Fuel 238 (2019) 240–247

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Full Length Article

# Comparative studies on liquefaction of low-lipid microalgae into bio-crude oil using varying reaction media

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ARTICLE INFO

*Keywords:*
Liquefaction
Low-lipid microalgae
Reaction medium
Bio-crude oil

ABSTRACT

This study aimed to determine the most effective reaction medium for producing bio-crude oil from low-lipid microalgae via liquefaction treatment. To this end, the screening tests were carried out at 275 °C for 60 min by employing varying reaction media including water, water with four acid catalysts (formic acid, acetic acid, sulfuric acid, and hydrochloride acid), and four different organic solvents (methanol, ethanol, ethyl acetate, and acetone) without acid catalyst. In view of the bio-crude oil yield, methanol as the most effective reaction medium was choses for the further investigation on the effects of residence time, biomass/solvent mass ratio, and reaction temperature on the products distribution. The results showed that liquefaction at 225 °C for 60 min with a 1:5 biomass/solvent mass ratio led to the highest bio-crude oil yield of 85.5 wt% and a higher heating value (HHV) of 30.6 MJ/kg. Finally, a series of analytical approaches (elemental, GPC, TGA, GC–MS, and FT-IR analysis) were conducted for characterizing bio-crude oil.

## 1. Introduction

Recently, microalgae have attracted much attention as a sustainable source for the 3rd generation bio-fuel production. Compared to lignocellulosic biomass, microalgae have unique advantages such as high lipid content, non-arable land use, and substantial environmental benefits through the capture of atmospheric $CO_2$ [1,2]. The commonly used conversion technique (i.e., transesterification) for microalgae requires high-lipid strains; however, these strains tend to have lower biomass productivities than those of low-lipid microalgae [3]. In order to obtain high lipid yield, a stressed condition, like nitrogen depletion, is a necessity for microalgae cultivation, which could not only lower biomass productivity and net energy yield but also make this process very susceptible to contamination [4]. Therefore, it would be beneficial to use high-productivity low-lipid microalgae as the feedstock for producing bio-crude oil. Another problem for transesterification is that only lipid fraction of microalgae can be converted, and thus protein and carbohydrates fraction will be ended up as waste. According to previous studies, the whole microalgae biomass including lipid, protein, and carbohydrates can contribute to the oil formation by thermochemical conversion technologies [5,6].

Pyrolysis and hydrothermal liquefaction are regarded as two major thermochemical conversion techniques for microalgae [7]. As a comparison, hydrothermal liquefaction (HTL) is a more suitable approach for wet biomass due to the non-requirement for feedstock drying/dewatering step [8]. In addition, the bio-crude oils obtained from pyrolysis often contain higher oxygen contents than those obtained from HTL process, which could negatively affect the energy density of oil products [9]. Nevertheless, there are several challenges of microalgal HTL still ahead, such as harsh reaction conditions, relatively lower oil yield, and poor bio-crude oil quality [10].

To address the above challenges, numerous catalysts have been applied in the HTL treatment [11,13–15]. The advantages of using catalyst for microalgal liquefaction may be summarized as follows: (i) promotes decomposition of biomass macromolecules into smaller molecules; and (ii) improves bio-crude oil properties [9]. Based on the previous literature, effect of catalyst on the quantity and quality of bio-crude oils is not only dependent on the type and dosage of the catalyst but also on the feedstock characteristics [5,11]. Biller and Ross [5] found that the use of sodium carbonate as a catalyst negatively affected bio-crude oil production obtained from low-lipid strain (*Porphyridium cruentum* and *Spirulina*). In contrast, Ross et al. [12] reported that the acid catalysts demonstrated positive effects on the yield and quality of bio-crude oil produced from HTL of *Chlorella vulgaris* (a low-lipid strain). As indicated by Yang et al. [1], the addition of organic/inorganic acid exhibited different catalytic performances through liquefaction treatment. In this present study, in consideration of low lipid content in the feedstock, four different acid catalysts including organic

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https://doi.org/10.1016/j.fuel.2018.10.124
Received 10 August 2018; Received in revised form 18 October 2018; Accepted 24 October 2018
Available online 29 October 2018
0016-2361/ 

and inorganic acids rather than base catalysts were adopted.

Apart from catalysts, various organic solvents (e.g., methanol, ethanol, and acetone) have been used as the reaction medium in the liquefaction process [16–18]. In literature, pure water is normally employed as the reaction medium in the microalgal liquefaction owing to its inherent benefits at higher temperatures and pressures, such as low dielectric constant and high ionic product [19]. However, Guo et al. [4] performed the mass balance analysis for the liquefaction in water, and the results showed that more than one-third of carbon in the feedstock were preferentially transferred into water phase rather than oily phase. Another advantage of using organic solvent as the reaction medium is that a higher bio-crude oil yield can be obtained at moderate reaction conditions [10]. The type of solvent, particularly its polarity, plays a significant role in algal liquefaction with respect to the products distribution and properties of bio-crude oils [10,17]. Furthermore, a variety of reaction conditions such as residence time, biomass/solvent mass ratio, and reaction temperature have significant effects on the microalgal liquefaction [18].

To the best of our knowledge, no systematic research has performed to identify the most effective reaction medium for converting low-lipid microalgae into bio-crude oil. Therefore, in this present study, nine different reaction media including water, water with four different acid catalysts (formic acid, acetic acid, sulfuric acid, and hydrochloride acid), and varying organic solvents (methanol, ethanol, ethyl acetate, and acetone) were investigated for the liquefaction of a low-lipid strain. The most effective reaction medium determined was then selected to explore the effects of residence time, biomass/solvent mass ratio, and reaction temperature on the products distribution. Furthermore, physical and chemical properties of bio-crude oil were characterized.

## 2. Materials and methods

### 2.1. Materials

*Chlorella* was purchased from PureBulk, Inc (Roseburg, USA) as food-grade powder. The microalgae sample had a moisture and ash content of 3.48 wt% and 7.15 wt%, respectively. The ultimate analysis on a dry basis was as follows: 46.54% C, 7.37% H, 8.59% N, 0.48% S, and 29.86% O (calculated by difference). The higher heating value of raw material was 20.97 MJ/kg. The biochemical composition of microalgae were lipid, protein, and carbohydrates with 6.10 wt%, 53.66 wt%, and 33.09 wt%, respectively. All chemicals used in this study were purchased from Caledon Laboratories Ltd (Georgetown, Canada), and used as received.

