

Article

Co-Solvent Assisted Hydrothermal Liquefaction of Algal Biomass and Biocrude Upgrading

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Abstract: This study reports the hydrothermal liquefaction (HTL) of microalgae *Spirulina platensis* in the presence of alcohol or formic acid co-solvents. HTL runs are performed in a 1.8-L batch reactor at 300 °C using an alcohol (methanol and ethanol) or formic acid co-solvent. Consequently, hydrodeoxygenation (HDO) of resultant algal biocrude is performed at 350 °C for 2 h under high hydrogen pressure (~725 psi) using the Ru/C catalyst. The HTL results are compared with the control HTL run performed in water only. The results of the study show that the addition of co-solvents leads to a 30–63% increased biocrude yield over the control HTL run. Formic acid results in a 59.0% yield of biocrude, the highest amongst all co-solvents tested. Resultant biocrudes from formic acid-assisted and ethanol-assisted HTL runs have 21.6% and 3.8–11.0% higher energy content, respectively, than that of the control run. However, that of the methanol-assisted HTL results in biocrude with 4.2–9.0% lower energy density. Viscosity of biocrude from methanol- or ethanol-assisted HTL is higher than the control HTL but formic acid-assisted HTL results in a less viscous biocrude product. In addition, the HDO study leads to a 40.6% yield of upgraded oil, which is characterized by a higher net energy content and lower O/C and N/C ratios when compared to the initial HTL biocrude.

Keywords: algae; hydrothermal liquefaction; biocrude; co-solvent; hydrodeoxygenation



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1. Introduction

Bio-based fuels are renewable in nature, carbon neutral, and have potential for fossil fuel replacements. Microalgae-derived biocrude is particularly of interest as a potential biofuel source, owing to its high energy content and low life-cycle emission of greenhouse gases [1]. In the last decade, hydrothermal liquefaction (HTL) has attracted significant attention due to its ability to process a wide range of wet biomass materials, including algae, aquatic plants, and organic waste streams and it produces an energy-dense “biocrude” along with nutrient-rich aqueous co-products, solid residue, and carbon dioxide-rich gases [2]. HTL of microalgae biomass is conducted in sub-critical water conditions (<374 °C, <3200 psi, i.e., <22.1 MPa) that have the advantage of the latent heat of water [3–5]. A review of scientific literature shows that HTL product yields are significantly affected by operating conditions (temperature, pressure, and residence time), feedstock type, catalysts, and use of reducing gases or hydrogen donors [6–8]. Earlier, the present authors conducted extensive studies on HTL of *Spirulina platensis* [9–12]. These studies focused on determining the optimum HTL operating conditions, using aqueous co-product as an input into algae cultivation, finding the impacts of catalysts on HTL product distribution, and examining the energy and mass balances in HTL and pyrolysis (a process that is performed at 400–600 °C and atmospheric pressure using dry biomass at <10% moisture content). These studies revealed that HTL is energetically more efficient than pyrolysis for conversion of wet algae [11]. The authors also reported that in HTL, the product distribution, and in particular the biocrude yield, is affected by a number of factors, temperature being the

most significant operating parameter when the process is conducted without the aid of chemical reagents/catalysts [9]. The above fact is also supported by studies reported by many other researchers [5,13]. HTL conducted at 350 °C produced the optimal biocrude yield of ~40% [9]. However, the corresponding operating pressure at this temperature is 2650–2900 psi (18–20 MPa), which could lead to huge capital and operating costs for the development of pressure vessels, valves, and other system components. One of the important parameters is reaction temperature, which dominates the process energetics of any thermochemical process. Thermodynamic calculations suggest that reductions in HTL reaction temperature from 350 to 300 °C could lead to 12.5% less consumption of heat energy (when operated at 20% solids content in the slurry).

To evolve HTL as a commercial-scale sustainable process for algae processing into biocrude, it is important to have minimal energy and capital cost requirements and hence the HTL temperature and pressure. Operating at low temperature favors economic engineering considerations [14,15]. In addition, previous studies have shown that the addition of chemical catalysts, co-solvents, and co-processing gases (example, H₂) can alter HTL reactions and result in higher biocrude yield [8,16–24]. Although the use of catalysts and H₂ gas can lead to additional costs (and hence, may not be economical), the addition of co-solvents in HTL remains an option for enhancing the HTL process and biocrude yield. Using alcohol as a co-solvent has been considered to have great potential in the HTL of biomass. The water–alcohol binary solvent has unique thermodynamic properties (Gibb’s free energy, enthalpy, and entropy) that allows certain reactions, such as hydrogenolysis, enhanced decarboxylation, and condensation, to occur at a faster rate, which is not possible in the single solvent (e.g., water). The addition of alcohol, such as ethanol, methanol, and propanol, to water can drastically reduce the critical temperature (T) and pressure (P) of the mixture (as shown in Table 1) while increasing the reactivity (alcohol being a good hydrogen donor in the hydrothermal reactions). This leads to improved biocrude yield and energy density [17,19].

Table 1. Critical values and operating conditions of water–ethanol solvent mixtures.

% Co-Solvent	T _c , °C	P _c , psi (MPa)	* T _{op} , °C	* P _{op} , psi (MPa)
0	374.0	3205 (22.1)	300	2668 (18.4)
10	363.1	2886 (19.9)	300	2421 (16.7)
50	314.1	1693 (11.67)	300	1914 (13.2)

T_c and P_c: Critical temperature and pressure; * T_{op} and P_{op}: Actual operating temperature and pressure in this experiment.

A review of the literature showed that numerous studies have been investigated using the co-solvent in HTL. For example, the use of ethanol and propanol as co-solvents has been investigated for the liquefaction of lignocellulosic feedstocks [17,19,23,25,26], algae feedstock [16,18,22,27–29], and yeast biomass [30]. The aforementioned studies have reported improved yield and quality of biocrude. Although there are limitations with the use of pure organic solvent in liquefaction of biomass [31,32], using a mixture of organic solvent and water (thus the term “co-solvent”) presents potential for synergies in a chemical reaction. Pan et al. reported that the mixture of ethanol and water led to a maximum conversion rate of 98.44% at 280 °C when compared to 83.47 wt% (at 300 °C) for water alone and 72.6 wt% for pure ethanol. This conversion led to corresponding biocrude yields of 62 wt%, 38 wt%, and 32 wt% for ethanol–water co-solvent, water, and pure ethanol, respectively [17]. In addition, Caporgno et al. [16] investigated the influence of different solvents on the liquefaction of *Nannochloropsis oceanica*. They reported that up to a 60% bio-oil yield was achieved with the addition of alcohol, and higher than 54% yield was obtained at the severe operating condition using water alone. Cui et al. [18] studied the influence of different alcohols as co-solvents on HTL of *Galdieria sulphuraria*, where bio-oil yield increased from 13 to 73% due to the addition of alcohol. The increases in bio-oil were attributed to the presence of alcohols, facilitating the transfer of algal protein-derived

fragments from the aqueous phase into the oil phase. The investigation of ethanol–water co-solvent HTL of the lignocellulose biomass suggested that water enhances hydrolysis of cellulose and hemicellulose [33], where ethanol was found to favor the dissolution of lignin-derived intermediates. Thus, preventing them from repolymerization and/or condensation reactions, which leads to the formation of higher molecular weight solids. The present study uses different co-solvents in HTL of a low-lipid algae feedstock to explore their influence on product yield and properties of biocrude.

