8.41% to 16.8%, which was lower than the typical range of 25%–53% from previous studies.

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## 1. Introduction

Algae are viewed as favorable next generation bioenergy feedstock because of their higher photosynthetic efficiency and

less competition for arable land, compared to other terrestrial plants (Tsukahara and Sawayama, 2005). Most of the previous algae-to-biofuel research focuses on algae species with high lipid content for extraction and transesterification to biodiesel (Luque

*Abbreviations:* Ash, ash fraction in the dry mass of AW (no units); AWVM, mass of volatile matter of mixed-culture algal biomass from wastewater treatment system (g); F, mass of feedstock (g); G, mass of gas products (g); MWI, mass of moisture in the water-insoluble products (g); R, raw oil (g); SR, mass of toluene-insoluble fraction in the water-insoluble products (g); VM, volatile matter (g); WI, mass of water-insoluble products (g).

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et al., 2010). However, low-lipid algae typically have higher total biomass productivity than high-lipid species. Furthermore, low-lipid algae are more common in wastewater cultivations, which can reduce algal biomass production costs and environmental pollution such as eutrophication (Chen et al., 2002). This paper investigates the potential for integration of algal wastewater treatment with bioenergy production via hydrothermal liquefaction (HTL), which is referred to here as the Environment-Enhancing Energy (E<sup>2</sup>-Energy) system. This system can uptake nutrients from wastewater and re-releases most of them after HTL to support multiple cycles of algae growth, which amplifies the biofuel potential of wastewater treatment (Yu et al., 2011a,b; Zhou et al., in press).

In an HTL process, macromolecules in biomass are depolymerized first into light molecules and then the unstable fraction of chemicals are repolymerized into oil compounds (Peterson et al., 2008). HTL is more suitable for treating wet feedstocks than other thermochemical conversion processes such as pyrolysis and gasification, which need dry feedstocks for a positive energy balance. Wet feedstocks can be treated directly by HTL without drying and energy-dense oil products self-separate from the water after HTL treatment. For example, one previous study showed that when HTL reaction temperatures reached 240 °C, bio-crude oil products began to form as self-separated bitumen-like products; below 240 °C, the feedstocks were not completely converted into bio-oil products (Yu, 2012). Retention time was another important factor in the formation of bio-crude oil. Typically, it takes at least ten minutes to form self-separating bioenergy products from algal biomass. As holding time increased, the bio-crude oil yield did not increase significantly, indicating that a long retention times was not an essential factor for the bio-crude oil formation. Past research also showed that initial pressure had little effect on the HTL products distribution and oil product composition under HTL conditions when the additional initial pressure was above the saturation pressure of water (Yu et al., 2011a,b). Consequently, this study specifically focuses on HTL of the mixed-culture algal biomass from a wastewater treatment system (AW) with temperatures ranging from 260 °C to 320 °C, retention times ranging from 0 to 1.5 h and an initial pressure of 0.69 MPa, which was the lowest pressure previously shown to produce a substantial oil product (Yu et al., 2011a,b).

Past work demonstrated that low-lipid algae species can be efficiently converted into bio-crude oil via HTL (Yu, 2012). In order to achieve the goal of positive energy output, it has been suggested to couple waste treatment with bio-energy production (Clarens et al., 2010). Producing bio-crude oil via HTL can not only provide bio-waste treatment but also saves great amounts of energy on dewatering algae. This study intends to examine the feasibility of using mixed-culture algal biomass (a by-product of wastewater treatment combining various species of algae, bacteria and other organisms) as the HTL feedstock. The effects of the reaction temperature and retention time on the bio-crude oil yields were analyzed. In addition, the liquefaction products were characterized via elemental analysis, GC-MS and TGA to examine nutrient recovery, physicochemical properties and possible reaction pathways for the bio-crude oil formation.

Although other studies have used waste-fed algae as HTL feedstocks (Roberts et al., 2013; Zhou et al., in press), which may still encounter the risk of contamination by competing microorganisms during the algae cultivation (National Research Council, 2012), this study appears to be the first of its kind to use mixed-culture algal biomass that was directly harvested from a full-scale operating wastewater treatment systems. The conversion of low-lipid, mixed-culture algal biomass into bioenergy products resolves the contamination issues associated with algal biofuels and allows for the full potential of E<sup>2</sup>-Energy technology to be realized.

## 2. Methods

### 2.1. Feedstock

The mixed-culture algal biomass (AW) was directly harvested from a wastewater treatment system (One Water Inc., Indianapolis, IN) and was comprised of microalgae, macroalgae, bacteria, and other organisms. AW was dried and pulverized with a commercial blender (MX 1000XT, Waring Commercial Inc., Torrington, CT) and then stored in a refrigerator below 4 °C. The dry solids content and the ash content of AW were measured as the weight fraction after drying at 105 °C and the residual fraction after combustion at 550 °C, respectively. Elemental analysis of feedstock was operated by a CHN analyzer (CE-440, Exeter Analytical Inc., North Chelmsford, MA). Other macromolecules and chemical compositions were analyzed according to the standard methods of the Association of Official Analytical Chemists (AOAC). The higher heating value of dry AW material was measured using an oxygen bomb calorimeter (Model 6200, Parr Instrument Co., Moline, IL). Detailed chemical composition of AW is summarized in Table 1.

### 2.2. HTL experiments

The HTL experiments were performed by using a stainless steel cylinder reactor of 100 ml capacity with a magnetic drive stirrer and removable vessel (Model 4593, Parr Instrument Co., Moline, IL) operated in a batch mode. Reaction temperatures ranged from 260 °C to 320 °C and retention times ranged from 0 h to 1.5 h. The 100 ml batch reactors used in the present work typically took about 0.5 h, 0.75 h, 1 h and 1.25 h to reach 260 °C, 280 °C, 300 °C and 320 °C, respectively. The retention times as used in this study do not include the heat-up times. After each test, the reactors were rapidly cooled down to room temperature within 0.5 h by circulating tap water through cooling coils located outside the reactors. A typical temperature profile is also presented in the Supplementary data. 30 g of slurry feedstock with 25 wt% total solid content of AW was used in each test. The reactor was subsequently sealed and the headspace was purged with nitrogen three times. Nitrogen gas was again added to the reactor to build up to 0.69 MPa gauge pressure to prevent the water from boiling during the experiments (Yu et al., 2011a,b). Initial/final pressures and temperatures were recorded.

