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Use of Co-Solvents in Hydrothermal Liquefaction (HTL) of Microalgae

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Received: 14 November 2019; Accepted: 22 December 2019; Published: 25 December 2019



Abstract: This study reviewed and summarized the literature regarding the use of alcohols during hydrothermal liquefaction (HTL) of algal biomass feedstocks. The use of both pure alcohols and alcohol-water co-solvents were considered. Based upon this review, laboratory experiments were conducted to investigate the impacts of different alcohol co-solvents (ethanol, isopropanol, ethylene glycol, and glycerol) on the HTL treatment of a specific saltwater microalga (*Tetraselmis* sp.) at two temperatures: 300 °C and 350 °C. Based on their performance, two co-solvents, isopropanol and ethylene glycol, were selected to explore the effects of varying solvent concentrations and reaction temperatures on product yields and biocrude properties. The type and amount of added alcohol did not significantly affect the biocrude yield or composition. Biocrude yields were in the range of 30–35%, while a nearly constant yield of 21% insoluble products was observed, largely resulting from ash constituents within the algal feedstock. The benefits of using alcohol co-solvents (especially isopropanol) were the reduced viscosity of the biocrude products and reduced rates of viscosity increase with biocrude aging. These effects were attributed mainly to the physical properties of the co-solvent mixtures (solubility, polarity, density, etc.) rather than chemical processes. Under the reaction conditions used, there was no evidence that the co-solvents participated in biocrude production by means of hydrogen donation or other chemical processes. Recovery and recycling of the co-solvent present various challenges, depending upon the type and amount of the co-solvent that is used. For example, glycol solvents are recovered nearly completely within the aqueous product stream, whereas simple alcohols are partitioned between the biocrude and aqueous product streams. In commercial applications, the slight benefits provided by the use of co-solvents must be balanced by the challenges of co-solvent recovery and recycling.

Keywords: hydrothermal liquefaction (HTL); *Tetraselmis* sp.; alcohols; glycols; co-solvents; microalgae

1. Introduction

The challenges of climate change and the need for energy, combined with the on-going depletion of fossil resources, have led to an interest in producing biofuels from biomass. Given its high photosynthesis efficiency, low land competition, and feasibility of cultivation in either fresh-/salt-water or wastewater, algal biomass is regarded as a promising feedstock for such biofuels. While early work focused on the extraction of algal lipids and their conversion into biodiesel fuel, more recent developments have shown that hydrothermal liquefaction (HTL) is an attractive process for converting whole, wet algae into a liquid biofuel precursor called biocrude.

The high moisture content and small particle size of microalgae make HTL a convenient conversion technique for producing biocrude [1]. Due to its ready availability, inexpensive cost, and favorable environmental factors, water is widely used as a liquefaction medium in the HTL process. However, HTL in various organic solvents, such as alcohols (methanol, ethanol, isopropanol, ethylene glycol, glycerol, etc.), phenol, acetone, tetralin, toluene, and others, has been shown to enhance biocrude yields and retard char formation for varying feedstocks (lignocellulosic, lignin, and microalgae), compared to water-only reactions [2–6]. In general, solvents with higher polarity give higher conversions [4,7].

The enhancement of biocrude yields when using organic co-solvents is generally associated with improved solubility of the organic products [8] and the feasibility of conducting the HTL process under milder reaction conditions, depending on the critical temperatures and pressures of the added organic solvent [9]. It has also been suggested that the hydrogen donation abilities of certain solvents can enhance biocrude yields. It is believed that these solvents act as both agents of hydrogenolysis (a combination of dehydration and hydrogenation) to depolymerize biomass, and as radical scavenging agents to reduce char formation [10,11]. It is hypothesized that the radicals ($\cdot\text{H}$ and $\cdot\text{CH}_3$) derived from organic solvents act as radical quenching agents to stabilize lignin-derived radicals and hence retard repolymerization and char formation [5]. Other studies point out that organic solvents, such as alcohols, help to dissolve and stabilize intermediate products, and hence reduce the condensation/repolymerization of these intermediates [12,13]. Thus, both physical processes (such as dissolution and extraction) and chemical processes (such as thermal degradation and radical scavenging) are likely affected by the use of organic co-solvents in the HTL treatment of biomass feedstocks.

Because the alcohols can serve as both solvents and reactants, their use as HTL reaction media has drawn considerable attention [14,15]. However, there is no clear literature consensus on the effects of pure alcohol on the yields or properties of biocrude obtained from the liquefaction of various feedstocks [16–19]. In addition, most alcohol co-solvent studies focused on HTL performance with woody biomass. Studies regarding HTL of algal biomass in pure alcohol or alcohol-water mixtures are discussed in detail in the following sections.

In this paper, we combined a literature review of alcohol co-solvent usage in HTL processing of algal biomass with our own experimental results. The principal aim of this paper is to explore the use of selected alcohol co-solvents in HTL treatment of *Tetraselmis* sp., a saltwater microalga grown in Qatar.