Algal biocrude upgrading and catalytic hydrodeoxygenation (HDO): Biocrude produced from the HTL of algae is a complex mixture of oxygenated (carboxylic acids, phenols, aldehydes, ketones, esters), nitrogenated (indole, pyrrolidines, amines, amides), and sulfur (thiophene) compounds [3,5,9]. High amounts of oxygenated (9–15%) and nitrogenated compounds (3–7%) in algal biocrude can significantly affect its energy content, storage and transportation stabilities, and emission properties when used as a fuel. Hence, the upgrading of biocrude is required in order to eliminate the O and N heteroatoms. The removal of or reduction in the heteroatoms from HTL biocrude can be achieved by using several methods including separation, hydrotreatment, esterification, catalytic cracking, or hybrid processes [2]. Hydrotreatment (often referred to as “hydroprocessing”) is the treatment of biocrude at 300–400 °C under high-pressure hydrogen atmosphere in the presence of a suitable catalyst [1,34–36]. Hydroprocessing promotes hydrodeoxygenation, hydro-denitrogenation, hydrodemetalization, hydro-desulfurization, and hydrogenation, occurring in a single reaction. Catalytic upgrading should hydrogenate and hydrocrack HTL biocrude while eliminating oxygen and nitrogen, thereby improving its stability. Most hydroprocessing catalysts reported in the literature are CoMo, NiMo, NiW, Co, Pd, Pt, and CuCrO to hydrogenate biocrude and model compounds phenol, p-cresol, ethyl-phenol, dimethyl-phenol, trimethyl-phenol, naphthol, and guaiacol (methoxyphenol) [1,35–40]. Hydrodeoxygenation of algal biocrude conducted at 400 °C over 1–8 h using Pd/C and Pt/C catalysts led to complete desulfurization, >50% reduction in N and O, and an overall improved quality of treated bio-oil over the HTL bio-oil [41,42]. Due to its high surface area and higher activity, Ru on carbon has been reported as the most effective HDO catalyst for algal biocrude obtained from a two-stage HTL, in which the minimal number of nitrogen heteroatoms were obtained in the treated biocrude [38]. Hydrotreatment of HTL-processed algal biocrude is of growing interest in recent times owing to the potential of algal biocrude as a future commodity fuel via co-processing in the existing refinery system. HTL biocrudes obtained from diverse types of algal feedstock can be different due to differences in heteroatom types and composition, and they pose challenges during the hydroprocessing step [40]. Therefore, investigation on HDO of the biocrude derived from the HTL of *Spirulina platensis* is necessary.

The primary objective of the present research is to conduct a detailed investigation on the effect of the addition of an alcohol or formic acid co-solvent in the HTL of *Spirulina platensis*. This study also makes a preliminary evaluation of catalytic HDO of biocrude obtained from the HTL of *S. platensis* without a co-solvent. HDO evaluation is limited to the reporting of the yield and the characterization of the upgraded product and catalyst.

2. Materials and Methods

2.1. Raw Materials

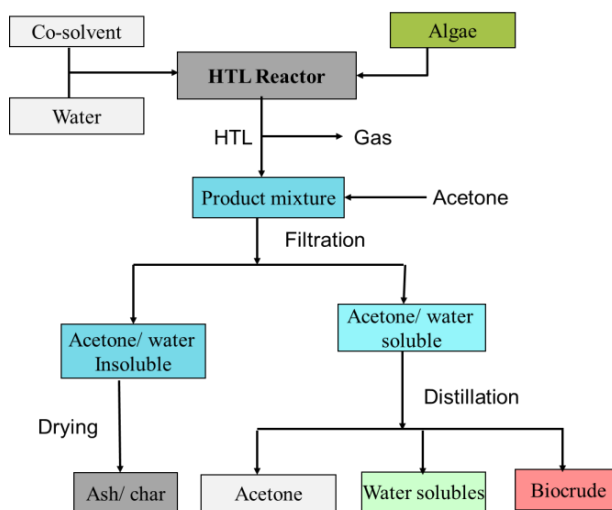
Freeze-dried *S. platensis* biomass was obtained from Earthrise Nutritionals LLC Calipatria, Irvine, USA. Methanol ($\geq 99.8\%$ purity), ethanol ($\geq 99.8\%$ purity), and formic acid ($\geq 95\%$ purity) and laboratory grade acetone ($\geq 99.5\%$ purity) were purchased from Sigma Aldrich, Burlington, USA. Ru/C (5 wt% of ruthenium, *w/w*) catalyst of 20 μm particle size was purchased from the Sigma Aldrich, Burlington, USA. Ru/C catalyst was chosen due to its high surface area and hence higher activity for hydrodeoxygenation³⁸ (had surface area and average pore sizes of 721 $\text{m}^2 \text{g}^{-1}$ and 11.4 Å, respectively). Nitrogen, hydrogen (99.99% purity), and helium (99.5% purity) were purchased from Air Gas, Radnor, PA, USA.

2.2. Hydrothermal Liquefaction (HTL) Experimentation and Product Separation

HTL experiments were performed in a batch type 1.8-L reactor system (Parr Instruments Co. Moline, PA, USA) (Figure 1a). As shown in the figure, the reactor is equipped with magnetic stirrer, solenoid valve assembly for temperature control, real-time sampling ports, process controllers, and data logger. All liquefaction runs (co-solvent HTL and control runs) were performed at a reaction temperature of 300 °C and for 30 min holding time using 20% algal solids loading, according to previous study [9,16,18]. Co-solvent HTL runs were performed at a solvent to water ratio of 1:10 (i.e., 10% *v/v* co-solvent) or 5:5 (i.e., 50% *v/v* co-solvent) using methanol, ethanol, and formic acid. In a typical experimental run, 120 g of dry *Spirulina platensis* powder was mixed with 480 g of water–solvent mixture. Then, the premixed algal slurry was loaded into the reactor. Thereafter, the reactor was sealed, thoroughly purged with nitrogen, and pressurized to 289 ± 3 psi (~ 2 Mpa) using nitrogen in order to prevent the vaporization of water. Then, the reactor was heated to 300 ± 3 °C temperature (autogenous working pressure was 1175 ± 90 psi, i.e., 8.1 ± 0.6 MPa) using an electrical heating jacket. After attaining desired temperature, the reaction was maintained for 30 min reaction time. During reaction, the reactor contents were continuously agitated (at 300 rpm) using an impeller type built-in magnetic stirrer to ensure homogenous reaction and avoid mass transfer limitation.