### 2.3. Analysis of products

After the reactor was cooled down, the gas products were sampled through a control valve into a Tedlar<sup>®</sup> gas sampling bag (CEL Scientific CORP., Cerritos, CA). The rest of the HTL reaction products were separated using a Whatman<sup>®</sup> glass-fiber filter. The aqueous portion was defined as the water-soluble portion (which can pass through the filter) while the rest of the filtration cake was defined as the raw-oil. The moisture content of the water-insoluble product was measured with a distillation apparatus based on ASTM Standard D95-99 (ASTM, 2004a,b,c) whereas the solid residue fraction of the raw oil product was determined via Soxhlet extraction according to ASTM Standards D473-02 and D4072-98 (ASTM, 2004b,c). The HTL product recovery procedures are summarized in Fig. 1.

The yields of the liquefaction products were calculated on the volatile matter (VM) basis of AW. The equations used for product distribution calculations can be found in Table 2. The gas yield was estimated by the ideal gas law using the initial/final temperature and pressure. The gas composition was analyzed in a Varian CP-3800 Gas Chromatograph equipped with an Alltech HayeSep D 100/120 column and a thermal conductivity detector (TCD). The gas composition of AW in this study was found to be

**Table 1**  
Proximate analysis of AW (wt% dry basis).

Chemical composition		Crude fat	Crude protein	Hemicellulose	Cellulose	Lignin	HHV (MJ/kg)	
VM <sup>a</sup>	Ash							
52.5	47.5	1.70	27.2	3.50	14.4	5.70	12.9	
Elemental composition		N	S	Na	Ca	P	Mg	O <sup>b</sup>
C	H							
27.9	3.01	3.90	0.40	0.36	16.5	0.93	0.37	65.2

<sup>a</sup> Volatile matter.

<sup>b</sup> O (wt%) = 100 – (C + H + N) (wt%).

**Table 2**  
Calculation equations for the liquefaction products distribution.

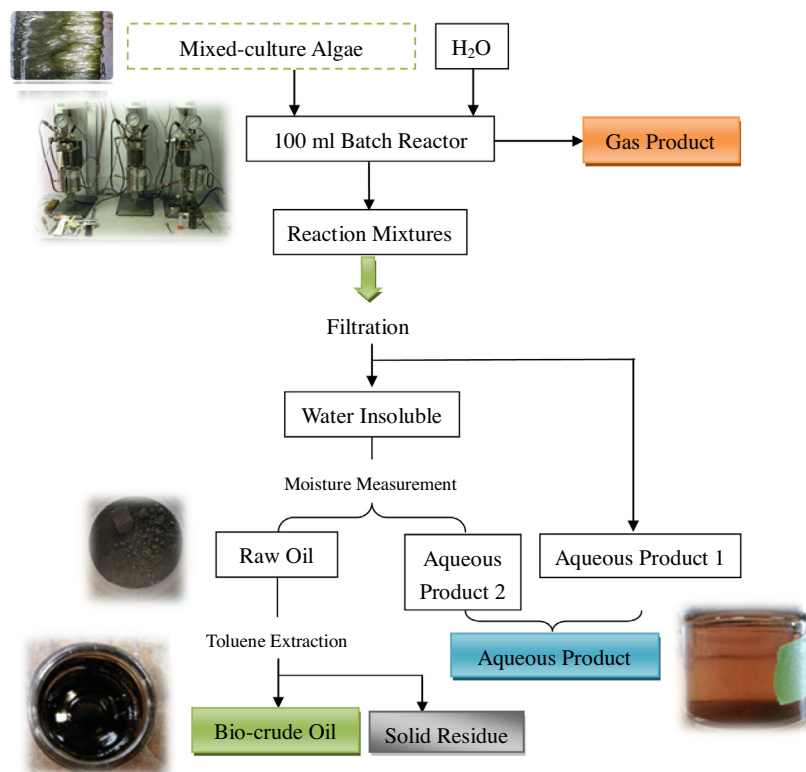
Product	Equation
Bio-crude oil yield (V.M.%)	$= \frac{WI - MWI - SR}{AWVM} \times 100$
Gas yield (V.M.%)	= Based on the ideal gas equation
Solid residue yield (V.M.%)	$= \frac{SR - F \times Ash}{AWVM} \times 100$
Aqueous product yield (V.M.%)	$= \frac{AWVM - G - Solid\ residue - Biocrude\ oil}{AWVM} \times 100$

97.8–99.9% CO<sub>2</sub> and 0.07–2.19% CO. This was similar to the previous studies (Wang, 2011; Yu, 2012).

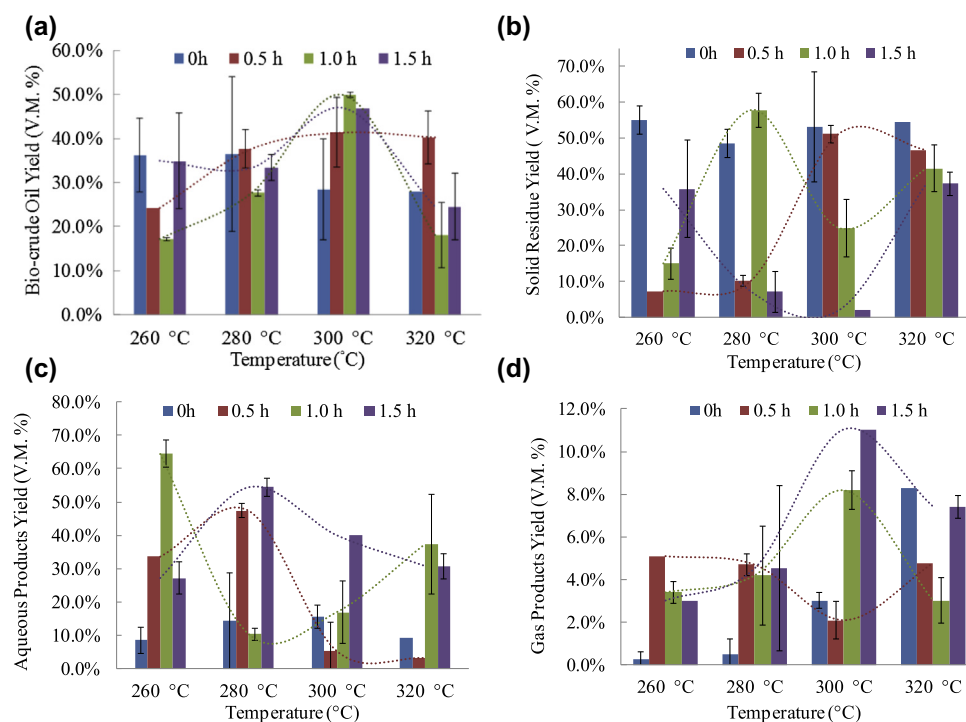
The elemental composition of the bio-crude oils and solid residues were determined with a CE 440 elemental analyzer (Exeter Analytical, Inc., North Chelmsford, MA) with duplicate measurements. Before elemental tests, the bio-crude oils were dried at room temperature in a fume hood, and the solid residues were dried at 105 °C in an oven for 24 h. The composition of oxygen was calculated by the equation O (dry wt%) = 100 – (C + H + N) (wt%). Carbon and nitrogen recoveries for liquefaction products were estimated according to previously described methods (Yu et al., 2011a,b). The higher heating value (HHV) of bio-crude oils

was calculated by the Dulong formula,  $HHV = 0.3383 \times C + 1.422 \times (H - O/8)$ , where C, H, and O are the carbon, hydrogen, and oxygen mass percentages of the dry material. Energy recovery was defined as the HHV of the bio-crude oils divided by that of AW (Yu et al., 2011a,b).