### 2.2. Liquefaction experiments

Liquefaction experiments were carried out using a 100 mL Micro-Bench top reactor equipped with a magnetic stirrer (Parr 4590, Illinois, USA). Throughout the liquefaction processes, temperature and pressure were monitored by a Type J thermocouple and Alloy 400 Pressure Gaga, respectively. Briefly, around 5.0 g of dried microalgal biomass and 25.0 g of water were introduced into the reactor without catalyst. For the catalytic HTL runs, the reactor was charged with 5.0 g of dried microalgal biomass and 25.0 g of water containing 1.5 g of acid catalyst (5 wt% of the total slurry). The tested acid catalysts in this work were formic acid, acetic acid, sulfuric acid, and hydrochloride acid. In the case of liquefaction experiments in organic solvent, 5.0 g of dried microalgal biomass and 25.0 g of a specific organic solvent (methanol, ethanol, ethyl acetate or acetone) were loaded into the reactor without catalyst. Afterwards, the reactor was sealed and flushed with pure nitrogen to displace the residual air inside the reactor. The reactor was then heated to the desired reaction temperature at a heating rate of approx. 5 °C/min. After that, this temperature was held for a pre-set residence time. The liquefaction experiments were carried out under varying reaction temperatures (175 °C, 225 °C, 275 °C, and 300 °C), residence times (30 min, 60 min, 90 min, and 120 min), and biomass/solvent mass ratios (1/2.5, 1/5, and 1/10).

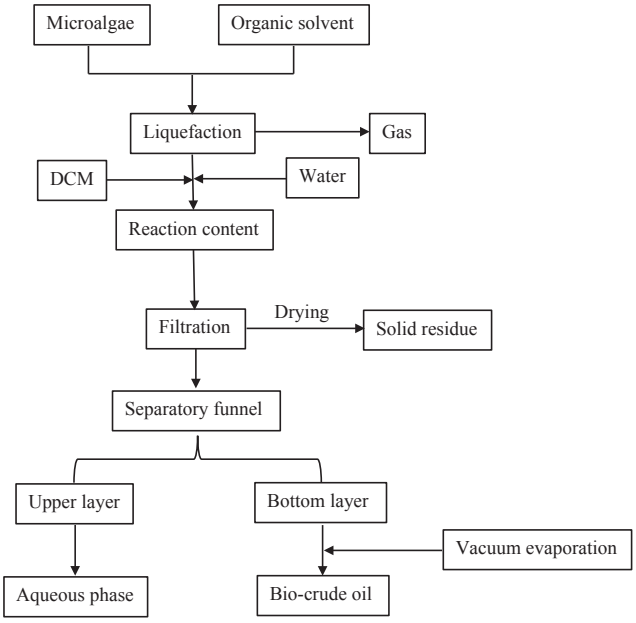


**Fig. 1.** The procedure of product separation and bio-crude oil recovery used for the liquefaction in organic solvent.

### 2.3. Product separation

The procedure used for the HTL in water and the catalytic HTL was fully described in our previous report [20]. Besides, the procedure to separate different product fractions for the liquefaction in organic solvent is shown in Fig. 1. After the reactor was cooled to room temperature, the gases were released into the fume hood. It shall be noted that the gas formation, mainly $CO_2$, was found to be negligible due to the low liquefaction temperatures used in this work, thus the gas yield was not quantified. After that, the reaction mixture was transferred into a 250 mL beaker. The reactor and stirrer were further washed using dichloromethane (DCM) and water, followed by filtration through a pre-weighed filter paper. The filter paper was then oven-dried at 105 °C for 24 h and weighed to determine the solid residue yield. Afterwards, the reaction mixture was transferred into a separatory funnel, and allow it to stand for at least 30 min to form two sperate layers. The bottom layer was transferred into a 500 mL pre-weighed round-bottom flask and evaporated at 45 °C under reduced pressure. The remaining dark material in the flask was weighed and denoted as bio-crude oil. The yields of bio-crude oil and solid residue were determined in wt%, in relation to dried mass of the feedstock, and the balance was calculated as the yield of other products (gases + aqueous phase).

### 2.4. Analysis

#### 2.4.1. Feedstock

The moisture content was determined by drying the sample in an oven at 105 °C for 24 h. The ash content was measured by ashing the dried microalgal biomass in a muffle furnace at 575 °C in air for 3 h until reaching a constant weight. The elemental compositions (C, H, N, and S) were analyzed using a CHNS [Cl] Elementar vario EL, while the O content was estimated by difference on dry basis (% O = 100% − %Ash − %C − %H − %N − %S). The higher heating value (HHV) was calculated by the Dulong equation [HHV (MJ/kg) = 0.338C + 1.428(H-O/8) + 0.095S]. The lipid content was determined by the Bligh & Dyer method [21]. The protein content was estimated by %N × 6.25 [22]. The carbohydrates content was

calculated by difference on dry basis (%Carbohydrates = 100% − %Ash − %Lipid − %Protein).

#### 2.4.2. Bio-crude oil

The elemental compositions and HHV were analyzed using the same method as earlier described for feedstock. The average molecular weight ($M_n$: number average; $M_w$: weight average) and polydispersity index (PDI = $M_w/M_n$) were determined using a Waters Breeze GPC-UV instrument, coupled with a Waters Styragel HR1 column. The boiling point distribution was estimated by a Pris TGA instrument (Massachusetts, USA), using a similar temperature program, as previously described by Ross et al. [12]. The key chemical components were determined by an Agilent 7890–5975 GC–MS equipped with a HP-5MS nonpolar capillary column (30 m × 0.25 mm × 0.25 μm). Pure helium was used as the carrier gas, with a flow rate of 2.64 mL/min. Prior to GC–MS analysis, the bio-crude oil sample was diluted in acetone. In a typical test, 1 μL diluted sample was injected at 280 °C with a split ratio of 20:1. The GC oven temperature was programmed as follows: held at 60 °C for 2 min, followed by heating at 20 °C/min to 280 °C and held for 10 min. The major components were identified by NIST (National Institute of Standards and Technology) database. The functional groups were characterized with a Nicolet 6700 Fourier Transform Infrared Spectroscopy (Thermo Fischer Scientific, Massachusetts, USA) in the range of 4000–600 $cm^{-1}$, with a resolution of 4 $cm^{-1}$.