(a)



(b)

Figure 1. (a) 1.8-L Parr reactor apparatus for hydrothermal liquefaction (HTL) and hydrodeoxygenation (HDO) experiments; (b) HTL products separation protocol.

Following a typical HTL experimental run and cooling of reactor, the process gas was sampled using Tedlar sampling bags for further analysis and the remaining gas was vented into the atmosphere. The gas samples were analyzed using GC-MS according to Jena et al. [9]. The liquid products mixture was transferred to a separating funnel and then separated into different product fractions through a series of filtration and distillation methods, as shown in Figure 1b. The filter paper and reactor wall were washed with acetone and vacuum filtered in order to recover retained water insoluble components (biocrude and solids). Then, the solids recovered on the filter paper were oven dried at 105 °C for 24 h. The acetone soluble fraction was vacuum evaporated at 55 °C to obtain the biocrude fraction, as shown in Figure 1b. Although water soluble components obtained as filtrates were sampled, they were not analyzed in this study. They have been extensively investigated and reported in previous studies by the authors [9,10,12].

2.3. Hydrodeoxygenation (HDO) Experimentation and Product Separation

Algal biocrude obtained from HTL at 300 °C (without co-solvent) was used for catalytic upgrading via hydrodeoxygenation (HDO). HDO experiments were conducted using the 1.8-L Parr reactor (Figure 1a) at 350 °C, high H₂ (99.99% purity) pressure, and 10% (*w/w*) Ru/C catalyst. In a typical HDO run, approximately 40 g of algal biocrude, 4 g of Ru/C, and 160 g fresh DI water were loaded into the reactor; the reactor head space was thoroughly purged with nitrogen twice and with H₂ three times before being pressurized with H₂ at 5 MPa (725 psi). The heating rate of the reactor was approximately 5–7 °C min⁻¹. Once the operating temperature reached 350 °C, the reaction was allowed to stand for 2 h while continuously agitating the reaction mixture at 500 rpm. After completion of reaction time, the reactor was cooled to room temperature at a cooling rate 15 °C min⁻¹. Gas sample was collected for further analyses, as mentioned previously. The hydrotreated products in the reactor were recovered and separated into upgraded oil, catalyst, coke, and water. This was achieved by pouring 200 mL of dichloromethane (DCM) into the reactor and washing the cooling coil. The solution was vacuum filtered through 0.45 µm Whatman filter. Then, the cake was rinsed with 25 mL additional DCM and dried at 105 °C for 4 h. The filtrate was subsequently transferred into a 1-L separating funnel for phase separation. The bottom phase (oil + DCM) was decanted and distilled using a Rotavap unit under vacuum pressure (40 mbar) and 37 °C water bath for 60–70 min until all the DCM was evaporated. The remaining product was weighed to determine the mass of the upgraded oil product. Only the upgraded HDO oil fraction was quantified in this study.

2.4. Analytical Methods

Yield of each product fraction was determined as the ratio of its weight to the initial weight of algal biomass load. The yield of upgraded HDO product was reported as the mass of the final product obtained per 100 g of the initial weight of biocrude fed into the reactor system. The number of gaseous products (denoted as “Gas”) was determined by measuring the weight difference of the reactor and contents before and after the experiment. Triplicate runs were conducted, and average yield was reported.

Ultimate analysis was performed to evaluate the elemental carbon, hydrogen, nitrogen, sulfur, and oxygen contents of the biocrude and solid (raw biomass, residual char) samples using a LECO brand (Model CHNS-932) following ASTM D 5291 and D 3176 methods. Sulfamethazine (C—51.78%, H—5.07%, N—20.13%, and S—11.52%) was used as a standard material for calibration. Composition of carbohydrate, protein, and lipid contents of algae feedstock has been reported previously [9]. Energy contents of solid and biocrude samples were evaluated by measuring the higher heating values (HHV) in accordance with Jena et al. [10]. The bomb calorimeter was calibrated using benzoic acid as the standard material. Water content of the biocrude samples was measured in accordance with a previous report¹. Gravimetric method was employed for specific gravity (g mL⁻¹) measurements using 2 mL Gay-Lussac pycnometers. A Brookfield DV-I + Viscometer (with a UL/YZ spindle adapter) followed a modified version of ASTM D 2983.

A Hewlett-Packard (Model HP-6890) gas chromatograph in conjunction with a Hewlett-Packard mass spectrometer (Model HP-5973) with a mass selective detector was used to analyze the composition of biocrude and HDO upgraded products. The HP-5 MS column specifications were 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness. The GC-MS analysis was performed following the method outlined in a previous article published by the authors [9] at inlet temperature detector temperature, of 230 °C and 280 °C, respectively, at 1 mL min⁻¹ He flow at mass spectrometer scan range of 15–500 mass units. The oven temperature was ramped from 40 to 250 °C at 8 °C min⁻¹ after holding for 2.5 min at 40 °C and holding at 250 °C for 5 min. Furthermore, 1 µL sample size was prepared by diluting the bio-oil to 2.5% (*v/v*) with acetone before injecting into the GC-MS, and the compounds were identified using probability-based matching (PBM) algorithm using National Institute of Standards and Technology’s 1998 library. An Agilent Technologies portable micro GC (3000A) equipped with a thermal conductivity detector was used to

measure gaseous products' composition, which used a molecular sieve column (5A PLOT) 10 m × 0.32 mm size helium (at 80 ± 2 psi pressure). Residual solid and aqueous co-phase products of HTL were not analyzed in this study; only their yields were reported for the purpose of mass balances. Catalyst surface area was measured by N₂ adsorption over a relative pressure range (P/P_0) of 0.05–0.35 using a 7-point BET analysis equation (Quantachrome AUTOSORB-1C; Boynton Beach, FL, USA) and average pore radius, and total pore volume was estimated from N₂ desorption curves.

Statistical relevance of data: One-way ANOVA was performed using IBM SPSS 20.0 statistical package to report the difference in mass yields of biocrudes (at a significance level $p = 0.05$). All HTL and HDO experiments were performed in triplicate.