The chemical composition of bio-crude oils and aqueous products were analyzed using a GC–MS (Agilent Technologies, Santa Clara, CA). A 2 μL sample was injected in a split mode (7:1) into the GC–MS system consisting of an Agilent 6890 (Agilent Inc, Palo Alto, CA.) gas chromatograph, an Agilent 5973 mass selective detector, and an Agilent 7683B autosampler. Gas chromatography was performed on a 15 m ZB-FFAP column with 0.25 mm inner diameter (I.D.) and 0.25 μm film thickness (Phenomenex, Torrance, CA), with an injection temperature of 250 °C, Mass Selective Detector transfer line at 250 °C, and the ion source adjusted to 230 °C. The helium carrier gas was set at a constant flow rate of 1.6 ml/min. The temperature program was 5 min at 50 °C, followed by an oven temperature ramp of 5 °C/min to 250 °C for the final 20 min. The mass spectrometer was operated in positive electron impact mode (EI) at 69.9 eV ionization energy in *m/z* 30–800 scan range. The spectra of all chromatogram peaks were evaluated using the HP Chem Station (Agilent, Palo Alto, CA) and AMDIS



**Fig. 1.** Experimental procedure for HTL process.



**Fig. 2.** Effect of reaction temperature and retention time on yields of liquefaction products: (a) bio-crude oil, (b) solid residue, (c) aqueous phase products, and (d) gas products.

(NIST, Gaithersburg, MD) programs. The spectra of all chromatogram peaks were compared with an electron impact mass spectrum from NIST Mass Spectral Database (NIST08) and W8N08 library (John Wiley & Sons, Inc., Hoboken, NJ). To allow comparison between samples, all data were normalized to internal standards: isopentanoic acid (0.1  $\mu\text{M}$ ) for aqueous extracts and pentadecanoic acid methyl ester (0.5  $\mu\text{M}$ ) for toluene extracts.

Thermogravimetric analysis (TGA) of bio-crude oils and solid residues were performed on a Q50 TGA (TA Instruments, Schaumburg, IL) from 110 °C to 800 °C in 60.0 ml/min  $\text{N}_2$  at 10 °C/min to estimate the boiling point distribution. Bio-crude oils and solid residues were dried naturally in the fume hood for 24 h and then subject to TGA analysis.

### 3. Results and discussion

#### 3.1. HTL products yields

In order to investigate the effect of temperature and retention time on products yields from HTL conversion of AW, temperatures ranging from 260 to 320 °C and retention times ranging from 0 to 1.5 h were applied to the HTL tests. As shown in Fig. 2, the bio-crude oil yields ranged from 17.2% to 49.9% (VM). The highest bio-crude oil yield was realized at 300 °C with 1 h retention time. Compared to other studies (Anastasakis and Ross, 2011; Roberts et al., 2013; Valdez et al., 2012; Vardon et al., 2011; Yu et al., 2011a,b), the highest bio-crude oil yield presented in this work was comparable but slightly higher. The bio-crude oil yields generally increased with reaction temperature. However, when the reaction temperature reached 320 °C, the bio-crude oil yields were similar to or lower than yields at 300 °C. This suggests that the bio-crude oil may be further decomposed into char/gas at higher temperatures. Similarly, increased retention times did not consistently increase bio-crude oil yields.

Fig. 2 indicates that at relatively low temperatures (260 and 280 °C), AW was likely transformed into solid residues or aqueous

products, instead of bio-crude oils. When the temperatures increased beyond 300 °C with retention times of 0 and 0.5 h, there were more bio-crude oils or solid residues rather than aqueous products. This inferred that the aqueous products may tend to be converted into bio-crude oils when temperature increases. This observation was also consistent with a previous study suggesting the formation of bio-crude oil from water-soluble products (Valdez et al., 2012). Since dry AW contains about 25% lignocellulose, bio-crude oils converted from this type of algal feedstock may require higher temperatures than those produced from high-protein or high-lipid algae. Similar results were found in a study transforming various model compounds into bio-crude oil via HTL (Wang, 2011). Liquefaction product distributions also illustrated that the reaction temperature significantly affected the liquefaction product yields.

The gas product yields were least affected by the retention time or reaction temperature in the present work. However, as the temperature exceeded a specific point (300 °C), the gas products greatly increased. One previous work showed that the gas product yields were significantly increased when the reaction temperature increased from 320 to 380 °C (Li et al., 2012). Notably, the gas yields in the current work were lower than several previous studies using pure species algae as HTL feedstock (Anastasakis and Ross, 2011; Yu, 2012). The existence of calcium carbonate in the feedstock may affect the solubility of carbon dioxide. Under HTL, carbon dioxide may partly dissolve in water and react with calcium carbonate to form a calcium bicarbonate solution. GC-MS analysis showed that the aqueous products contain carbon dioxide.

#### 3.2. Analysis of bio-crude oils

##### 3.2.1. Elemental analysis and higher heating values (HHVs)

Fig. 2 reveals that the bio-crude oil yields were maximized with a retention time of 1 h and a reaction temperature of 300 °C. Therefore, bio-crude oils produced at 300 °C or with a 1 h retention time were selected for further characterization to elucidate HTL reaction efficacy. Table 3 summarizes the results of ultimate analysis, high-

er heating values (HHVs) of bio-crude oils and the toluene solubility of raw oils converted from AW. With a 1 h retention time, the carbon, hydrogen, and nitrogen contents as well as the HHV of bio-crude oils first slightly decreased and then increased as the temperature increased from 260 to 320 °C. In contrast, the toluene solubility of raw oils showed the opposite trend. The elemental analysis of bio-crude oils implies that the hydrolysis of proteins may begin before 260 °C; the deamination and decarboxylation of proteins became dominant from 260 to 300 °C; and repolymerization governed beyond 300 °C. Yet, there was a trade-off between HHV and toluene solubility of raw oil, implying that two competitive reactions – producing volatile matter and char – may take place as the reaction temperature increased (Zhang, 2010). At 300 °C, the carbon, hydrogen, and nitrogen contents as well as the HHV of bio-crude oils first increased with retention time and then decreased as the retention time exceeded 0.5 h (Table 3). When the retention time exceeded 1.5 h, these values increased again. On the contrary, the oxygen contents presented a reverse trend; the toluene solubility of raw oils increased with retention time and then leveled off after 1 h. The above results indicate that the biomass may be depolymerized to small compounds initially and then these compounds began to rearrange through condensation, cyclization, and repolymerization to form new compounds (Peterson et al., 2008).