1.1. HTL of Algal Biomass in Pure Alcohols

The use of simple alcohols as the sole HTL reaction medium restricts the maximum allowable reaction temperature, due to the low critical temperatures of these alcohols ($T_c = 239\text{ }^\circ\text{C}$ for methanol and $241\text{ }^\circ\text{C}$ for ethanol). In addition, a pure alcohol reaction medium requires the complete drying of the feedstock, which entails significant energy penalties. In HTL treatment of *Sargassum tenerrimum* algae at $280\text{ }^\circ\text{C}$, Biswas et al. found biocrude yield increased from 16.33% to 22.8% and 23.8% when changing liquefaction media from water to methanol and ethanol, respectively [20]. The use of alcohol solvents also increased the aliphatic ester content of the biocrude products. Similarly, Huang et al. found fatty acid ethyl esters to be the dominant compounds in biocrude produced from HTL treatment of *Spirulina* in ethanol [21]. While this in-situ transesterification process produces esters similar to those found in biodiesel, it should be pointed out that considerable additional processing of the biocrude would be required to produce an acceptable final fuel.

Recently, several studies have been published in which supercritical alcohols were used to treat algal pastes, containing 80%–90% moisture content [22–24]. The so-called in situ technology enables simultaneous oil extraction and transesterification of the algal biomass. Unlike conventional biodiesel production processes in which strict feedstock quality is required, this one-step in situ transesterification treatment of algae can be applied to feedstocks of variable quality. Compared to conventional HTL treatment, in situ transesterifications of algae are usually conducted at milder reaction temperatures ($<250\text{ }^\circ\text{C}$) and longer reaction times. In this type of co-solvent system, a large amount of alcohol

(ethanol or methanol) is required since it plays roles both as a solvent for lipid extraction and as a reactant for transesterification.

The transesterified products include fatty acid methyl esters (FAME) when methanol is used as a solvent, and fatty acid ethyl esters (FAEE) when ethanol is used. It has been shown that the use of too much methanol can result in reduced FAME yield. In addition, the use of excess alcohol makes the final product separation procedure more energy-intensive [25]. While this direct supercritical FAME production process can utilize wet algal biomass, it relies on high lipid content algae as feedstock, requires large amounts of alcohol, and requires expensive product separation and cleanup to produce acceptable fuels.

Most experiments mentioned above were carried out using high concentrations of alcohol solvents and even pure alcohols. The use of high alcohol concentrations requires additional drying of the algal feedstock, or it results in a lower concentration of algal slurry loaded into the reactor. Both approaches involve economic and energetic penalties. Furthermore, based on the literature reports reviewed above, it is not clear that increased biocrude yield will result from the use of excess alcohol solvent.

1.2. HTL of Algal Biomass with Alcohol Co-Solvents

To overcome the energy-intensive drying process for algal feedstocks, using a water-organic solvent mixture (co-solvent) is an alternative in HTL treatment. Solvent penetration helps to extract lipids from algal cells, and the biphasic mixture enables hydrophilic ions to remain in the aqueous phase, reducing post-treatment steps [26]. Moreover, the role of water as a reactant in the HTL process is critical, and the solubility of products in the water is important. In co-solvent systems, the dielectric constant is high enough to dissolve and even ionize salts, but low enough to dissolve some non-polar compounds. The ionic product is typically high enough to favor ionic reactions, which contribute to biocrude conversion, rather than radical reactions, which promote solids and gaseous products [27].

In addition, given that the critical temperature and critical pressure of alcohol are different from those of water, the reaction pressure can be manipulated by adjusting the amount of alcohol in the co-solvent mixture. Effects of reaction pressure on biocrude yield have been extensively studied by others who varied the initial pressure with either inert gases (nitrogen or helium) or reducing gases [hydrogen or carbon monoxide (CO)] to replace air inside the reactor [28–30]. With inert gases, the main purpose of the initial pressure application is to maintain water in the liquid phase, thereby reducing the enthalpy of phase change. With reducing gases, increased pressure enhances mass transfer between the reductant and the biomass, thereby promoting desired chemical reduction reactions. Regardless of the initial pressurization, the HTL operating pressure is primarily determined by the vapor pressure of water at the selected reaction temperature. Gaseous products produced during HTL treatment can also influence the total operating pressure. Reaction pressure affects several properties of water, including density, dielectric constant, and ion product. Higher reaction pressure promotes decomposition and extraction of biomass [31]. However, there is no strong consensus about the overall effects of initial pressure and gas type on HTL treatment of microalgae, with or without the use of catalysts. Zhang's group investigated the effects of HTL processing parameters on the batch treatment of *Chlorella pyrenoidosa* [14,28]. Varying initial nitrogen pressure did not have a significant effect on the conversion of *C. pyrenoidosa* [28]. Similarly, when conducting HTL treatment of the same feedstock in pure ethanol, with and without heterogeneous catalysts, hydrogen pressurize did not significantly affect biocrude yield [14]. The same research group also conducted HTL treatment of swine manure in a continuous reactor system and found that biocrude yield decreased with CO pressurization [32].

The liquefaction process of algal biomass using different alcohols mixed with water as co-solvent has been studied extensively. A summary of published studies is given below.