## 3. Results and discussion

### 3.1. HTL media screening

Initial studies were carried out at 275 °C for 60 min, with a biomass/solvent mass ratio of 1/5 for screening nine different reaction media including water, water with acid catalyst (formic acid, acetic acid, sulfuric acid, or hydrochloride acid), or organic solvent (methanol, ethanol, ethyl acetate, or acetone).

As shown in Fig. 2, the type of reaction medium considerably affected the products yield. For the organic acid-catalyzed HTL, the bio-crude oil yield was significantly higher (38.0 wt% with formic acid and 32.6 wt% with acetic acid) compared to that obtained from HTL in water without catalyst (20.3 wt%). This trend was consistent with the previous study on the acid-catalyzed HTL [23]. The increased oil yield may be attributed to the acid-catalyzed degradation of macromolecules into small molecules during liquefaction process. Besides, the yield of solid residue drastically reduced from 21.0 wt% (no catalyst) to 3.3 wt % (formic acid) and 9.8 wt% (acetic acid). In the case of catalytic HTL with hydrochloride acid, only a slightly higher yield of bio-crude oil (22.1 wt%) was observed compared to that obtained without catalyst (20.3 wt%). Surprisingly, the addition of sulfuric acid led to a decrease in the oil yield (12.6 wt%) when compared with that obtained without catalyst (20.3 wt%). This reduced bio-crude oil yield may be due to the sulfuric acid-catalyzed degradation or condensation of oily compounds to yield water-solubles at high temperatures (i.e., 275 °C). Previously, Zou et al. [24] studied the effect of sulfuric acid on the HTL of *D. tertiolecta* (a low-lipid strain), and an increased bio-crude oil yield was observed at a low temperature of 170 °C with the addition of sulfuric acid.

As clearly shown in Fig. 2, the bio-crude oil yield was consistently higher when using organic solvent as the reaction medium (26.2 wt%, 40.1 wt%, 62.7 wt%, and 68.3 wt% with ethyl acetate, acetone, ethanol, and methanol, respectively) than that from liquefaction in water (20.3 wt%). This result is in good agreement with the findings of many previous literature reports [18,19,25]. This increased bio-crude oil yield could be attributed to the low dielectric constant of organic solvents and thus allow them to dissolve or stabilize the weak polar or even non-polar intermediate products [10]. However, it should be noted that the reaction medium employed in this study might react with liquefaction intermediates and/or final products, which might result in a higher bio-crude oil yield. In order to elucidate, the carbon balance for microalgal liquefaction in various organic solvents at 275 °C for 60 min and a biomass/solvent mass ratio of 1/5 was carried out. As

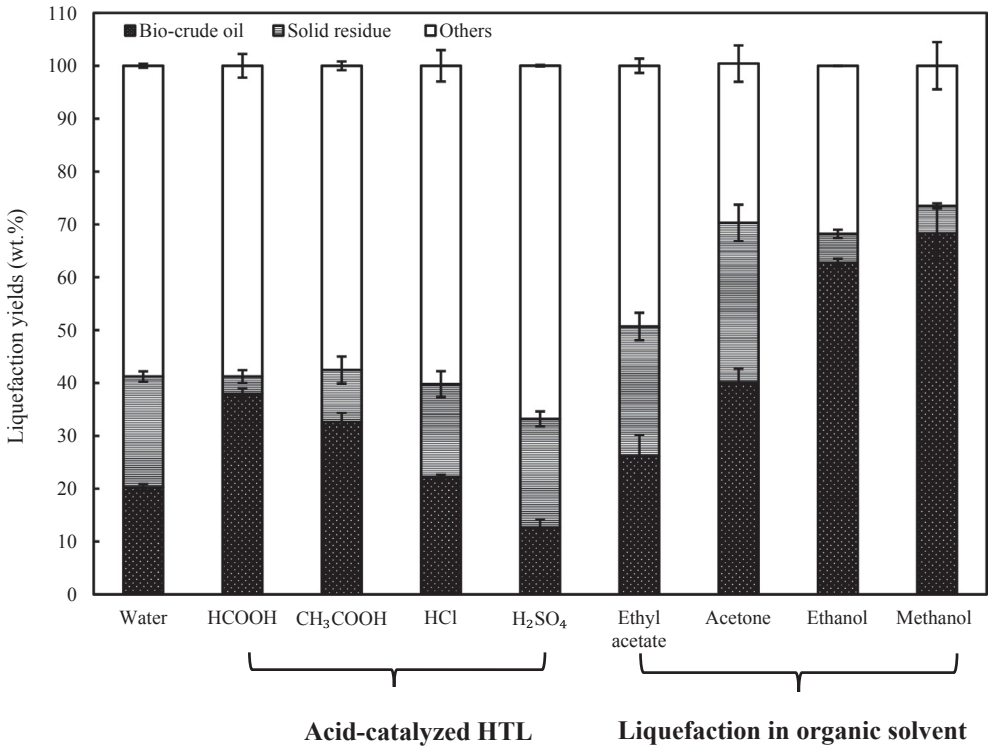


**Fig. 2.** The products distribution obtained from nine different reaction media at 275 °C for 60 min, with a biomass/solvent mass ratio of 1/5 (Note: No catalyst was used for HTL in water; Catalytic HTL were performed in pure water).

**Table 1**
Carbon balance for liquefaction treatment of microalgae in various reaction medium at 275 °C for 60 min, with a biomass/solvent mass ratio of 1/5, assuming 100 g of total amount of biomass in each test.

| | Methanol | Ethanol | Ethyl acetate | Acetone |
|---|---|---|---|---|
| *Liquefaction yields (wt%)* | | | | |
| Bio-crude oil | 68.34 | 62.70 | 26.15 | 40.16 |
| Solid residue | 5.13 | 5.50 | 24.51 | 30.13 |
| Water-solubles[a] | 26.53 | 31.80 | 49.34 | 30.13 |
| *C content in the liquefaction products (wt%)*[b] | | | | |
| Bio-crude oil | 69.41 | 71.85 | 63.13 | 71.57 |
| Solid residue | 19.87 | 8.94 | 63.47 | 67.46 |
| Water-solubles | 26.07 | 20.14 | 23.22 | 20.66 |
| Total C amount in the products (g) | 55.37 | 51.95 | 43.52 | 55.29 |
| C content in the feedstocks (wt%) | 46.54 | 46.54 | 46.54 | 46.54 |
| Total C amount in the feedstocks (g) | 46.54 | 46.54 | 46.54 | 46.54 |
| Carbon balance (%) | 119% | 112% | 94% | 119% |

[a] Water-solubles were obtained by removing water from the aqueous phase using a rotary evaporator at 70 °C under reduced pressure.
[b] C content of bio-crude oil, solid residue, and water-solubles was measured by an elemental analyzer (Vario EL Cube, Elementar, Germany).

shown in Table 1, the resulting carbon balance for all liquefaction runs ranged between 94% and 119%. In consideration of experimental error (approximately ± 10%), the extent of the organic solvent incorporating into the final oil products was insignificant.