Since the quality of bio-crude oils is significantly affected by its H/C and O/C ratios, the classifications of feedstocks and bio-crude oils gained at various reaction conditions were summarized in a Van Krevelen diagram (Fig. 3). Gasoline, coal, biodiesel, and one

previous study using low-lipid microalgae as HTL feedstock (Speight, 2008; Yu et al., 2011a,b) were also reported for comparison. It seems that the highest temperatures and shorter holding times used in this study were not advantageous in terms of bio-crude oil quality: the corresponding H/C or O/C ratios were located in the undesired area as transportation fuel. The surface response methodology is suggested to determine optimal conditions for HTL conversion of AW feedstock into bio-crude oil. Compared with the original AW biomass, the N/C ratios of bio-crude oils were greatly reduced because of the hydrolysis of proteins during HTL. Nevertheless, the N/C ratios increased with reaction temperatures and retention times. At 320 °C, this change was apparent, indicating repolymerization may progress aggressively at a higher temperature. In contrast to several previous studies using pure algae as HTL feedstock (Valdez et al., 2012; Vardon et al., 2011; Yu et al., 2011a,b), the N/C ratio (about 0.050) of bio-crude oils produced in this study were lower and the O/C (about 0.30) ratios were higher. This may be due to that AW contains less protein and more lignocellulose and ash than axenically-cultured microalgae. In terms of the H/C ratios, the bio-crude oils obtained in this study were comparable to those of biodiesel and gasoline, but the O/C and N/C ratios of these bio-crude oils need to be improved.

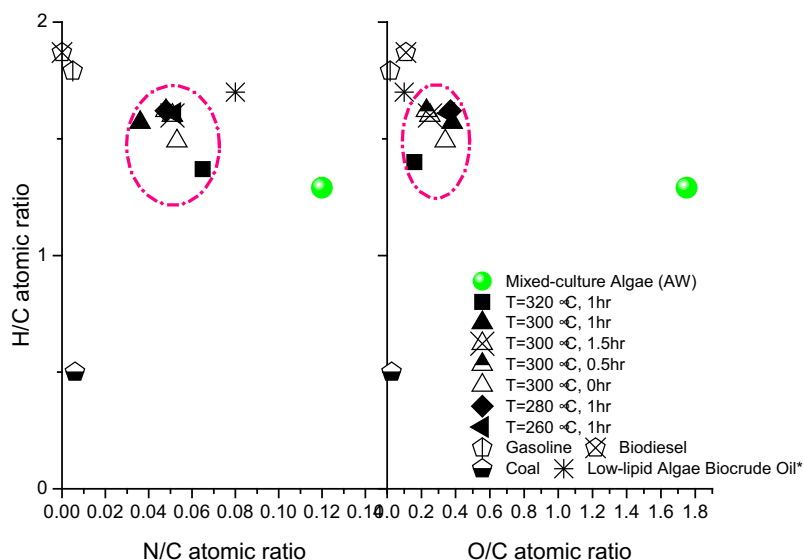
### 3.2.2. GC–MS analysis

Extracted bio-crude oils contain a complex mixture of hydrocarbons. GC–MS analysis can help explore the general chemical properties. In order to establish the major reaction pathways for different biomolecules under hydrothermal conditions,

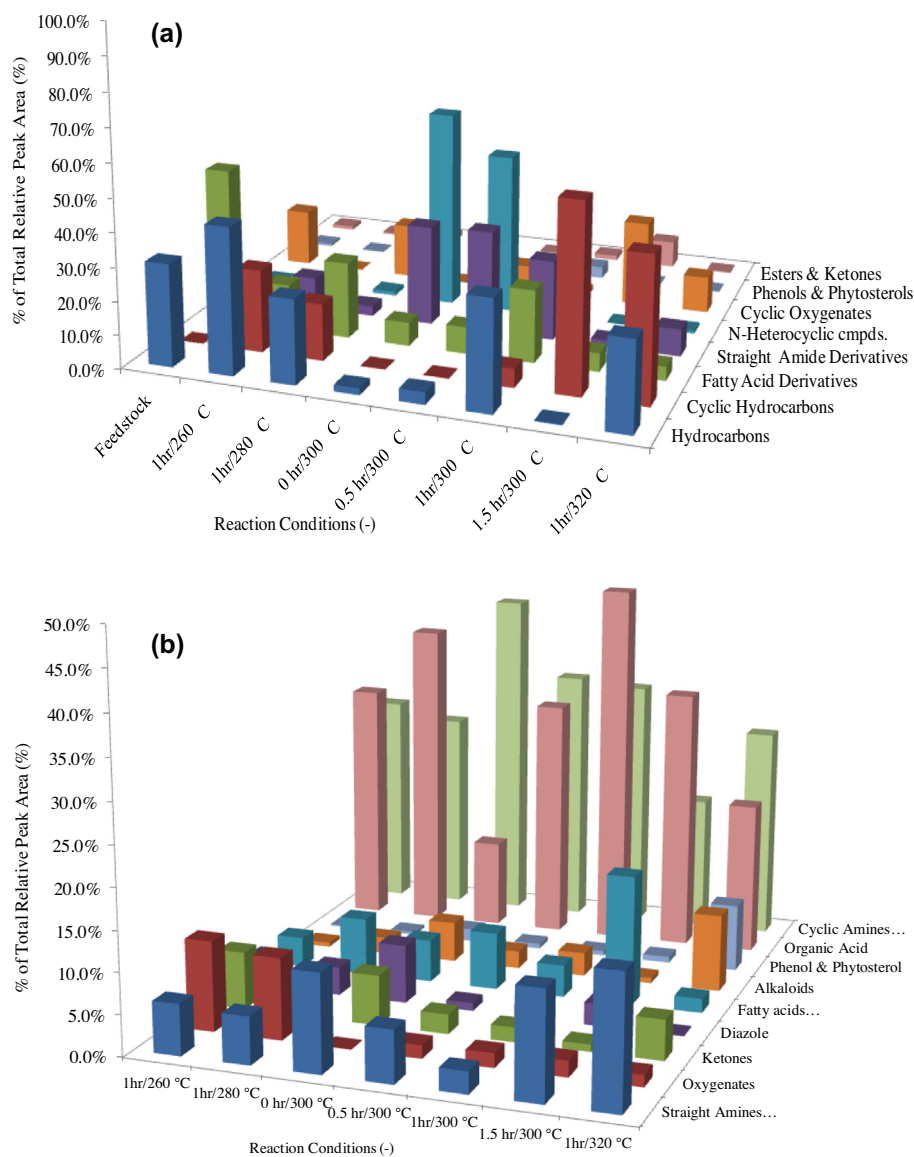
**Table 3**  
Ultimate analysis and higher heating value (HHV) of the bio-crude oils; the toluene solubility of raw oils.