3. Results and Discussion

3.1. Biomass Characterization

S. platensis biomass had over 93% of organic matter (78.1% volatiles and 15.2% fixed carbon) and 6.6% ash content. The HHV was measured as 20.5 MJ kg⁻¹, which is higher than that of many herbaceous biomass and energy crops (12–17 MJ kg⁻¹) [17,32]. Detailed characterization of *S. platensis* used in this study has been described elsewhere [9]. High organic content, low ash content, and high energy content make algae biomass an excellent feedstock for biofuel conversion compared to its counterparts, such as lignocellulosic feedstocks, sewage sludge, animal manure, and municipal solid wastes [43–49]. A large amount of protein (49.2%) and small amount of lipid (11.1%) make *S. platensis* unsuitable for biodiesel or renewable diesel production, unlike some of the high-lipid algal species.

3.2. HTL Product Distribution and Characteristics

The distribution of HTL products obtained from *S. platensis* is presented in Figure 2. Generally, the yield in biocrude was 36.2–59.0 wt%, which was much higher than the starting lipid content (11.1%) of the feedstock. This suggests that it is not only lipids but other components, such as protein, that are converted into biocrude. HTL conversion could be a more attractive option for this algal biomass than the lipid conversion pathway, such as transesterification. The addition of a co-solvent resulted in a significantly higher yield of biocrude than the HTL performed without a co-solvent addition ($p = 0.001$). These results were found to be in good agreement with the previous studies investigating solvent assisted HTL. These studies reported increased biocrude yield for co-solvent HTL of lignocellulosic biomass [25,29], algae [18,50,51], and yeast biomass [30] in HTL depolymerization reactions through the solvolysis of macromolecules that largely depends on the solvent used [52].

The interactions between solvent and substrate increase when the solvent is a product of the biomass itself—such as alcohols, acids, and derivatives. During HTL, decomposition of algae biomass proceeds through several reactions, including cracking, isomerization, defragmentation, and rearrangement, which are very much related to the polarity [53]. Methanol, ethanol, and formic acid are polar solvents. Therefore, the solvents tested in the present study have been highly effective in increasing the biocrude yields. Co-solvent hydrothermal liquefaction (CSHTL) using formic acid led to a maximum increase in biocrude yield and with 62.8% higher yield than that of the non-co-solvent HTL. The use of methanol or ethanol as a co-solvent led to 30–33% more biocrude yield over the control HTL run (without co-solvent) (Figure 2a).

Furthermore, similar yields in biocrude were obtained using different concentrations of methanol or ethanol. CSHTL with 50% concentration of methanol and ethanol resulted in more biocrude yields when compared with 10% concentration. As shown in Figure 2b, a biocrude yield of 54–57% was obtained from 50% co-solvent whereas 47–48% was obtained when 10% co-solvent was used. This suggests that methanol and ethanol have similar influence in HTL, and higher concentrations favor decomposition of biomass macromolecules, leading to more yields in biocrude. Previous reports on co-solvent HTL reported that using an alcohol–water or formic acid–water binary solvents resulted in increased biocrude yield [18,19]. Moreover, addition of alcoholic co-solvent (iso-propanol) was shown earlier

to significantly increase biocrude yield in HTL of yeast [30]. This suggest that the data obtained in the present study could be useful in future applications.

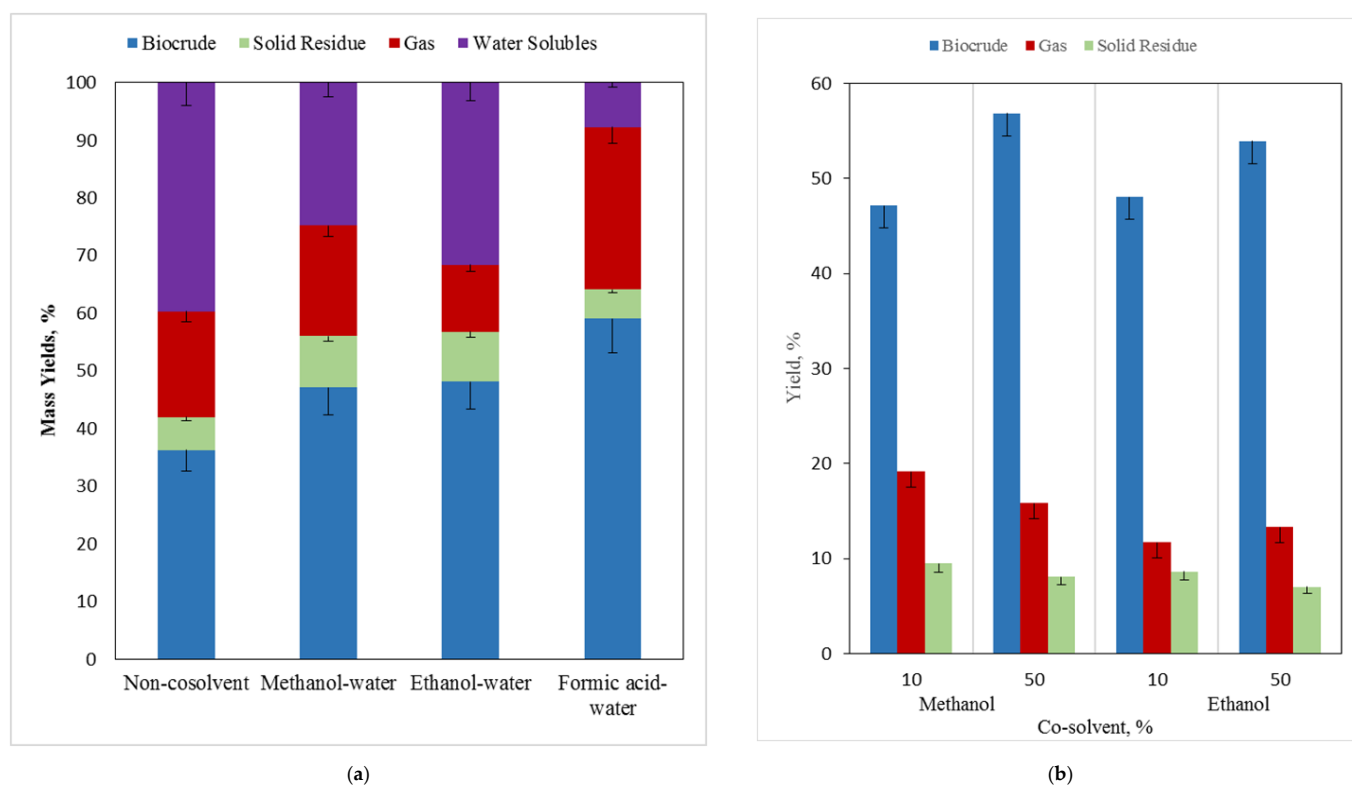


Figure 2. Co-solvent HTL of *S. platensis*: (a) effect of solvent types on product mass distribution in different solvents (at 1:10 co-solvent to water ratio), (b) effect of co-solvent amounts (%) on yields of biocrude, gas, and solids. (HTL experiments were conducted at 300 °C, 30 min time, and 20% solids loads).