Additionally, Fig. 2 shows that the type of organic solvent played an important role in the products distribution. Specifically, the yield of bio-crude oil obtained using a polar protic solvent (methanol or ethanol) as the reaction medium was significantly higher than that obtained using a polar aprotic solvent (ethyl acetate or acetone). One possible reason could be that the hot-compressed methanol/ethanol can serve as a hydrogen donor solvent. The released hydrogen free radical (H·) could promote the hydrocracking of macromolecules into small molecules. Meanwhile, the fragments/intermediates from liquefaction can be stabilized in the presence of hydrogen donor solvent, thereby preventing char formation and improving bio-crude oil yield [26]. This could be confirmed with the extremely low yield of solid residue (~5 wt%) with these alcohols. In contrast, the solid residue yield obtained in ethyl acetate (25.4 wt%) or acetone (30.1 wt%) was found to be significantly higher than those obtained in an alcohol solvent (methanol or ethanol).

According to the results as described above, methanol was identified as the most effective reaction medium for converting low-lipid microalgal biomass into bio-crude oil. Following this, the effects of residence time, biomass/solvent mass ratio, and reaction temperature on the products distribution were investigated.

### 3.2. Effects of operating conditions on products distribution

Effects of residence time on the products distribution were investigated at 275 °C for 30–120 min, with a biomass/methanol mass ratio of 1:5. As shown in Fig. 3, the bio-crude oil yield increased from 59.0 wt% to 68.3 wt% while prolonging residence time from 30 min to 60 min, and thereafter dropped to 41.1 wt% at 120 min. A similar trend was reported by Duan et al. [10] and Ji et al. [18]. The reduction of bio-crude oil yield at extended residence time may be due to the further decomposition of oil products into gases and/or water-solubles, as evidence by a sharp increase in the others yield (gases + aqueous products) from 26.5 wt% at 60 min to 54.2 wt% at 120 min. On the contrary, it was observed that the residence time had no significant effect on the solid residue yield. This phenomenon may be attributed to the role of methanol as a hydrogen donor solvent in the liquefaction process [26]. The optimal residence time for producing bio-crude oil

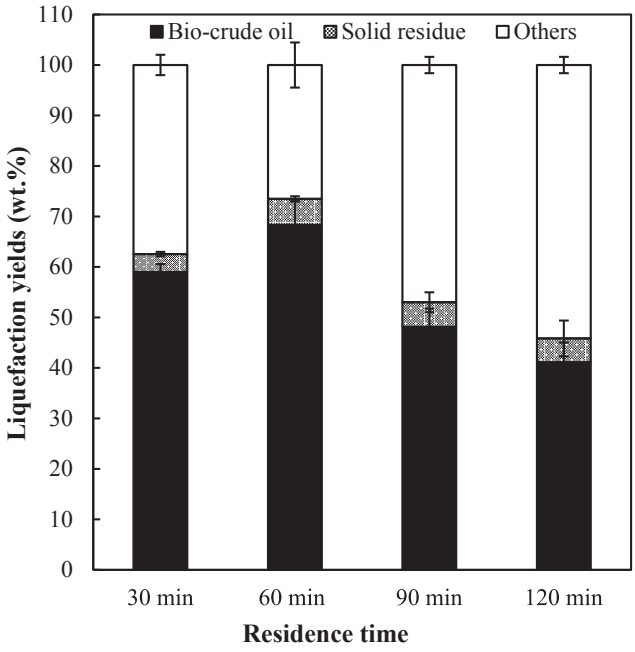


**Fig. 3.** Effects of residence time on the products distribution obtained in methanol (Other reaction conditions: 275 °C; biomass/solvent mass ratio of 1/5).

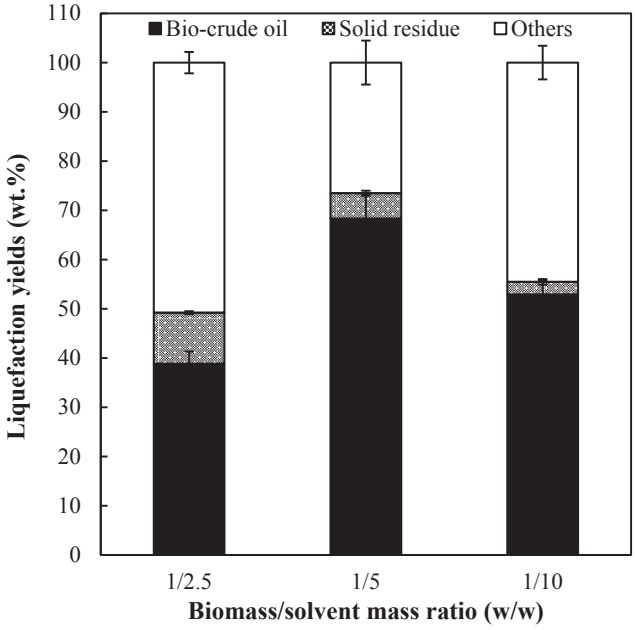


**Fig. 4.** Effects of biomass/solvent mass ratio on the products distribution obtained in methanol (Other reaction conditions: 275 °C; residence time of 60 min).

was observed to be 60 min for the given reaction conditions. Therefore, this residence time was chosen for investigating the effect of biomass/solvent mass ratio on the products distribution.