Component (d.w.%) <sup>a</sup>	1 h Retention time				Temperature at 300 °C			
	260 °C	280 °C	300 °C	320 °C	0 h	0.5 h	1 h	1.5 h
C	59.8 ± 0.5	59.1 ± 0.4	59.4 ± 0.05	71.4 ± 0.3	61.0	66.7 ± 0.8	59.4 ± 0.05	65.7 ± 0.02
H	8.03 ± 0.04	7.98 ± 0.02	7.79 ± 0.02	8.36 ± 0.03	7.58	8.98 ± 0.09	7.79 ± 0.02	8.76 ± 0.03
N	3.62 ± 0.09	3.34 ± 0.02	2.50 ± 0.1	4.92 ± 0.07	3.76	3.71 ± 0.08	2.50 ± 0.1	3.93 ± 0.03
O	28.5	29.6	30.3	15.4	27.6	20.7	30.3	21.7
Heating value (MJ/kg)	26.6	26.1	25.8	33.3	26.5	31.2	25.8	30.0
Toluene solubility	14.4	25.4	35.5	10.3	14.3	28.5	35.5	34.1

<sup>a</sup> Dry weight basis.



**Fig. 3.** Van Krevelen diagram of bio-crude oils gained at various temperatures with 1 h retention time and at 300 °C with different retention time (\*adopted from one previous study (Yu et al., 2011a,b)).



**Fig. 4.** Effect of reaction temperature and retention time on the composition of (a) bio-crude oils and (b) aqueous products.

components characterized by GC–MS were classified into several groups such as hydrocarbons and (N–/O–/S–) heterocyclic compounds. Compounds containing more than one functional group were classified into only one category. For example, methane, isocyanato—, consisting of both hydrocarbon and C=N functional group, was classified as straight amide derivatives based on its chemical property. For each bio-crude oil sample, about 92% of the identified components were categorized according to the above criteria. GC–MS spectra and the major components (>1% relative total peak area) of bio-crude oils were also listed in the [Supplementary data](#).

**Fig. 4a** summarizes the major chemical groups in the bio-crude oils for various reaction conditions. AW was extracted with toluene for comparison. Initially, there were no cyclic hydrocarbons and amine derivatives in the feedstock, inferring the decomposition of proteins and the formation of cyclic hydrocarbons may cross over a high energy barrier. As the reaction approached 260 °C, more hydrocarbon derivatives were produced via the decarboxylation of fatty acids while the cyclic oxygenates were greatly decreased. This reveals that the hydrolysis of lignocellulosic materials occurred before 260 °C (Liu et al., 2013). When

temperature increased, the corresponding products such as furan derivatives may be decomposed due to high temperature or long residence time. Additionally, the change of pH may affect the products derived from lignocelluloses (Zhang, 2010). Similar changes were found in previous work converting microalgae into bio-crude oil (Yu, 2012). Yet, as the temperature increased from 260 to 320 °C, cyclic hydrocarbons, amine derivatives, and cyclic oxygenate derivatives were significantly enhanced, showing that recombination and repolymerization may become more intense under hydrothermal conditions, partly because water molecules can also participate in chemical reactions (Peterson et al., 2008). Notably, the decomposition for various amino acids may take place at different temperatures. For instance, L-Methionine decomposes at around 289 °C while L-Proline degrades at about 231 °C (Olafsson and Bryan, 1970). Hence, it was difficult to fine tune the production of amine derivatives in this work. Further characterization such as matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) of feedstock may help address this concern.

With 0 and 0.5 h retention times, there were few hydrocarbons and cyclic hydrocarbons, but the two groups drastically increased

**Table 4**  
Boiling point distribution of bio-crude oils (%).

Distillate range (°C)	Coke oil typical application <sup>a</sup>	Feedstock	1 h Retention time				Temperature at 300 °C			
			260 °C	280 °C	300 °C	320 °C	0 h	0.5 h	1 h	1.5 h
25–110	Bottle gas and chemicals	4.92	1.18	1.58	0.79	3.60	1.62	1.18	0.79	3.22
110–200	Gasoline	1.27	3.83	8.71	5.13	18.2	7.90	4.62	5.13	19.3
200–300	Jet fuel, fuel for stoves, and diesel oil	15.8	23.0	23.8	26.3	23.3	32.1	24.0	26.3	29.9
300–400	Lubricating oil for engines, fuel for ships, and machines	14.9	20.1	13.5	19.0	12.3	18.1	16.9	19.0	15.6
400–550	Lubricants and candles, fuel for ships	8.32	22.9	20.7	19.2	10.5	18.4	22.5	19.2	11.78
550–700	Fuel for ships, factories, and central heating	9.40	1.45	2.11	1.91	0.74	1.00	2.02	1.91	2.92
700–800	Asphalt and roofing	3.22	0.74	1.32	1.03	0.53	0.71	1.05	1.03	1.98
>800	Residues	42.1	26.8	28.3	26.7	30.9	20.2	27.8	26.7	15.4

<sup>a</sup> Adopted from *Handbook of Petroleum Product Analysis* (Speight and Speight, 2002).

when holding time lasted for 1 and 1.5 h, respectively. Typically, decarboxylation of fatty acids does not readily proceed. On the contrary, amine derivatives, and N-containing heterocyclic compounds were abundant at the very beginning and then reduced when reaction held for more than 0.5 h. Amine groups may be used to form cyclic amine/amides, which would subsequently distribute to the aqueous products. Similarly, 1H-Pyrrole-3-carbonitrile may serve as an intermediate for other energetically-favorable reactions and, therefore, disappeared as retention time increased. The —CN bond on this compound is very active and could lead to further chemical reaction such as ring-opening (Kvaskoff et al., 2010). Besides, these heterocyclic compounds may be degraded into solid residues as retention time increases (Peterson et al., 2008; Zhang et al., 2013).