1.2.1. Ethanol

Given its ready availability and its production from potential carbon-neutral sources, ethanol has been widely used as liquefaction medium with varying feedstocks [17,33,34]. With its lower

critical temperature and pressure compared to water, milder reaction conditions can be achieved with ethanol-water as the liquefaction medium. Due to its lower dielectric constant, ethanol can also dissolve relatively high-molecular-weight products derived from HTL treatment of microalgae. However, excessive amounts of ethanol in co-solvent mixtures apparently do not contribute to higher biocrude yield. When conducting HTL of *Dunaliella tertiolecta* with varying ethanol concentrations in co-solvent media, Chen et al. found that biocrude yield peaked with a 40 vol% ethanol solution [35]. Higher ethanol percentages promoted increased yield of insoluble products. In conducting co-liquefaction of mixed feedstocks (algal and lignocellulosic biomass), Feng et al. [36] found that biocrude yield was enhanced when less than 50 vol% ethanol was added as a co-solvent. Ji et al. [37] found that condensation reactions dominated when using higher ethanol content mixtures (>75 vol%), and that hydrolysis reactions were inhibited. This resulted in the biocrude yield decreasing, and the solid yield increasing.

Improvements in biocrude quality when using alcohol co-solvents are most commonly determined by calculated HHV values of the biocrude [35,38,39]. However, these values often have considerable uncertainty, especially if the oxygen content of the biocrude is estimated by mass difference. Furthermore, although a biocrude's calorific value may increase when using alcohol co-solvents, this is often accompanied by increased nitrogen content and decreased H/C ratio, both of which are undesirable with respect to biocrude quality [33,39,40]. In addition, Reduced H/C ratios are inconsistent with the often mentioned "hydrogen donor effect" of added ethanol.

In addition, ethanol exhibits a significant degree of self-association even at high reaction temperatures, which may affect the formation of solvent-solute hydrogen bonds. With increased pressure, hydrogen-bonds between ethanol molecules are more readily formed, which reduces the hydrogen donating ability as a solvent [41]. From control experiments using individual pure alcohols at 400 °C for 60 min, Jo et al. [42] found the self-decomposition of methanol, ethanol, and isopropanol to be only 0.1%, 3.2%, and 4.8%, respectively. In solvothermal liquefaction experiments with ethanol, Bondesgaard et al. [43] found only a slight formation of by-products below the critical temperature of ethanol but observed a sharp increase in the number of by-products (71 species) at 450 °C and a total ethanol decomposition of 4.86%.

1.2.2. Isopropyl Alcohol (IPA)

IPA and tetralin have been used widely as hydrogen donor solvents in catalytic transfer hydrogenation reactions of unsaturated organic compounds [44]. For example, in the presence of Ru/C catalysts, IPA was used as a hydrogen source for the conversion of furfural to methyl furan [11] and the conversion of 5-(hydroxymethyl) furfural to 2,5-dimethyl furan [45]. Despite its reported hydrogen donor ability, the application of IPA as solvent or co-solvent in the HTL process is still rare. IPA is reported to be more stable than ethanol under liquefaction treatment because the ketone derived from dehydrogenation of isopropanol (acetone) is more resistant towards decomposition than is the aldehyde derived from ethanol (acetaldehyde) [43].

Wangner et al. [9] conducted HTL treatment of lipid-extracted microalgae hydrolysate using varying concentrations of IPA (0%, 10%, 20%, 50% *v/v*) in water at 310 °C. The inclusion of IPA resulted in a significant enhancement of liquefaction yields and allowed the reaction to proceed under milder conditions compared to the pure water reaction. The maximum biocrude yield was achieved using 50 vol% IPA, although the biocrude quality was reduced due to increased nitrogen and oxygen contents. When using pure IPA, three product phases were collected from the reactor—solid, biocrude, and gas. Under these reaction conditions, the solid yields increased more than two-fold compared to conditions using IPA: water at 50:50. The increase in solids when using pure IPA is attributed largely to water-soluble ash, which comprised 50 wt% of the raw feedstock. When water was included as reaction media, these salts were dissolved and removed with the aqueous phase products; but in the absence of water, they remained part of the solid product fraction.

1.3. HTL of Algal Biomass with Glycol Co-Solvents

1.3.1. Ethylene Glycol (EG)

Ethylene glycol (EG) has been used widely in the solvolysis of wood biomass [46,47]. It was found that the dissolution of lignin is much higher with EG than with simple alcohols from methanol to n-hexanol. The total conversion of wood biomass with EG is almost the same as with the best solvent candidate—phenol.

A few recent studies have utilized EG for algal biomass liquefaction. Zou et al. [48] investigated catalytic thermal liquefaction of *Dunaliella tetiolecta* in pure EG acidified with H₂SO₄ as catalyst at 120 °C–200 °C in a flask reactor at atmospheric pressure. In this study, only the conversion rate (defined as 100%-solid yield) was reported. The conversion rate as high as 97.05% was observed. However, the biocrude obtained from this process included considerable oxygen functional groups, such as ketones, phenols, and esters, which resulted in higher total oxygen content (32%) than that in biocrude from pyrolysis of the same feedstock.

Duan et al. [4] conducted a comprehensive study on HTL conversion of *Chlorella pyrenoidosa* at 350 °C in 11 different pure solvents (water, EG, methanol, ethanol, n-propanol, isopropanol, acetone, ethyl acetate, 1,4-dioxane, tetralin, and benzene). The biocrude products were extracted by dichloromethane and recovered by rotary evaporation at 65 °C under a vacuum condition. The authors pointed out that organic solvents with high boiling points (EG and tetralin) cannot be completely recovered under these conditions. Thus, the biocrude yields using tetralin and EG were calculated by subtracting the initial loading amount of these two solvents from the remaining materials after evaporation.