Fig. 4 demonstrates the effect of biomass/methanol mass ratio on the products distribution at 275 °C for 60 min. Variations of biomass/solvent mass ratio were obtained by mixing a fixed feedstock loading (5.0 g on dry basis) with different solvent loading amounts from 12.5 g to 50 g, corresponding to a biomass/solvent mass ratio from 1:2.5, 1:5 to 1:10, respectively. As shown in Fig. 4, the bio-crude oil yield sharply increased from 38.9 wt% to 68.3 wt% when increasing the solvent loading from 12.5 g to 25 g, while, further increasing the solvent loading decreased the bio-crude oil yield. This result was similar with

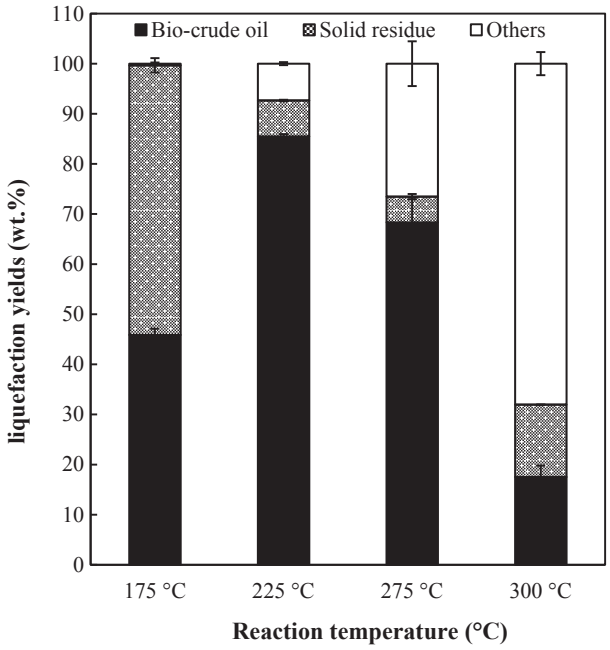


**Fig. 5.** Effects of reaction temperature on the products distribution obtained in methanol (Other reaction conditions: residence time of 60 min; biomass/solvent mass ratio of 1/5).

that reported in the literature regarding liquefaction of microalgae [9,18]. The increased bio-crude oil yield at a higher solvent loading could be due to the improved mass and heat transfer during microalgal liquefaction [25]. Nevertheless, the oil compounds tended to partially decompose into gaseous/water-soluble products when the solvent loading was too high (e.g., solvent loading = 50.0 g), which can be confirmed with an increase in the yield of others (gas + aqueous phase). Besides, the yield of solid residue gradually decreased from 10.3 wt% to 2.5 wt% with increasing solvent loading, which might suggest the promoted degradation of macromolecules into bio-crude oil, gases, or water-solubles. It should however be noted that very low solid residue yield (approx. 3–5 wt% on dry basis) was obtained at a higher solvent loading, even smaller than the ash content (7.2 wt%) of the original feedstock. This was possibly caused by the dissolution of some inorganic salts of the ash fraction into the water phase, thereby reducing the yield of solid residue [10]. With respect to bio-crude oil yield, the optimal biomass/solvent mass ratio of 1:5 was selected for studying on the effect of reaction temperature on the products distribution.

Fig. 5 shows the effect of reaction temperature on the products distribution obtained in methanol at 175–300 °C for 60 min, with a biomass/solvent mass ratio of 1:5. The critical temperature and pressure of methanol are 240 °C and 8.1 MPa, respectively. Hence, the liquefaction experiments were carried out in sub-/super-critical methanol. As indicated in Fig. 5, the bio-crude oil yield was increased with increasing reaction temperature, reaching its maximum yield of 85.5 wt % at 225 °C. Whereas, further increase in the reaction temperature until 300 °C actually led to a drastic reduction of bio-crude oil yield. Such strong dependency of bio-crude oil yield on the reaction temperature was commonly observed in many previous studies, e.g., Li et al. [6], Brown et al. [8], Shakya et al. [11], and Zhou et al. [16]. The increase in the oil yield could be caused by the following two reasons: (i) the presence of organic solvent could promote the degradation of large molecules into small molecules [17]; and (ii) organic solvent can dissolve or stabilize the weak polar or even non-polar intermediates during liquefaction process, resulting from a lower dielectric constant than that of water [10]. Previously, Chen et al. [32] stated that the use of alcohol as the reaction medium may react with the decomposed intermediates via esterification and this might be another reason for the increased oil yield. The possible interactions between methanol and liquefaction intermediates and/or final products might be confirmed with the subsequent GC–MS and FT-IR analysis. On the other hand, the reduced bio-crude oil yield at higher temperatures might be attributed to: (i) the further decomposition of oil intermediates into gases and/or water-solubles; and (ii) the re-polymerization of light volatile oily compounds into solid products [27]. Moreover, the yield of solid residue drastically decreased with increasing temperature from 175 °C to 275 °C, and thereafter increased at 300 °C. This behaviour could be due to the endothermic nature of the biomass macromolecules degradation, which could indicate that more fraction of biomass macromolecules could be liquefied and converted into bio-crude oil at higher temperatures. From the liquefaction results above, the optimal reaction temperature was observed to be 225 °C under the given conditions.

**Table 2**
The elemental composition and higher heating value of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5.

| Elemental composition (wt%) | Water | Methanol |
|---|---|---|
| C | 65.09 | 62.41 |
| H | 8.69 | 9.12 |
| O[a] | 19.54 | 19.83 |
| N | 6.17 | 8.39 |
| S | 0.51 | 0.25 |
| HHV (MJ/kg)[b] | 30.97 | 30.60 |

[a] Determined by difference.
[b] Calculated by HHV (MJ/kg) = 0.338C + 1.428(H-O/8) + 0.095S.

### 3.3. Bio-crude oil analysis

The elemental composition and higher heating value (HHV) of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5 were presented in Table 2. When compared with original biomass, the O content in bio-crude oil samples was reduced from 29.86% to a range of 19.54–19.83% and the HHV was greatly increased. Besides, a high N content (6.17–8.39 wt%) was observed in the bio-crude oils, due to the higher protein content in the feedstock (approx. 54 wt%), typical of microalgae-derived bio-crude oils [24]. It was found that both of bio-crude oil samples contained a lower S content between 0.25 wt% and 0.51 wt%, which is comparable to that of petroleum crude oil (0.1–2%) [27]. As shown in Table 2, the bio-crude oil obtained in methanol contained higher C content than that obtained in water. While, no big difference was observed in the O and S content of two oil products. In addition, the use of methanol resulted in a higher N-containing bio-crude oil compared to liquefaction in pure water. Dote et al. [28] investigated the reaction pathway of protein (the major source of N content in the bio-crude oil) in hot-compressed water, and the results showed that around 80% of N in the feedstock tended to migrate into aqueous phase. Hence, the presence of less water in the reaction medium would result in more N-containing compounds transfer into oil phase. Previous literature suggest that a high N content of bio-crude oil would result in $NO_x$ emission upon combustion [9]. Additionally, the presence of basic N-containing compounds (e.g., pyridines and amines) in the microalgae-derived bio-crude oil could deactivate the catalyst during the subsequent crude oil upgrading process by neutralizing the acid sites of the catalyst. These basic oil components could also react with the acids present in the bio-crude oil via acid-base reactions, thereby causing fouling of the equipment [34]. Therefore, N removal treatment is required for microalgae-derived bio-crude oils prior to the downstream catalytic upgrading. Although some recent studies investigated the adsorptive and extractive approaches for removing N from model fuel and light cycle oil [35], to the best of our knowledge, adsorptive and extractive denitrogenation is yet to be