### 3.2.3. TG analysis

The boiling point distribution of bio-crude oils were evaluated using thermal gravimetric analysis (TGA), which can be viewed as a miniature “distillation”. Table 4 showed that the weight loss of bio-crude oils before 110 °C was about 2%, revealing the drying process efficiently removed the toluene. As the reaction temperature increased, bio-crude oils distilled in the range of 110–200 °C also increased, while the fraction distilled between 300 and 550 °C decreased, and the distillate fraction between 200 and 300 °C remained unchanged. The bio-crude oils generated at 320 °C displayed an obvious weight loss peak at around 170 °C (Fig. S.3), which was the lowest temperature compared to other peaks. However, the bio-crude oil yield obtained at 320 °C was relatively low, suggesting that there was a trade-off between bio-crude oil quality and bio-crude oil yield.

With retention times of 1.5 h, about 20% of the bio-crude oil was distilled in the range of 110–200 °C, which reveals that increasing retention times may lead the bio-crude oil to undergo further cracking, forming smaller molecular compounds. On the contrary, the bio-crude oil produced with 0 h retention time seems to show a preferred boiling distribution but the bio-crude oil yield was very low at this condition. This indicates that bio-crude oils have already begun to form even before the temperature reached the set point. This may be due to the warm-up phase of the reactors. In contrast to the bio-crude oils converted from pure algae, the bio-crude oils obtained in this study contained more distillate fraction between 110 and 300 °C and were comparable for the distillate fraction between 300 and 550 °C (Anastasakis and Ross, 2011; Vardon et al., 2011; Yu, 2012). Compared to Illinois shale oil, the AW-based bio-crude oils had less distillate material between 110–200 °C and 300–400 °C (Vardon et al., 2011).

### 3.3. Analysis of the solid residue

Table 5 demonstrated that the carbon, hydrogen, and nitrogen contents of the solid residue were all decreased at 1 h and then

increased with longer retention time. This indicates that the decomposition and repolymerization processes may occur simultaneously as the retention time increased. It was also found that about 12%–18% of the carbon remained in the solid residue. Meanwhile, TG analysis reveals that the solid residues (Table 6) could be potentially used as fuels for central heating or asphalt. Table 6 showed that the solid residues contained about 26%–32% of components with the boiling point range of 550–800 °C, suggesting further treatments such as cracking are beneficial. The TG analysis of solid residues also indicates there would be more cracking with longer retention times. With 1.5 h retention times, the solid residue consisted of about 40% of total distillates.

### 3.4. Analysis of aqueous phase products

The pH values of the aqueous products were measured right after HTL tests (Supplementary data). The pH of the aqueous products remained almost constant (7.39–7.95) as the reaction temperature increased while it increased from 7.39 to 8.60 as the retention time held for more than 1 h. Fig. 4b illustrated the major groups of organic aqueous products generated at various reaction conditions. GC–MS spectra and the major components (>1% relative total peak area) of the aqueous products are also listed in Supplementary data. Similar to bio-crude oils, aqueous products were grouped according to the chemical properties of different compounds. The major groups appearing in the aqueous phase were organic acids (excluding fatty acids but including amino acids) and cyclic amine derivatives. It is generally preferable to have nitrogenous organics in the aqueous products rather than the bio-crude oil because less acid and nitrogen compounds results in less upgrading work for transportation fuels. It was observed that carbon dioxide partly dissolved in water but remained primarily in the gas phase as reaction temperature increased from 280 to 320 °C.

As the reaction temperature increased from 260 to 320 °C, organic acids, and cyclic amine derivatives first increased and then decreased while amine derivatives and ketones showed the opposite trend. In contrast, cyclic hydrocarbons and fatty acid derivatives remained almost constant regardless of the temperature change, inferring that these two groups tended to partition to the oil phase. The increase in organic acids may be brought about by the decomposition of various amino acids. Several amino acids such as prolines and leucines were observed in the aqueous products. Ketones may transform between alcohols and acids and, thus, they were less stable under hydrothermal conditions. Besides, ketones may be consumed for the formation of cyclic amine derivatives. The substantial increase of amine derivatives appeared at 320 °C, which can be explained mainly by formation of urea. Under high pressure and temperature, urea can be synthesized from ammonia and carbon dioxide (Krase and Gaddy, 1922). Alkaloids and phenol derivatives also increased when the reaction temperature reached 320 °C. This may be that the decomposition of

**Table 5**  
Ultimate analysis of the solid residue.

Component (d.w.%) <sup>a</sup>	1 h Retention time				Temperature at 300 °C			
	260 °C	280 °C	300 °C	320 °C	0 h	0.5 h	1 h	1.5h
C	18.2 ± 0.9	15.2 ± 0.2	15.9 ± 0.2	12.0 ± 0.3	15.6 ± 0.2	17.3 ± 0.6	15.9 ± 0.2	18.4 ± 0.1
H	0.94 ± 0.1	0.51 ± 0.02	0.58 ± 0.01	0.21 ± 0.05	0.83 ± 0.04	0.6 ± 0.1	0.58 ± 0.01	1.10 ± 0.03
N	1.20 ± 0.2	0.76 ± 0.02	0.74 ± 0.02	0.20 ± 0.02	0.58 ± 0.02	0.86 ± 0.1	0.74 ± 0.01	0.78 ± 0.06
Ash & O <sup>b</sup>	79.7	83.5	82.8	87.6	83.0	81.2	82.8	79.7

<sup>a</sup> Dry weight basis.<sup>b</sup> Calculated by difference.**Table 6**  
Boiling point distribution of solid residue (%).

Distillate range (°C)	Coke oil typical application <sup>a</sup>	Feedstock	1 h Retention time				Temperature at 300 °C			
			260 °C	280 °C	300 °C	320 °C	0 h	0.5 h	1 h	1.5 h
25–100	Bottle gas and chemicals	4.92	1.07	0.57	0.82	0.62	0.13	0.32	0.82	0.32
100–200	Gasoline	1.27	0.30	0.15	0.19	0.16	0.22	0.11	0.19	0.27
200–300	Jet fuel, fuel for stoves, and diesel oil	15.9	1.50	0.75	1.02	0.39	0.83	0.36	1.02	1.13
300–400	Lubricating oil for engines, fuel for ships, and machines	14.9	4.32	2.00	2.30	1.07	2.26	0.85	2.30	2.55
400–550	Lubricants and candles, fuel for ships	8.32	4.63	2.63	4.73	2.24	3.35	1.83	4.73	5.56
550–700	Fuel for ships, factories, and central heating	9.40	12.3	11.5	16.1	8.69	9.50	7.01	16.1	8.41
700–800	Asphalt and roofing	3.22	14.0	21.6	10.1	22.2	21.0	23.2	10.1	21.6
>800	Residues	42.1	61.9	60.8	64.8	64.8	62.7	66.4	64.8	60.2

<sup>a</sup> Adopted from *Handbook of Petroleum Product Analysis* (Speight and Speight, 2002).

cellulose crystallinity takes place at about 320 °C and the degradation of lignin structure is also favored by temperatures around 280–300 °C (Peterson et al., 2008).