As shown in Table 1, the binary alcohol–water solvent mixture has lower critical temperature (T) and pressure (P) (~311.3 °C and 10.2 MPa or ~1484 psi) than water alone (374.1 °C and 22.1 MPa or ~3200 psi) [22] and approaches the supercritical state under the T, P conditions used in this study, making it a highly reactive medium that favors increased biocrude yield during HTL. Formic acid may enhance hydrogen donation and hydroxyalkylation, thus helping in alcoholysis and other HTL reactions [20]. The addition of methanol and ethanol led to increased solids yield; however, there was no significant difference in solids yield when formic acid was used as a co-solvent. The use of formic acid as a co-solvent led to maximum biomass conversion rate ~95%, with consequent increase in biocrude and gaseous yields when compared with other CSHTL runs (Figure 2a)

Formic acid is believed to serve as a hydrogen donor influencing the product distribution positively. Hydrogen donor solvents are known to show significant improvement in product distribution to liquid as well as in quality of biocrude in terms of lower oxygen content. This is mostly as a result of improved hydrogenation and hydrocracking reactions with inhibition of polycondensation [54]. This reaffirmed that the oxygen content of biocrude obtained using formic acid was significantly lower (12.69%) when compared to that of the control run or with other co-solvent runs (16.8–26.2%), as presented in Table 2. This is further discussed in the biocrude characterization (Section 3.2.1).

Table 2. Properties of biocrude obtained from co-solvent assisted HTL compared to the control HTL run.

	Control Run (No Co-Solvent)	Methanol-Assisted		Ethanol-Assisted		Formic Acid-Assisted (10%)
		10%	50%	10%	50%	
<i>Physical properties</i>						
Color	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Odor	Light smoky	Light smoky	Light smoky	Light smoky	Light smoky	Light smoky
Sp. Gr.	1.05	1.11	1.08	1.10	1.09	1.13
pH	7.96	8.62	8.90	8.90	9.80	7.32
Viscosity (cP)	67.8	114.8	117.0	n.d.	117.6	54.0
<i>Elemental analyses</i>						
C, %	62.42	57.20	59.65	61.35	68.31	69.02
H, %	8.24	8.20	8.25	9.36	8.36	9.92
N, %	6.92	7.90	7.67	7.13	5.90	7.71
S, %	0.52	0.41	0.49	0.63	0.67	0.66
[†] O, %	21.9	26.29	23.89	21.53	16.80	12.69
O/C	0.26	0.34	0.30	0.26	0.18	0.14
N/C	0.10	0.12	0.11	0.10	0.07	0.10
H/C	1.57	1.71	1.65	1.82	1.46	1.71
HHV, MJ kg ⁻¹	28.92	26.32	27.71	30.02	32.11	35.18

[†] By difference, All HTL runs were performed for 30 min residence time (Feedstock properties of *S. platensis*: C: 46.90%; H: 7.00%; N: 10.7%; S: 0.6%; O: 34.90%; HHV: 20.50 MJ kg⁻¹); n.d.: not determined.

3.2.1. Characterization of Biocrude

The chemical and physical properties of biocrude produced are presented in Table 2. Irrespective of operating media, biocrudes obtained from all HTL runs were dark brown in color and also characterized with light smoky odor. The specific gravity was within the range of 1.05–1.13. The HHVs of biocrudes were between 26.3 and 35.2 MJ kg⁻¹. Although HHVs of resultant biocrude are significantly higher than the original feedstock (20.5 MJ kg⁻¹), they are still lower than the 42.9 MJ kg⁻¹ of petroleum-crude. This variation is mostly due to the presence of large amounts of O and N heteroatoms, suggesting upgrading of HTL-biocrude. Among the used co-solvents, formic acid resulted in a significantly lower amount of elemental oxygen when compared to other HTL runs. Consequently, formic acid biocrude had higher energy density. The higher energy dense biocrude from formic acid assisted HTL could be due to an enhanced deoxygenation reaction, such as decarboxylation, decarbonylation, and carbon addition reactions (alkylation), which lead to high carbon and low oxygen contents. Methanol-assisted (10%) HTL had the lowest HHV of 26.32 MJ Kg⁻¹, which corresponded with higher oxygen content. Removal of these undesired heteroatoms from biocrude via catalytic hydrodeoxygenation could alter its chemical composition, hence improving the quality of biocrude.

Moreover, it was found that HHVs of biocrudes obtained in the present study were in good agreement with earlier scientific studies [3,4,55,56]. HHV increased with the addition of co-solvents, except methanol. Generally, biocrudes obtained at all HTL runs had higher carbon (55–83%) and hydrogen content (8.3–9.9%) and lower oxygen content than that of the original feed stock (Table 1), as mentioned previously. The specific gravities of biocrudes ranged between 1.05 and 1.13, which was higher than that of petroleum crude (0.86). The biocrudes obtained from all HTL conditions were dark brown in color with a light smoky odor and they had an alkaline pH (7.96–9.80). The viscosity of biocrudes (measured at 60 °C) was found to be in the range of 67.8–117.6 cP (Table 2). Although these values were significantly higher than that for light (5.14 cP) and medium (22.7 cP) petroleum crude, they were still lower than the 88.03 cP of heavy petroleum crude [57]. Viscosities of algal biocrude observed in this study were found to be within the range of the previously reported values [9,11]. Higher viscosity data suggested that algal biocrudes needed suitable modification for engine uses.

The chemical composition of biocrude obtained from different co-solvents is presented in Table 3. Out of over a hundred compounds identified in the GC-MS, only the selected ones are listed in Table 3. It should be noted that compounds with relative abundance

<0.5% are not reported here. The main types of compounds identified in this study include ketones (pentanones), esters (methyl-, dimethyl- and butyl-), higher alkanes (heptadecane, hexadecane, pentadecanes), carboxylic acids (hexadecanoic acids, octadecanoic acids, phthalic acids), and aromatics. The addition of the co-solvent reported a relatively larger abundance of ketones, carboxylic acids, and alkanes than that of the HTL run without the aid of any co-solvent. Hot, compressed water aided by the polar solvents (alcohol or formic acid) enhances cracking, isomerization, defragmentation, and rearrangement reactions of the biomass macromolecules that result in the higher abundance of oxygenated heteroatoms (ketones and carboxylic acids) [58,59].

Table 3. GC-MS identification of compounds in biocrude obtained from different co-solvent HTL experiments and the control HTL run.