**Table 3**
The average molecular weight and polydispersity index of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5.

| Molecular weight (g/mol) | Water | Methanol |
|---|---|---|
| $M_n$ | 207 ± 25 | 235 ± 71 |
| $M_w$ | 362 ± 52 | 378 ± 17 |
| PDI[a] | 1.75 ± 0.04 | 1.68 ± 0.44 |

[a] Determined by PDI = $M_w/M_n$.

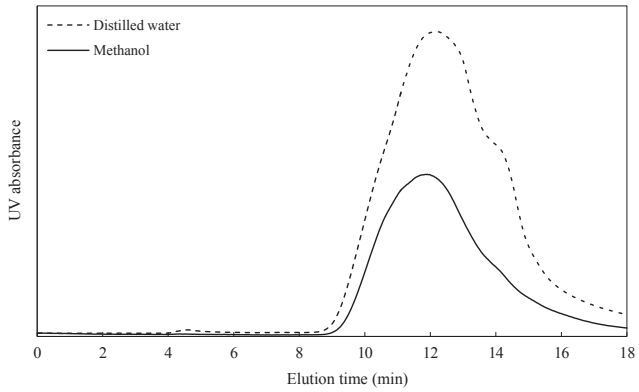


**Fig. 6.** The average molecular weigh of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5.

demonstrated for microalgal bio-crude oils. Hence, as s future study the N removal from microalgae-derived bio-crude oils by adsorption or extraction will be carried out.

Table 3 shows the average molecular weight ($M_n$ and $M_w$) and polydispersity index (PDI) of bio-crude oils obtained from liquefaction in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5. Fig. 6 presents the GPC curves of bio-crude oil samples. In general, $M_n$ of two bio-crude oil samples was observed to be comparable to that of petroleum crude oils (~$M_n$ = 250 g/mol) [29]. As indicated in Table 3, the bio-crude oil obtained in methanol had a lower $M_n$ (207 g/mol) and $M_w$ (362 g/mol) compared to those of bio-crude oil obtained in water ($M_n$ = 235 g/mol; $M_w$ = 378 g/mol). Moreover, polydispersity index (PDI) is an indicator of the differences in the minimum and maximum molecular weights [29]. Table 3 shows that no big differences in the PDI was observed between two bio-crude oil samples.

The boiling point distribution of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5 was presented in Table 4. The bio-crude oil obtained in methanol contained a higher percentage of low boiling point compounds (52.14 wt%, bp < 343 °C) and high boiling point compounds (25.51 wt %, bp > 538 °C), but a lower percentage of mild boiling point compounds (22.35 wt%, bp 343–538 °C). In general, the distillable fraction (bp < 538 °C) of two bio-crude oil samples were in the range of

**Table 4**
The boiling point distribution of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5.

| Distillate range (°C)[a] | Boiling point distribution (wt%) | |
|---|---|---|
| | Water | Methanol |
| < 193 (Heavy naphtha) | 12.13 | 11.87 |
| 193–271 (Kerosene) | 14.09 | 21.82 |
| 271–343 (Gas oil) | 23.69 | 18.44 |
| 343–538 (Vac gas oil) | 27.17 | 22.35 |
| > 538 (Residues) | 22.91 | 25.51 |

[a] Reference from Vardon et al. (2011).

**Table 5**
The major chemical compounds in the bio-crude oil obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5.

| RT (min)[a] | Compounds | Peak area (%) | |
|---|---|---|---|
| | | Water | Methanol |
| *N-containing compounds* | | | |
| 3.54 | Pyrazine, methyl- | 1.02 | |
| 11.16 | 2,4(1H,3H)-Pyrimidinedione, 1,3-dimethyl- | | 1.26 |
| 12.32 | (S)-6,6-Dimethyl-2-azaspiro[4.4]non-1-ene | 1.07 | |
| 13.17 | 3,6-Diisopropylpiperazin-2,5-dione | 11.54 | |
| 13.32 | 2,4,5-Trihydroxypyrimidine | 8.20 | |
| 13.40 | Hydrouracil, 1-methyl- | 3.08 | |
| 13.75 | N,3-Diethyl-3-octanamine | 3.85 | |
| 14.04 | 3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione | 4.23 | |
| 14.05 | Caffeine | | 4.19 |
| 14.08 | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)- | 9.92 | |
| 14.29 | 2-Butenamide, N,N-diisopropyl-, (E)- | 1.56 | |
| 14.34 | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- | 9.33 | 2.43 |
| 14.72 | 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1′,2′-d]pyrazine | 5.50 | |
| 15.67 | 2,5-Piperazinedione, 3-methyl-6-(phenylmethyl)- | 2.95 | |
| 15.97 | dl-Alanyl-l-phenylalanine | 1.64 | |
| 16.04 | 2,5-Piperazinedione, 3-benzyl-6-isopropyl- | 4.34 | |
| 16.61 | 2-Piperidinone, 1-methyl- | 3.86 | |
| 16.78 | Glyoxime, 1-cyano- | 1.56 | |
| 17.24 | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- | 2.39 | |
| *Esters* | | | |
| 10.89 | DL-Proline, 5-oxo-, methyl ester | | 3.18 |
| 12.87 | Hexadecanoic acid, methyl ester | 2.44 | 27.04 |
| 13.63 | l-Proline, N-propoxycarbonyl-, isobutyl ester | | 2.08 |
| 13.82 | 8-Octadecenoic acid, methyl ester | | 2.76 |
| 13.88 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 2.22 | 30.01 |
| 13.98 | 6,9-Octadecadienoic acid, methyl ester | | 1.24 |
| 14.50 | L-Leucine, N-cyclopropylcarbonyl-, methyl ester | 4.50 | |
| *Other O-containing compounds* | | | |
| 12.04 | Phytol | | |
| 12.32 | 2-hexadecen-1-ol, 3,7,11,15-tetramethyl | | |
| 12.32 | Myristaldehyde | | |
| 13.36 | 5-Hydroxy-2,4,4-trimethyl-cyclopentane-1,3-dione | | |
| 13.36 | Cyclohexane, ethoxy- | | 1.98 |
| 13.55 | 4-Methyl-2-pentanone | 1.18 | |
| *Cyclic oxygenates* | | | |
| 13.49 | 3-Ethoxy-4-methoxyphenol | 1.59 | |
| 13.84 | 6-Methylene-1,4-dioxaspiro[4.5]decane | 3.52 | |
| Total | | 91.49 | 76.17 |

[a] Retention time (min).