1.3.2. Glycerol

Glycerol is the main by-product in the conversion of fats and vegetable oils to fatty acid derivatives in the food, cosmetics, and biodiesel industries. Due to growth in these markets, glycerol has become readily available, and its use as a “green solvent” has increased [49]. In both its crude form and purified form, glycerol has been widely used as liquefaction media for treating wood and manure biomass [10,50].

Kim et al. [51], conducted solvothermal liquefaction of oven-dried *Tetraselmis* sp. in a mixture of polyethylene glycol 400 (PEG#400) and glycerol under mild reaction conditions (from 110 °C to 210 °C) with sulfuric acid as a catalyst. The optimal liquefaction condition, where biomass conversion was 88.5%, was 190 °C for 60 min with a ratio of PEG#400/glycerol = 60/40, 5% acid loading, and 20% biomass loading.

Hu et al. [52] examined HTL treatment of *Chlorella vulgaris* powder at 275 °C for 50 min, using recycled aqueous products as the liquefaction medium. Adding glycerol to the reaction medium reduced biocrude yields compared to that obtained from HTL in pure water. Although the biocrude nitrogen content was reduced when using glycerol, the overall biocrude quality was diminished due to decreased carbon content and increased oxygen content. The authors postulated that the lower biocrude yield (and changes in composition) may result from the reduced polarity of the glycerol-containing co-solvent mixture, which changes the partitioning of organic products between the aqueous and biocrude phases.

Crude glycerol, which is a bulk emulsion of glycerol, catalyst (salts), and transesterification residues (free fatty acids and trapped fatty acid methyl esters), has also been used as a co-solvent and feedstock in the HTL process. Lu et al. [53] conducted HTL treatments of dry powder macroalgae *Enteromorpha profifera* (Ep) in water, neat glycerol, and crude glycerol. A separate aqueous product layer was observed in all cases. The highest biocrude yield (43.22%) was obtained when using a 1:5 mass ratio of algae to crude glycerol at 320 °C with 30 min reaction time. Under the same conditions, a biocrude yield of 13.35% was obtained when treating Ep in pure water. When treating crude glycerol alone (without algae feedstock), a biocrude yield of 32.35% was obtained. Thus, it was concluded that the synergetic effects between crude glycerol and feedstock did not occur, and it was not cost-effective

to liquefy algal biomass in crude glycerol. Suppression of biocrude yields when using neat glycerol (as compared to water) as liquefaction medium was reported at both mild (240 °C to 280 °C) and severe reaction condition (360 °C). At intermediate temperature (320 °C), the use of both neat and crude glycerol increased oxygen content and decreased carbon content of the biocrude product, while nitrogen content was reduced significantly in all cases. The low biocrude yields when using pure glycerol at mild reaction conditions could be attributed to the corresponding low reaction pressures. Reduced pressure may lead to incomplete reactions, especially when the feedstock solubility in pure glycerol is low.

Among the numerous reports of HTL using co-solvents, only a few mention solvent recycling [10, 47,52,54]. In these studies, the focus is on isolation and re-use of the aqueous products as a liquefaction medium rather than on the recovery of the alcohol solvents from the aqueous products. In fact, alcohol recovery is energy and cost-intensive. For the simple alcohols (ethanol and IPA), unreacted solvents can be stripped off under reduced pressure during a rotary evaporation process, along with the extraction solvent (typically DCM) and light organic products. Alternatively, in a distillation process, the solvent can be collected along with light oil products [55]. In some studies, recovered biocrude is simply placed in a drying oven to evaporate excess alcohol co-solvent, without any attempt to recover the alcohol [40]. For polyalcohols (EG and glycerol), their high boiling points make recovery difficult through either rotary evaporation or distillation. In these co-solvent cases, it is economically desirable to conduct the HTL process using an alcohol/water ratio as small as possible and to leave the unreacted co-solvent as part of the biocrude product.

In the experimental work described below, four different alcohols (ethanol, IPA, EG, and glycerol) were utilized as co-solvent candidates. The two principal aims of the present study were to investigate the effects of different alcohol-water mixtures on HTL performance and product distributions; and to investigate the impacts of varying alcohol concentrations on product yields and properties.

2. Materials and Methods

2.1. Materials

Tetraselmis sp. dry powder was provided by Qatar University [56]. The sun-dried powder was stored at 4 °C in a refrigerator before use. The proximate analysis and ultimate analysis results were described in a previous study [57]. Pure glycerol, ethanol, isopropyl alcohol, and ethylene glycol were purchased from a chemical supplier, VWR™ International (www.vwr.com). All chemicals were of reagent grade. The important physical properties of the different HTL solvents are listed in Table 1.

Table 1. Physical properties of solvents used in hydrothermal liquefaction (HTL) treatment of microalgae.

Solvent	Density, g/cm ³	Boiling Point, °C	Critical Temperature, °C	Critical Pressure		Solubility with DCM	Relative Polarity [58]
				psi	MPa		
Water	0.998	100	374	3205	22	Immiscible	1.000
Ethanol	0.79	78.3	241	913	6.3	Miscible	0.654
IPA	0.79	82.3	235	690	4.8	Miscible	0.546
EG	1.11	197.3	447	1189	8.2	Immiscible	0.790
Glycerol	1.26	290.0	577	1087	7.5	Immiscible	0.812

2.2. HTL Process

Reactions were conducted in a 1.2 L Parr stainless steel batch reactor (Model 4522, Parr Instruments, Moline, IL, USA), as described previously [57] and shown in Figure 1. The reactor was charged with 30 g of dry algal feedstock, and 270 g of the liquid medium to yield a 10 wt% loading ratio. For co-solvent HTL experiments, the alcohol concentration was calculated by the mass ratio of added alcohol to the total contents charged in the reactor. For example, 30 g of IPA and 240 g of de-ionized water together with 30 g of feedstock resulted in a 10% IPA co-solvent treatment.