Surprisingly, the fatty acid derivatives noticeably increased when retention time increased while organic acids presented an opposite trend. In the aqueous product obtained with 1.5 h at 300 °C, octadecanoic acids were greatly increased and acetic acids were diminished. Notably, glycerol was also reduced at this condition. The above observation suggests that fatty acids and lipids may be produced with the increasing retention times. One study on hydrothermal vents (McCullom et al., 1999) noted that, Fischer–Tropsch type (FTT) reaction can occur under hydrothermal conditions, with water or other short carbon chain organic acids acting as hydrogen sources. CO can then apparently react with H<sub>2</sub> to produce lipids (such as *n*-alkanols and *n*-alkanoic acids), *n*-alkenes, *n*-alkanes, and alkanones under milder temperatures (150–250 °C) in a stainless steel reactor (Peterson et al., 2008; Yu et al., 2011a,b). Previous work also showed that lipid compounds can be synthesized through FTT-type reactions from oxalate acids or formic acids at temperatures as low as 175 °C (Peterson et al., 2008). In this study, oxalate acids were also found in the aqueous product at 260 °C with 1 h.

It was previously shown that cyclic amine derivatives were not stable under hydrothermal conditions because the formation of their reactants, such as hydroxy acids and lactams, was strongly dependent on the applied conditions (Decker et al., 2004). Also, competition may exist among the formation of cyclic amines and straight amine derivatives when retention times exceeded 1 h. With 1.5 h at 300 °C, the growth of straight amine groups consisted of the formation of hydrazines, amino diols, and tertiary amides. Hydrazines can be synthesized from ammonia and peroxide while tertiary amides can be produced by the dehydration of primary amides (Maxwell, 2004). Overall, the generation of these three straight amine derivatives may compete with cyclization for the same reactants and, thus, bring about the reduction of cyclic amine groups. Ketones and diazoles may serve as intermediates for other reactions such as ring-opening since they were less stable during HTL (Kvaskoff et al., 2010).

The analysis of the aqueous phase demonstrates that recycling and re-use of these aqueous products is warranted. For instance,

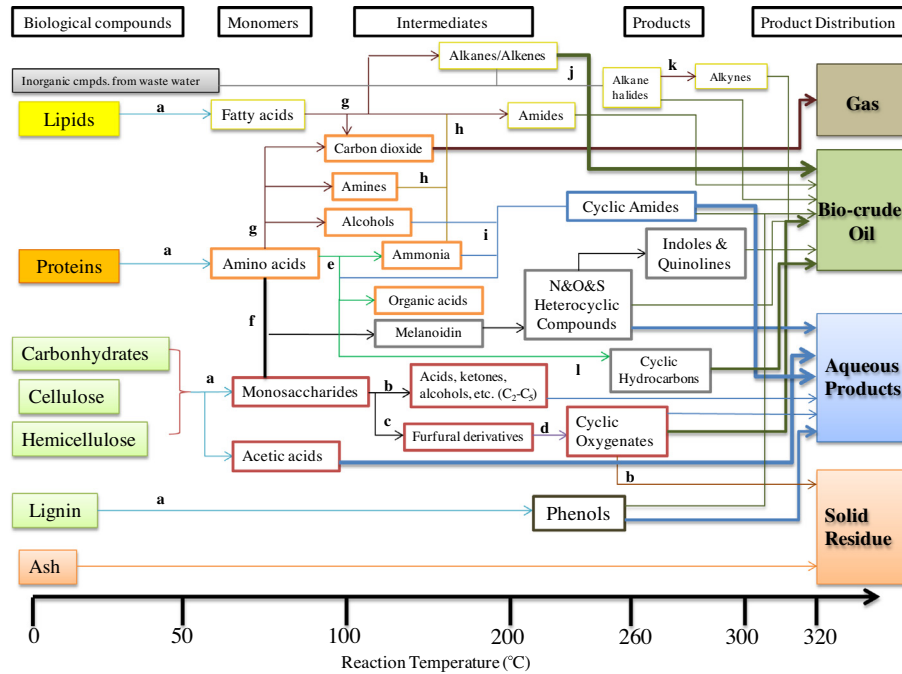
there was a relative abundant amount of straight amide derivatives such as urea and hydrazine. Urea is known to be a good culture media for algae and microorganisms while hydrazine can be used as rocket fuel.

### 3.5. Potential reaction pathways for HTL of AW

Based on the GC–MS results and possible reaction pathways described in the literature (Peterson et al., 2008; Wang, 2011; Yu, 2012; Zhang et al., 2013), a potential HTL reaction scheme for AW biomass was proposed in Fig. 5. The thickness of arrows represent the relative amount of products distributed to different phases. Initially, biological compounds including lipids, proteins, and fibers were hydrolyzed into their corresponding monomers such as fatty acids, amino acids, and glucose at lower temperatures. As the reaction temperatures increased, these monomers were decomposed. Nitrogen and oxygen were removed from the carboxyl and amine groups via decarboxylation and deamination, respectively. Meanwhile, the Maillard reactions took place, combining amino acids with reducing sugars to produce Melanoidin. Aminolysis can occur when the temperatures were higher than 220 °C (Roe et al., 1952; Wang, 2011). Carboxylic acid derivatives from fatty acids or amino acids can react with amines or ammonia to form amides. However, few amides were measured in this work, indicating there may be competition between hydrolytic and ammonolytic reactions (Roe et al., 1952). Cyclization involving alcohols, ammonia, and amino acids can occur and cyclic amine derivatives can be generated under hydrothermal conditions of temperature >250 °C (Decker et al., 2004). Simultaneously, condensation and halogenation can take place. Alkane halides and cyclic hydrocarbons such as cycloheptatrienes were formed, respectively (Foote et al., 2011). Dehydrohalogenation of alkane halides subsequently occurred at elevated reaction temperatures and alkynes were, thus, generated at about 300 °C.