74–77 wt%, which is significantly higher than that of North American tar sand bitumen (44–65 wt%) [20].

The major chemical compounds (i.e., the relative percentage of peak areas over than 1%) in the bio-crude oil obtained from liquefaction in water or methanol at 225 °C for 60 min with a biomass/solvent mass ratio of 1/5 were identified by GC–MS analysis and summarized in Table 5. However, it should be noted that some oil compounds can be detected due to: (i) some light oil compounds might be lost during solvent evaporation; and (ii) the heavy compounds cannot be eluted through GC column [8]. As indicated in Fig. 7, some distinct differences can be observed in the relative concentrations of compounds (based on peak areas) between two bio-crude oil samples. The major compounds in bio-crude oil obtained in water were N-containing compounds (76.0%), while esters (66.3%) were the most abundant compounds in the bio-crude oil obtained in methanol. As given in Table 5, the content of hexadecanoic acid, methyl ester identified in the oil product increased from 2.4% in water to 27.0% in methanol, and 9,12-octadecadienoic acid (Z,Z)-, methyl ester was also much higher in the oil

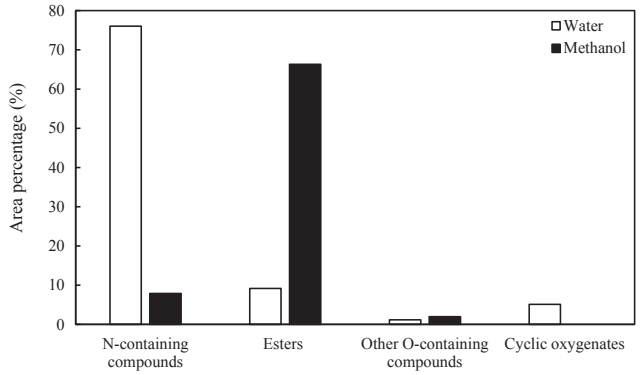


**Fig. 7.** The major chemical compounds of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5.

obtained in methanol (30.0%) than that of oil obtained in water (2.2%). The above results evidence that the interaction between fatty acids from microalgal biomass and alcohol via esterification reactions. In fact, the most abundant compounds in the biodiesel are $C_{16-20}$ fatty acid methyl/ethyl esters, similar to the esters detected in the bio-crude oil obtained from the liquefaction of low-lipid microalgae in methanol [16]. Except that, some nitrogen-containing esters like DL-proline, 5-oxo-, methyl ester were also observed in the bio-crude oils obtained in methanol, which was likely resulted from the reactions of amino acids with alcohols via esterification reactions [16].

The FT-IR spectra of bio-crude oil samples obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5 are displayed in Fig. 8. The interpretation of major peaks was performed according to Socrates [30]. A broad absorption band from 3700 to 3100 $cm^{-1}$ can be assigned to O—H and N—H stretching vibration. The peaks at 2944, 2925, and 2853 $cm^{-1}$ can be ascribed to C—H asymmetrical and symmetrical stretching vibration, indicating the presence of alkyl C—H in the bio-crude oils. Another peak at 1742 $cm^{-1}$ could be related to C═O carbonyl group, suggesting the presence of carboxylic acid, ketones, or esters in the bio-crude oil. The peak at 1666 $cm^{-1}$ represents the C═O stretching vibration from primary amide compounds. Besides, the peak at 1440 $cm^{-1}$ could be attributed to C—H vibration in methyl groups. The peaks at 1199 and 1031 $cm^{-1}$ can be related to the C—O stretching vibration. Finally, a strong peak at 753 $cm^{-1}$ indicated the presence of aromatic C—H in the bio-crude oil. Although the FT-IR spectra of two bio-crude oils obtained in different reaction media (water and methanol) showed similar functionalities, some evident differences between oil samples were observed in Fig. 8. Firstly, the stretching vibrations of carbonyl C═O at 1742 $cm^{-1}$ and

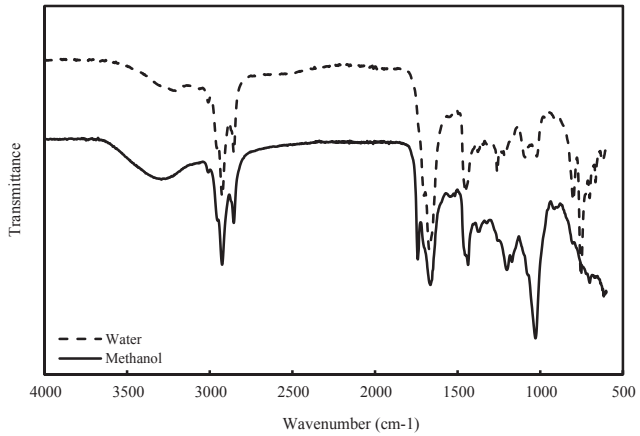


**Fig. 8.** FT-IR of bio-crude oils obtained in water or methanol at 225 °C for 60 min, with a biomass/solvent mass ratio of 1/5.

C—O at 1029 $cm^{-1}$ were observed to be more intensive in the bio-crude oil obtained in methanol, which may indicate the formation of esters between acid intermediates and alcohol. In addition, the peak at 753 $cm^{-1}$ representing aromatic C—H vibration was weaker in the bio-crude oil obtained in methanol, implying enhanced hydrogenation of the oil products.