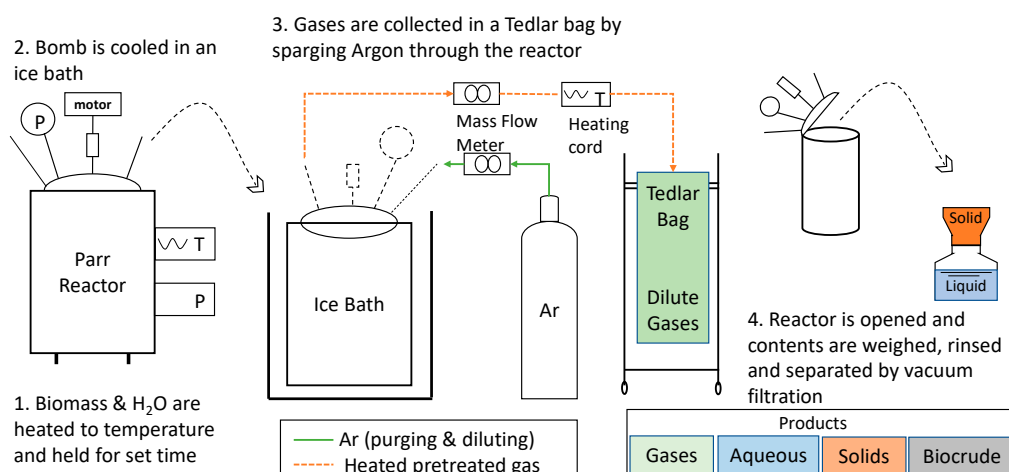


Figure 1. Flow diagram of the HTL process and product separation.

The reactor vessel was first sealed, de-oxygenated (by purging with Argon), and then heated to the desired temperature while stirring (160 rpm). The reaction parameters of temperature pressure and stirring speed were continuously recorded using a reactor controller (Model 4547, Parr Instruments, Moline, IL, USA). The product separation and isolation procedures were similar to those reported previously [57], except that more aggressive rotary evaporation conditions were required to remove IPA from the biocrude product in experiments where high concentrations of IPA co-solvent were used. In most cases, an emulsion layer was observed in the separatory funnel used during the liquid-liquid extraction process. This layer was combined with the aqueous products (AP) layer and was vacuum filtered to obtain a clear, single aqueous product solution.

All HTL experiments and results reported here were based on the products recovered when using a processing temperature of 350 °C, which was previously determined to be the optimal reaction temperature for *Tetraselmis* sp. Results from HTL processing at 300 °C are included in the Supplementary Material. For all experiments, the residence time at full reaction temperature was held at 30 min.

2.3. Characterization of Products

Methods used to characterize biocrude, gaseous, aqueous, and insoluble products from HTL processing of *Tetraselmis* sp. have been reported in detail previously [57]. Briefly, similar analyses were performed on insoluble products and feedstock. The insoluble products collected from the HTL reactor were air-dried overnight in a fume hood. The calorific energy content of samples was measured as a higher heating value (HHV) using a Parr 6200 calorimeter, equipped with a Parr 6510 water handling system. A Thermo Nicolet 6700 FTIR instrument, equipped with a diamond attenuated total reflection (ATR) crystal, which was used for infrared (IR) spectroscopic analysis. Spectra were acquired over the wavenumber range of 500 to 4000 cm⁻¹.

Biocrude products were characterized with respect to energy content using the same methods as for feedstock and insoluble products. Information about organic functional groups and chemical structure within the biocrude was obtained using the FTIR instrument mentioned above and a Varian VNMRS 500 MHz spectrometer for proton nuclear magnetic resonance (¹H NMR) spectra. Biocrude viscosity at 40 °C was determined using 2 different capillary-type viscometers (a BS/IP/RF U-Tube Reverse Flow Viscometer and a Zeitfuchs Cross-Arm Viscometer) according to the ASTM International standards D445 and D446 [59,60]. When using the BS/IP/RF viscometer, about 12 mL of the biocrude sample remained in the viscometer after the first measurement. To assess the impact of biocrude aging upon viscosity, the sample was left in the viscometer at room temperature, for the desired period of time. Then the sample was re-heated to 40 °C, and a vacuum was applied to draw the biocrude sample back to the starting level, at which point gravity flow was used to take another measurement

of viscosity. When using the Zeitfuchs cross-arm viscometer, 1–3 mL of fresh biocrude sample was introduced each time a measurement was to be made, with the remainder of the biocrude being stored (at room temperature under nitrogen) in a well-sealed vessel. The use of these 2 viscometers was meant to examine the viscosity impacts of biocrude aging under 2 different storage approaches, one involving air exposures; the other involving inert conditions.