Compounds	RT, min	Relative Abundance, %			
		No Co-Solvent	Methanol	Ethanol	Formic Acid
4-Penten-2-one, 4-methyl-	2.79	9.17	2.87	5.78	12.85
3-Penten-2-one, 4-methyl-	3.59	57.38		46.55	50.69
2-Pentanone, 4-hydroxy-4-methyl-	4.46	29.50		24.60	14.54
Acetic acid, 1,1-dimethyl ester	4.51				5.90
Heptadecane	19.76	0.74	3.00	1.94	4.27
2-Hexadecane	21.53				4.00
9-Hexadecenoic acid, methyl ester, (Z)-	22.47		3.57		
Pentadecane		1.14			
n-Hexadecanoic acid	23.45			4.68	7.73
Hexadecanoic acid, methyl ester		0.51	29.91		
n-Hexadecanoic acid			29.20		
8-Octadecenoic acid, methyl ester	24.81		12.06		
Hexanal, O-methyloxime			7.08	4.22	
Phthalic acid, butyl ester, ester with butyl glycolate	27.23	0.62	3.20	0.95	
5-(2'-Chlorophenyl)-7-chloro-1,3-dihydro-1,4-benzodiazepine-2H-thione	29.88	2.54	4.56	3.30	1.58
1H-Pyrazole, 1-(9-borabicyclo [3.3.1]non-9-yl)-3-methyl-5-phenyl-	30.00		3.94		

Note: compounds with relative abundance <0.50% are not reported in this table.

3.2.2. Characterization of HTL Co-Product Gases

The gaseous products obtained from this present liquefaction study were sampled and analyzed. The majority of gaseous products consisted primarily of CO₂ (16–22 mol%) and small amounts of H₂, CO, CH₄, and higher hydrocarbon gases (C₂–C₅) (Table 4). The addition of methanol led to an increased CO₂ yield (23.8 mol%) compared to the control HTL run without any co-solvent (22.0 mol%), whereas the addition of ethanol and formic acid led to decreased CO₂ yield. Formic acid, being a hydrogen donor solvent, had more influence in the present study. This consequently led to higher yields of combustible gases (H₂, CO, CH₄, and C₂–C₅ gases). In addition, it resulted in the net higher fractional yields of gases and biocrude (Figure 2a). Due to decarboxylation and dehydration reactions, formic acid is believed to enhance breakdown of biomass macromolecules to H₂, CO, and CO₂ under hydrothermal conditions [56].

Furthermore, the used solvent (e.g., formic acid) could have acted as a hydrogen donor (HCOOH → H₂ + CO₂), thereby increasing efficiency of hydrogen production [58,59]. This condition suppresses condensation, cyclization, and repolymerization of free radicals that result in residue formation and hence increased gas formation. The higher hydrocarbon gases identified were: ethane, propane, butane, pentane, acetylene, butane, ethylene, and propylene. Generally, the addition of a co-solvent resulted in the higher amounts of hydrocarbon (C₂–C₅) gases (Table 4). The yield of C₂–C₅ gases was higher in the CSHTL and was the highest when formic acid was used as a co-solvent (2.59 mol% compared to only 0.45 mol% in HTL without any co-solvent). This could be due to the solvent having

lower dielectric constant and critical temperature when compared with water, consequently leading to more efficient decomposition of organic compounds [60,61]. The formation of CO, CO₂, and hydrocarbon gases could be due to a combination of hydrothermal reactions, such as decarboxylation, water gas shift reaction, methane forming reaction, and gasification of solid residues (char), which are favored by a highly reactive hot compressed water–solvent mixture. The data obtained from the gaseous composition from both co-solvent and control HTL runs were found to be similar to those from previous studies on algae [9].

Table 4. Concentration of gaseous species (relative to N₂) in co-solvent assisted HTL of *S. platensis* (at 300 °C, 30 min time, 20% solids concentration, solvent/water ratio 1:10, and 2 MPa initial N₂ pressure).

Co-Solvent Type	Yield of Gas Species (mol%)				
	H ₂	CH ₄	CO	CO ₂	^a C ₂ -C ₅
None	0.34	0.00	0.10	22.00	0.45
Methanol	0.25	0.68	1.05	23.81	1.10
Ethanol	0.51	0.96	1.04	20.09	1.67
Formic acid	2.14	1.67	1.10	16.06	2.59

^a Higher hydrocarbon gases: Ethane, propane, butane, pentane, acetylene, butane, ethylene, propylene.

This study has shown that the organic solvent could improve yield and quality of bio-oil. HTL yield was in the trend of methanol < ethanol < formic acid- assisted HTL, which suggests that biomass decomposition varies with different solvent system. In addition, based on obtained data, it could be reaffirmed that the organic solvent led to the reduction in oxygen atoms in the form of CO via decarbonylation, CO₂ through decarboxylation, and H₂O by hydrodeoxygenation reactions (in presence of catalysts), thus leading to higher energy dense biocrudes compared with non-assisted solvent liquefaction. However, the resultant biocrude cannot be used directly, as it still contains undesired heteroatoms; hence, it requires hydrotreatment. Whereas CSHTL focused on the influence of different co-solvents on HTL, HDO of algal biocrude (obtained from control run) was conducted to demonstrate the characteristics of the upgraded product.

3.3. Hydrodeoxygenation (HDO) Results

Catalytic HDO was performed using the algal biocrude generated from the control HTL run (at 300 °C, 60 min) without the addition of any co-solvent. The process water, HDO upgraded oil, and solids (catalyst and coke) from HDO were separated as outlined in the methodology. The solid residue and gas phases were not quantified in the present study. Table 5 shows the results of the HDO product oil yield and properties. The yield of HDO upgraded oil was about 40.6% (based on biocrude weight), which was ~15% of the mass of the starting algae feedstock. Studies on algal biocrude hydroprocessing suggest a product yield in the range of 41–89% [14,37,40,62], as shown in Table 5. Higher yields are generally obtained at more severe hydro-treating catalytic conditions, such as higher hydrogen pressure (>1000 psi), higher hydroprocessing temperature (>400 °C), and longer reaction times (>4 h). Catalyst type and loadings could also influence deoxygenation of molecules. In the present study, HDO was performed on the biocrude obtained from HTL at 300 °C compared to the one obtained at ≥350 °C in the other studies, and the HDO conditions were milder (725 psi H₂, 350 °C and 2 h). Although the HDO product oil yield was lower (40.6%), it is similar to the 41% reported by Biller et al. [36] for hydrotreating *Chlorella* HTL biocrude at 405 °C for 2 h using the Co/Mo catalyst. Biller et al. hydrotreated biocrude using the CoMo and NiMo catalysts at 350 and 405 °C. Operating at 350 °C led to a maximum upgraded biocrude yield of 93% but at the expense of lower HHV of 41.5 MJ kg⁻¹. A lower oil yield of 41% was achieved at 405 °C but with complete desulphurization and improved higher heating value of 44.9 MJ/kg. Importantly, there was a 60% reduction in nitrogen whereas a 85% reduction was found for oxygen content. In addition, a similar amount of 41% upgraded biocrude was obtained using CoMo at 450 °C