### 3.4. Reaction pathway

Microalgae is primarily composed of protein, carbohydrates, and lipid. Hence, the conversion of microalgae into bio-crude oil is actually the reactions of these molecules. It should be noted that the heating rate for all liquefaction runs was low (~5 °C/min) in this study, thereby requiring a longer heating time to reach the set-point of the reaction temperature. As a result, hydrolyzing reactions of those macromolecules might start during the pre-heating stage [19]. Therefore, the effect of pre-heating on the overall reaction mechanism of microalgal liquefaction should be considered. In this regard, possible reaction pathways for liquefaction of microalgae in methanol were proposed based on the temperature range and feedstock composition.

As shown in Fig. 9, protein is readily hydrolyzed into amino acids at 0–100 °C. In the meantime, lipid is hydrolyzed into long-chain fatty acids and glycerol, and carbohydrates into monosaccharides (e.g., glucose and fructose). These intermediate products (amino acids, long-chain fatty acids, and monosaccharides) will undergo further degradation at 100 °C–200 °C. Typically, amino acids will decompose to form carboxylic acids and amine by deamination and decarboxylation reaction, respectively. Although deamination and decarboxylation are two main pathways in the amino acids degradation, the ratio of deamination/decarboxylation is determined by varying factors, such as the type of amino acid, pH of reaction medium, and other reaction conditions [31]. Additionally, the long-chain fatty acids could be converted into alkanes and alkenes via decarboxylation reactions. In the temperature range of 100–200 °C, some cyclic oxygenates could be produced from the degradation of monosaccharides. When temperatures above 200 °C, a fraction of long-chain fatty acids might react with ammonia from amino acid to form amides. Another fraction of long-chain fatty acids can react with alcohol from either the reaction medium or the reduction of carboxylic acids to produce fatty acid esters. Besides, the formation of N&O-heterocyclics (e.g., pyrrolidinone and pyrrolidine) resulting from Maillard reactions between amino acids and reducing sugars (e.g., glucose and fructose) can be observed in the same temperature range. When the temperature is higher than 300 °C, the N&O-heterocyclics from Maillard reaction might react with long-chain fatty acids to form pyrrolidine derivatives of fatty acids. Meanwhile, phenols and phenol derivatives could be produced from glucose or fructose via ring opening/closure reactions [33].

## 4. Conclusions

In this study, the effects of different reaction media (including water, water with formic acid/acetic acid/sulfuric acid/hydrochloric acid, methanol, ethanol, acetone, and ethyl acetate) on the products distribution from low-lipid microalgae were investigated. The results showed that methanol was identified as the most effective reaction medium for the production of bio-crude oil from low-lipid microalgae. Afterwards, the effects of other reaction conditions (residence time, biomass/solvent mass ratio, and reaction temperature) on the liquefaction in methanol were examined. Liquefaction experiments at 225 °C for 60 min with 1:5 biomass/solvent mass ratio resulted in the highest bio-crude oil yield of 85.5 wt%, along with a higher heating value (HHV) of 30.6 MJ/kg.

## Acknowledgements

The authors would like to acknowledge the funding from

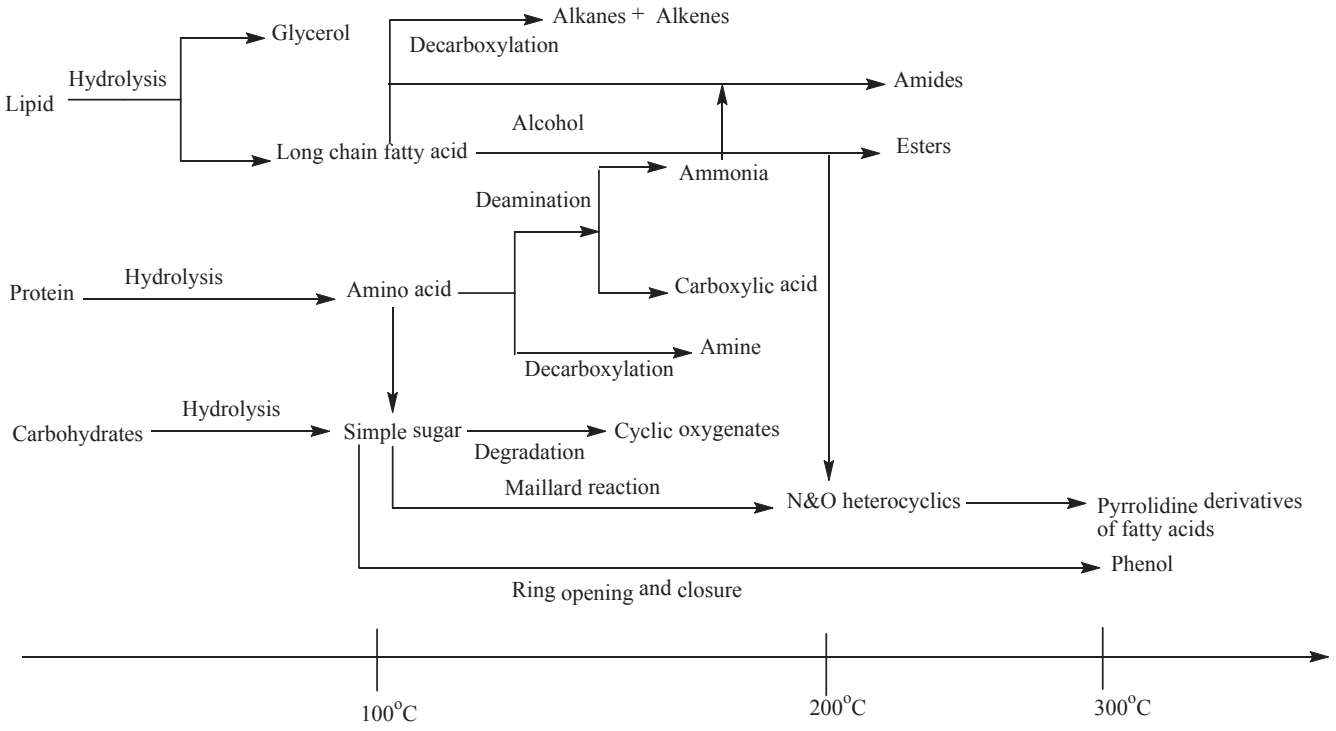


**Fig. 9.** The proposed reaction pathway for the liquefaction of low-lipid microalgae in methanol.

BioFuelNet Canada, a Network of Centres of Excellence and from NSERC through the Discovery Grants awarded to Dr. Xu and Dr. Bassi.

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