The discussion above suggests that operation of HTL with AW biomass is most advantageous in a range of 300–320 °C for 0.5–1 h. The bio-crude oils obtained with higher temperatures had lower boiling points, indicating less upgrading work would be required for transportation fuels. Meanwhile, the nitrogen-containing compounds can be further degraded into valuable





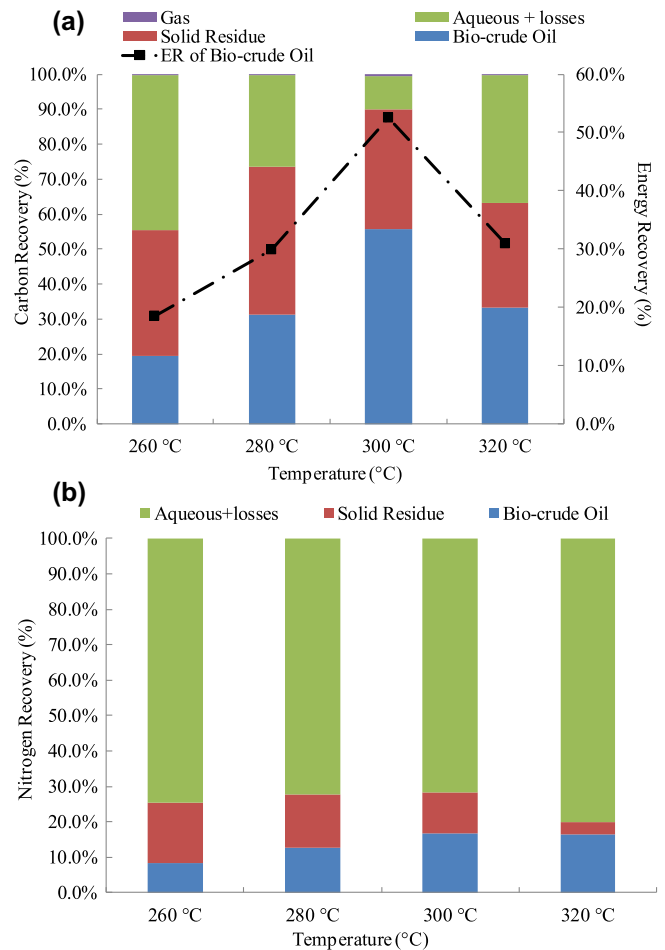
**Fig. 5.** Potential reaction pathways for HTL of AW: (a) hydrolysis; (b) decomposition; (c) dehydration; (d) polymerization; (e) deamination; (f) Maillard reaction; (g) decarboxylation; (h) Aminolysis; (i) cyclization; (j) halogenations; (k) dehydrohalogenation; (l) condensation + pyrolysis.

chemicals such as urea and distributed to the aqueous stream for recycling to support additional algal or microbial biomass production.

### 3.6. Nutrients recovery of AW

Since reaction temperature played a more influential role on liquefaction product yields, elemental nutrient recoveries were reported for a range of temperatures and 1 h of retention time (Fig. 6). Regardless of the temperature, the largest portion of nitrogen recovery (NR) occurred in the aqueous products. This indicates that **deamination**, bringing about the nitrogen recovered by organic compounds with the presence of organic acids and amino acids, may take place under hydrothermal conditions (Dote et al., 1998). Meanwhile, some amino acids may undergo degradation at about 300 °C or even higher temperatures (Sato et al., 2004). Therefore, the nitrogen content of the bio-crude oil at relatively high temperature may be slightly decreased. The present study demonstrated relatively low NR in the bio-crude oils (8.41%–16.8%) in comparison to other previous studies using microalgae or axenically-cultured algae as an HTL feedstock, which typically resulted in a NR for bio-crude oils between 25% and 53% (Anastasakis and Ross, 2011; Roberts et al., 2013; Valdez et al., 2012; Yu et al., 2011a,b).

The lower NR achieved in this study may potentially be explained by a relatively high content of calcium carbonate (16.5%) in the dry AW biomass, which may help capture nitrogen-containing compounds. A test was conducted to examine the effect of calcium carbonate on decreasing the NR of bio-crude oil. The amount of calcium carbonate of AW was reduced from 16.5% (d.w.) to 11.0% (d.w.) by screening out larger **flakes** of snail shell material that was present in the AW feedstock and consists of mostly CaCO<sub>3</sub>. An HTL test was operated at 300 °C with 1 h retention time with the reduced calcium feedstock. By removing 5.5% of the calcium carbonate from the feedstock, the nitrogen content of bio-crude oil increased from 2.77% to 5.08% (d.w.), **and the NR increased from 16.8% to 34.3% (d.w.)**, while the bio-crude oil yields remained constant. The calcium carbonate concentration in the HTL feedstock



**Fig. 6.** Effects of reaction temperature on (a) energy recovery of bio-crude oils along with carbon recovery and (b) nitrogen recovery, distributed in various liquefaction products with 1 h retention time.

was inversely correlated with nitrogen contents in HTL bio-crude oil products. **Previous research has shown that calcium carbonate can adsorb and co-precipitate proteins** (Sukhorukov et al., 2004). However, how calcium carbonates interact with nitrogen-containing compounds under HTL requires additional research. Nonetheless, the data presented in this study suggests that the bio-crude oils obtained with AW biomass may be easier to upgrade than other algal feedstock. Thus, it does appear advantageous to exploit AW as an HTL feedstock for bio-crude oil production.

From the perspective of carbon recovery of liquefaction products, it was found that it mostly occurred in the bio-crude oil at 300 °C and in the aqueous products at 260 °C. Besides, about 30.0%–42.3% of carbon recovery was distributed to solid residues, inferring that searching for an economical approach, such as the concept of E<sup>2</sup>-Energy, to extract and recover carbon (and nutrients) from the solid residues or aqueous products is necessary. The highest energy recovery of bio-crude oil was 52.5% (300 °C), which was comparable to three previous studies using pure algae as HTL feedstock (Li et al., 2012; Vardon et al., 2011; Yu et al., 2011a,b).

#### 4. Conclusion

This study demonstrated a high feasibility of using AW biomass as an HTL feedstock. The bio-crude oil yields and energy recovery were comparable to those converted from pure algae. The nitrogen recovery (NR) distributed to the bio-crude oils in the present work (8.41%–16.8%) was significantly lower than previous works (25%–53%). TGA results showed that the bio-crude oils could be used for jet fuel or lubricant oil while the solid residue may be used as asphalt. The TGA analysis also implied that possible target products should include both heavy crude products but also light oils.

#### Acknowledgments

The first author appreciates the financial support from Ministry of Education of Republic of China (Taiwan) and ERM (Environmental Resource Management) foundation. The authors are sincerely grateful for the Inventor Endowment for providing the experimental supplies for the research. Acknowledgment also goes to Mr. Gen-Shen Chen and Ms. Mei-Hsiu Lai for further assistance. The authors greatly appreciate Dr. Alexander Ulanov of the Roy J. Carver Biotechnology Center (Urbana, IL), Ms. Marie Keel and Mr. Rudiger Lauffhutte in the Microanalysis Laboratory (Urbana, IL) for their help on GC–MS and elemental analysis, respectively. Sincere appreciation also goes to Chih-Ting Kuo, Peng Li, and Yan Zhou for their help.

#### 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.2013.10.111>.

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