for 2 h [36] and 16–41% using NiMo/Al-SBA-15, Al-SBA-15 in Si/Al ratio 10–75 operating at 425 °C for 15 min for the treatment of *Chlorococcum* sp HTL biocrude [14]. However, the resultant upgraded biocrude was lower than 68.5% when Ru/C was used to treat bio-crude produced from *Chlorella pyrenoidosa*, which was lower than the 63–93% using HZSM-5, NiMo/Al₂O₃ catalyst. Using a similar catalyst (Ru/C), Bai et al. [37] reported an upgraded oil yield of 68.5%, characterized by an energy density of 45.3 MJ kg⁻¹ and hydrogen-to-carbon ratio of 1.68. The variation in their results could be mostly due to the fact the biocrude was pretreated at 350 °C (which could have enhanced its properties such as HHV and reduction in acid number), 4 h prior to hydro-treatment at 450 °C, 4 h. Based on the data presented, it could be concluded that Ru/C favored deoxygenation when compared to denitrogenation.

Furthermore, the use of Ru/C led to a 70% and 37% reduction in oxygen and nitrogen contents, respectively. Bai et al. reported similar findings, with a 69.4% reduction in oxygen content and a 40% in nitrogen content [37]. They also presented a full mass balance of their hydrotreating studies. The gaseous product fraction ranged from 7 to 14% and 14 to 29% for coke, and there were small amounts of water and losses. In the present study, water and coke formation was observed but these fractions were not quantified, as mentioned previously. Hydrotreating of biocrude over HZSM-5 catalysts at 400–500 °C in Li and Savage's study led to an upgraded oil yield of 75%, which reduced to 42% at higher temperatures [62]. Hydroprocessing of biocrude obtained from *Chlorella* at 400 °C with an H₂ atmosphere using γ -Al₂O₃ supported NiMoW, NiCoMo, CoMoW, and CoNiMoW catalysts showed that CoMoW/ γ -Al₂O₃ led to a refined oil yield of over 70 wt%. The upgraded oil was characterized with the maximum amount of C (78.6 wt%) and H (9.4 wt%) contents with a HHV of 38.7 MJ·kg⁻¹ [63]. Dirgarini et al. investigated the use of the Al-SBA-15 and NiMo/Al-SBA-15 catalysts, in the presence of Si/Al in different ratios (10–75) for hydroprocessing of HTL biocrude obtained from *Chlorococcum* sp. [14]. Their results showed that upgrading led to improved elemental species of 77.9–80.9% carbon, 9.4–10.8% hydrogen, 2.7–3.2% nitrogen, 5–9.2% oxygen, and an energy density of 38.2–42.1 MJ kg⁻¹ of the upgraded oil. In addition, there was a 50% reduction in the content of nitrogen in the upgraded oil fraction. The use of Al-SBA-15 alone led to a decrease in the upgraded oil yield and enhanced coke formation. However, the use of NiMo/Al-SBA-15, and Si/Al ratio of 10, 20 led to an increase in yields to a maximum of 65 wt%. There was no substantial effect of H₂ pressure on the yields of upgraded oil fraction, but the gas yields decreased at a higher operating pressure of 6 and 9 MPa.

Table 5 also shows the elemental composition of the upgraded oil, specific gravity, and calculated higher heating value. As shown in the table, the carbon content increased from 62.42 of the original biocrude to 77.92% of the upgraded oil. Similarly, the hydrogen content increased from 8.24% to 11.37%. There was a small reduction in N and a substantial reduction in oxygen observed. Sulphur was found to be below the detection limit in the upgraded oil. These values were found to be within the range of previous reports [14,36,59,63], as shown in Table 5. Previous studies [37,38] have shown that Ru/C performed best amongst the tested catalysts for deoxygenation of bio-oil, where it produced upgraded oil with a maximum carbon content, the lowest O/C molar ratio, and the highest HHV. This finding suggests that carbon favors the removal of oxygenates atoms from fatty acids. Compared to the untreated bio-oil use of Ru/C, there is reduced oxygen content in upgraded oil, which is in agreement with previous studies using a carbon-supported metal catalyst, as shown in Table 5.

The results indicate that HDO using Ru/C could significantly improve the net carbon (and hence the H/C ratio), and the net oxygen in the upgraded oil with an O/C ratio of 0.06 compared to 0.26 for the untreated biocrude. The removal of significant amounts of oxygen resulted in a more energy-dense product (41.6 MJ kg⁻¹), which was very close to fossil derived heavy fuel oil (42 MJ kg⁻¹). In addition, the HHV obtained in the present study was higher than 34.2 MJ kg⁻¹, using γ -Al₂O₃ as a catalyst for treating bio-oil produced from *Spirulina* sp. [36], and it was within the range of 38.2–45.3 MJ kg⁻¹ of the previous reports [14,36,39,40,50]. Furthermore, it used different catalyst support, as shown in Table 5. This finding suggests that Ru/C could also be used to hydrotreat bio-oil and to improve its fuel properties.

Table 5. Properties of upgraded products from HDO of algal biocrude using Ru/C.