After the aqueous and biocrude products were separated, the pH level and non-volatile residue (NVR) content of the aqueous solutions were measured immediately. The pH of the liquids was measured using a Hanna Instrument HI 8424 portable pH and temperature meter. The amount of NVR of the aqueous products (AP) was quantified by determining the mass difference of an aliquot of AP before and after overnight oven-drying at 105 °C. This NVR method was not reliable for experiments in which glycerol was used as a co-solvent. Due to the solubility differences, glycerol was partitioned nearly completely into the aqueous phase, with very little in the biocrude phase. However, due to its high boiling point, glycerol was not removed by overnight oven drying. In fact, by careful monitoring of weight loss, it was observed that stable weight was attained only after approximately one month.

Gaseous products from each HTL treatment were collected in a Tedlar bag. The gas composition was quantified on a mass basis using a GC analysis as described previously [57].

3. Results

3.1. Effect of Co-Solvent Inclusion on Product Distributions

Figure 2 shows the effects of adding 10 wt% of different alcohols as co-solvents on the product distributions (a) and pressure profiles (b) from HTL treatment of *Tetraselmis* sp. at 350 °C. Similar results when using a reaction temperature of 300 °C are presented in Figure S1 in the Supplementary Materials. Under both reaction temperatures, the reaction pressures varied with the type of co-solvent added. Specifically, the inclusion of the low boiling point alcohols—IPA and ethanol—increased reaction pressure, while high boiling point alcohols (EG and glycerol) decreased reaction pressure, as compared to the base case without co-solvent.

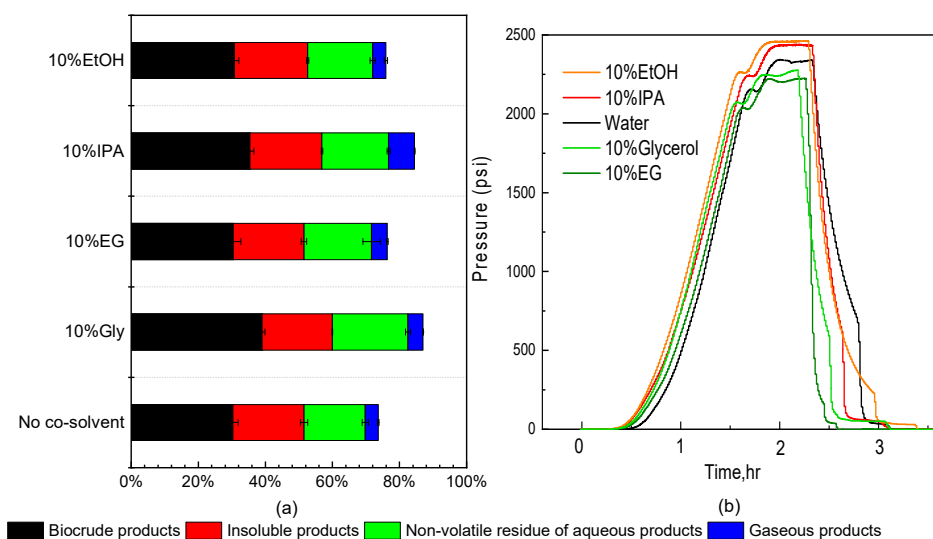


Figure 2. Effects of co-solvents on product yields (a) and reaction pressures (b) during liquefaction of *Tetraselmis* sp. at 350 °C (IPA: Isopropyl alcohol; EG: Ethylene glycol; EtOH: Ethanol; GLY: Glycerol). Error bars represent standard deviations from duplicate experiments.

Table 2 shows the yields of four HTL product streams and physical properties of these products when using different co-solvents. For comparison, the baseline results obtained without the inclusion of the co-solvent are also shown in Table 2. Similar results from HTL treatment at 300 °C are shown in Table S1 of the Supplementary Materials.

Table 2. Co-solvent effects on product yields (wt%, dry basis) and properties from HTL treatment of *Tetraselmis* sp. at 350 °C.

Co-Solvent	Biocrude Product Yield, %	Insoluble Product Yield, %	Yield of NVR of Aqueous Product, %	Gaseous Product Yield, %	Total Yield, %	pH of Aqueous Products	HHV of Biocrude, (MJ/kg)	HHV of Insolubles, (MJ/kg)	Viscosity of Biocrude ¹ , (mm ² /s @40 °C)
None	31.0 ± 2.1	21.2 ± 1.1	18.3 ± 0.9	3.9 ± 0.1	74.4	8.5 ± 0.1	33.3 ± 2.1	5.7 ± 0.9	14.7
10% IPA	35.4 ± 1.1	21.4 ± 0.2	19.8 ± 0.5	7.8 ± 0.1	84.5	8.5 ± 0.3	32.9 ± 2.3	4.6 ± 1.1	5.3 ± 0.6
10% EG	30.4 ± 2.3	21.1 ± 0.8	20.2 ± 2.6	4.7 ± 0.3	76.3	8.3 ± 0.2	34.4 ± 4.1	5.6 ± 0.3	26.1
10% EtOH	30.7 ± 1.4	21.9 ± 0.3	19.4 ± 0.8	4.0 ± 0.5	75.9	8.5 ± 0.0	34.2 ± 0.0	5.4 ± 1.2	24.5
10% GLY	39.0 ± 0.8	20.9 ± 0.1	22.6 ± 0.8	4.5 ± 0.1	87.0	8.1 ± 0.0	35.3 ± 1.8	4.8 ± 0.4	32.9±13.4

Table notes: NVR—Non-volatile residue; HHV—Higher heating value; IPA—Isopropyl alcohol; EG—Ethylene glycol; EtOH—Ethanol; GLY—Glycerol. ¹ Viscosity was measured by a Zeitfuchs cross-arm flow viscometer at 40 °C.