Microalgae	Biocrude Properties					HHV, MJ/kg	Sp. g.	Upgrading Process Parameters			Upgraded Oil Yield and Elemental Composition							References	
	C	H	N	S	O			T °C, time	Catalyst	Atmosphere	Yield, %	Sp.g	C	H	N	S	O		HHV, MJ kg ⁻¹
<i>Spirulina platensis</i>	62.42	8.24	6.92	0.52	21.9	28.9	1.05	350, 2 h	Ru/C	H ₂ , 5 MPa	40.6	0.95	77.92	11.37	4.37	bdl	6.45	41.60	This study
<i>Chlorococcum</i> sp.	75.5	9.7	7.8	nd	5.7	38.6	Nr	425, 15 min	NiMo/Al-SBA-15, Al-SBA-15 in Si/Al ratio 10-75	H ₂ , 3–9 MPa	16–43	-	77.9–80.9	9.4–10.8	2.7–3.2	-	5–9.2	38.2–42.1	[14]
<i>Spirulina</i> sp.	75	10.4	7.7	Nr	6.9	37.7	Nr	350, 4 h	NiMo/Al ₂ O ₃	H ₂ 4 MPa	75	-	82.2	11.1	5.4	nd	1.3	41.6	[40]
	75	10.4	7.7	Nr	6.9	37.7	Nr	400, 4 h	NiMo/Al ₂ O ₃	H ₂ , 8 MPa	63	-	83.7	12.3	4.1	nr	0.0	43.5	[40]
	69.9	7.7	6.5	0.37	8.36	31.9	Nr	400, 4 h	γ-Al ₂ O ₃	H ₂	-	-	76.1	7.1	6.3	0.55	9.90	34.2	[63]
<i>Chlorella</i>	72.8	9.4	6.0	0.8	11.1	36.1	Nr	350, 2 h	CoMo	H ₂ , DMS	93	-	80.4	10.5	4.7	0.20	4.2	41.5	[36]
	72.8	9.4	6.0	0.8	11.1	36.1	Nr	450, 2	CoMo	H ₂ , DMS	41	-	84.5	11.6	2.4	0.00	1.5	44.9	[36]
<i>Chlorella pyrenoidosa</i> .	79.2	10.8	8.0	-	2.1	41.8	Nr	400, 4 h	Ru/C	H ₂ , 6 MPa	68.5	-	84.5	11.8	2.6	-	1.1	45.3	[37]
<i>Nanno.</i> sp.	76.1	9.7	5.3	0.6	8.4	38.4	Nr	400–500, 3–8 h	HZSM-5	H ₂	75	-	84.8	10.7	1.69	nr	2.81	43.4	[62]

nd: not detected; nr: not reported; bdl: below detection limit; DMS: dimethyldisulfide.

Moreover, HDO could also significantly reduce the nitrogen content with the N/C ratio of 0.05 for the HDO upgraded oil compared to 0.10 for untreated bio-oil. At room temperature, the upgraded HDO oil was found to be much lighter in color and free flowing when compared to the original bio-crude, which is in agreement with previous findings [36,37].

The analysis of the chemical composition shows the presence of alkanes, straight-chain alkanes (C₁₅-C₁₇), aromatics, and aromatic compounds in the upgraded biocrude (Figure 3). The untreated bio-oil had several long-chain saturated and unsaturated hydrocarbons, such as naphthalene, pyrrolidine derivatives, piperidine derivatives, amides, and other N-containing compounds.

Although neither a detailed characterization of the spent catalyst nor its reuse was a focus of the present study, measurements of surface properties revealed that there was a significant change in the catalyst surface properties. Even after one catalytic HDO run, the surface area and pore volume were reduced by 62.6% and 33.3%, respectively (Table 6), suggesting that there was probably a good amount of coking during the HDO run and it should be further analyzed.

As shown in Table 6, the hydroprocessing catalyst Ru/C showed the extent of coking after upgrading the fuel properties of the biocrude. Further study on the recovery and reuse of the spent catalyst is necessary. The recovery of the spent hydroprocessing catalyst has attracted considerable attention due to environmental regulations which register the spent catalyst as a hazardous waste material [64,65] Although their disposal could cause environmental issues, spent catalysts containing high metal concentrations can serve as secondary raw materials. Several approaches have been proposed for the recovery of metals from spent hydroprocessing catalysts [66], which include direct melting, calcination, and melting, chlorination, and salt roasting [67,68]. In addition, some reagents, such as NH₃, NaOH, H₂SO₄, (NH₄)₂SO₄, and oxalic acid with H₂O₂, and Fe(NO₃)₂ have been investigated [69,70].

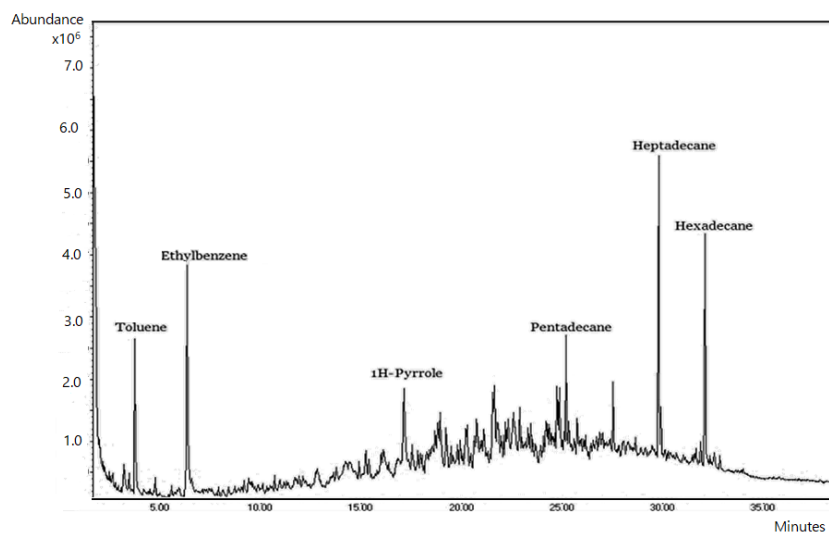


Figure 3. GC-MS analysis of compounds in the HDO upgraded product obtained from algal biocrude using Ru/C catalyst (HDO conditions: 350 °C, 2 h, 10 wt% Ru/C).

Table 6. N₂ adsorption data for fresh and used catalyst obtained from a hydrothreatment run (conducted at 350 °C, 725 psi H₂ pressure, for 2 h). Data are provided as an average of three measurements.

	Ru/C Catalyst	
	Fresh	Used
DBET surface area (m ² g ⁻¹)	721.0	269.1
Average pore size (Å)	14.4	33.0
Total pore volume (cm ³ g ⁻¹)	0.51	0.34

4. Conclusions

This study reports the experimental investigation of co-solvent use in hydrothermal liquefaction and opens a new avenue for efficiently utilizing a lipid-poor wastewater grown algae. The results show that a co-solvent influenced the HTL product yields. Biocrude yield is in the trend of methanol < ethanol < formic acid assisted HTL, which suggests that biomass decomposition varies with different solvent systems. For alcohol-assisted HTL, 50% solvent addition (5:5 solvent to water ratio) results in higher yield when compared to 10% (1:1 solvent to water ratio). The resultant biocrude from co-solvent HTL has higher energy content than that of the control run (without any co-solvent addition) and has significant amounts of oxygenated and nitrogenated compounds. This includes ketones, acids, ester, pyrroles, and small amounts of alkane-derived compounds. The hydrodeoxygenation of biocrude obtained from the control HTL run reveals that it is possible to significantly reduce the oxygen and nitrogen heteroatoms in the upgraded product oil. The significance of this work is that it shows the potential for HTL process simplification by lowering the HTL operating temperature (and pressure); hence, this research can help in building a complete flow diagram for the utilization of algae in the creation of an upgraded fuel product.

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