Apparently, the reaction pressure is not major parameter affecting biocrude yield in this study. The inclusion of 10 wt% glycerol and 10 wt% IPA as co-solvents both promoted biocrude production at 350 °C. However, reaction pressure increased with IPA as a co-solvent, but decreased with glycerol. The addition of ethanol and EG as co-solvents had no significant effect on biocrude yield. In previous HTL studies involving alcohol co-solvent treatment, observed increases of biocrude yield were attributed to synergistic effects between water and alcohol [17] or to increased formation of ester compounds resulting from the reaction of alcohol with amides and/or acids in biocrude [35]. The increased biocrude yield observed here with IPA and glycerol inclusion could be due to both physical processes (dissolution or extraction) and chemical processes. It is likely that the solubility of reaction intermediates and biocrude products was enhanced in reaction media containing these co-solvents.

During product separation steps, an emulsified layer was observed between the biocrude and aqueous phases. This emulsion layer was noticeably larger in experiments conducted under mild reaction conditions (300 °C). Co-solvent addition reduced the amount of this emulsion layer under both reaction temperatures. Similar emulsions have been observed in HTL treatment of lignocellulosic feedstock (corn stover) and were thought to originate from ash constituents in the feedstock [61]. As shown in Table 2, the pH of the AP ranged from 8.1 to 8.5. The addition of IPA and ethanol as co-solvent had no observable effect on the pH value compared to the water only baseline case, whereas the addition of EG and glycerol decreased the pH value slightly.

Regardless of co-solvent inclusion, the insoluble product yield from all experiments was nearly constant at 21%. These insolubles consisted largely of ash constituents from the raw feedstock. As reported previously, the ash content of the *Tetraselmis* sp. feedstock was approximately 16% [57].

Gaseous product yields ranged from 4% to 5% in most experiments, with CO₂ being the dominant constituent. The exception was the IPA case, where an 8% gaseous product yield was obtained, and the dominant constituent was propene (Figure 3). The produced propene was likely derived from dehydration of IPA. When the HTL process was conducted at a lower temperature (300 °C rather than 350 °C), the total gas yield was reduced, and the composition of the gases was less complex (compare Figure S2 with Figure 3). In particular, the formation of trace hydrocarbons (methane, ethene, and ethane) was enhanced at a higher reaction temperature. As previously described [57], dehydration of IPA to produce propene was enhanced by the presence of algal feedstock as compared to blank experiments using only co-solvent mixtures. It was evident that the co-solvents participated in chemical processes involved in liquefaction of the algal feedstock. This participation, which is often attributed to an alcohol's "hydrogen donor ability", appears to be more complex than simple dehydration or dehydrogenation.

3.2. Effect of Co-Solvent Inclusion on Biocrude Properties

A notable effect of co-solvent inclusion during HTL treatment of *Tetraselmis* sp. was the reduction in viscosity of the biocrude product, especially when using IPA. Figure 4 shows that the viscosity of the biocrude produced at 350 °C increased with aging, although it remained fluid for several weeks. The measured viscosities of fresh biocrude produced from various co-solvent inclusion conditions were quite consistent between the two viscometers and were all below 50 mm²/s. However, viscosities measured by the BS/IP/RF U-Tube reverse flow viscometer increased more sharply than those from the Zeitfuchs cross-arm viscometer as the biocrude aged. One likely reason for this difference was the oxidation of the biocrude in the BS/IP/RF U-Tube reverse flow viscometer, as the sample being measured was left in the viscometer (under air) for the entire week. In contrast, an aliquot of 'fresh' biocrude (stored under N₂) was used for each viscosity measurement in the Zeitfuchs cross-arm viscometer. Another reason for the accelerated aging of biocrude in the reverse flow viscometer was the loss of volatile compounds during the week-long measurement period. Although rubber stoppers were used at both ends of the viscometer to minimize evaporative losses, we still observed approximately 10%–15% mass loss at the end of a week compared to the initial loading mass.

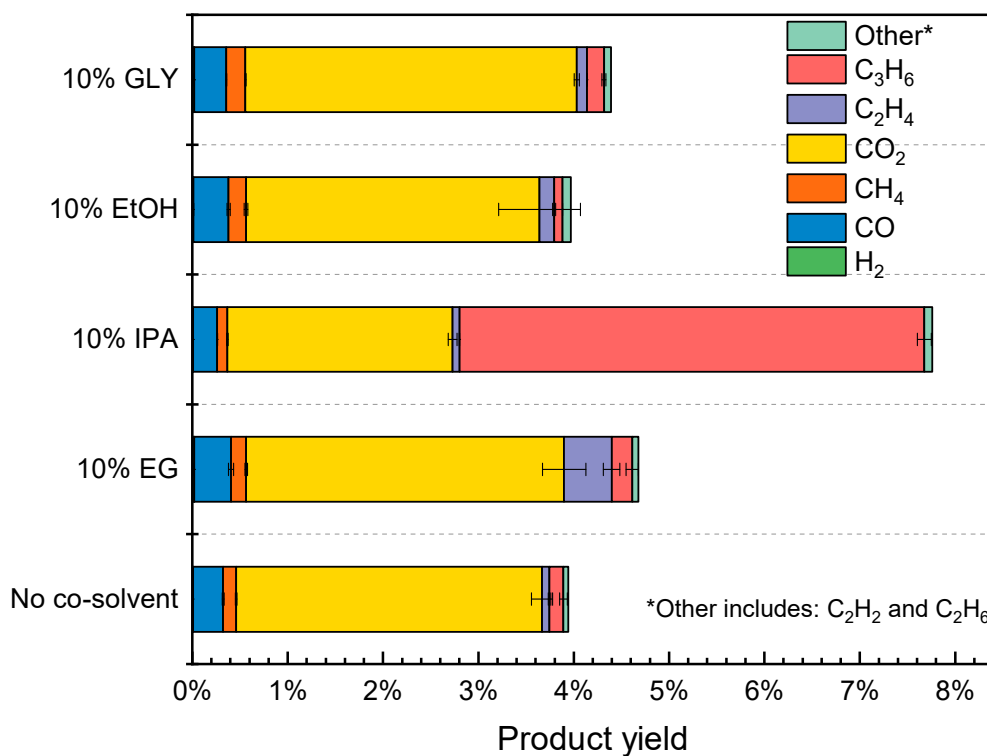


Figure 3. Gaseous product compositions and yields from HTL treatment of *Tetraselmis* sp. at 350 °C with different co-solvent inclusion (IPA: Isopropyl alcohol; EG: Ethylene glycol; EtOH: Ethanol; GLY: Glycerol). Error bars represent standard deviations from duplicate experiments.

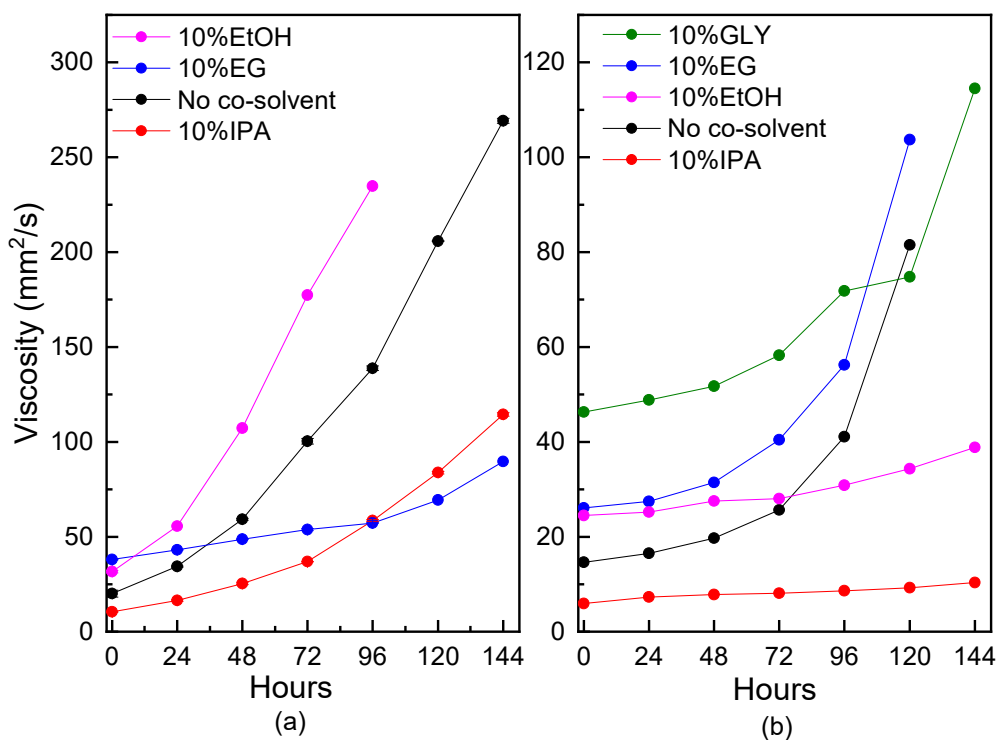


Figure 4. Biocrude viscosity measured with a BS/IP/RF U-Tube reverse flow viscometer (a) and a Zeitfuchs cross-arm viscometer (b).

The use of IPA produced a biocrude having a lower viscosity than the use of the other co-solvents (or no co-solvent). We believe this was due, in part, to the different solubilities of the co-solvents with the extraction solvent and to the rotary evaporation process used in isolating the biocrude. For example, whereas all co-solvents were miscible with water, only IPA and ethanol were also miscible with DCM. Therefore, unreacted EG and glycerol co-solvents were easily removed from the biocrude due to their solubility in water, while some of the unreacted IPA and ethanol were collected with the biocrude and required removal by rotary evaporation. Incomplete removal of IPA or ethanol can leave small amounts of the co-solvent in the recovered biocrude, which lowered the viscosity and acted as a stabilizer. Results from our previous study indicate that the use of IPA as a co-solvent did not significantly change the HHV or the elemental composition of the biocrude product. The constant H/C ratio with and without co-solvent inclusion suggested that IPA did not function as a significant hydrogen donor in this HTL process.

^1H NMR spectroscopy was used to help characterize the biocrude products and examine subtle differences among the various co-solvent treatments. As shown in Figure 5a, spectra of all co-solvent-derived biocrudes were quite different from the baseline biocrude spectra. Sharper peaks were observed in all co-solvent cases, whereas broadened peaks with low intensity were seen in the baseline case without co-solvent. Typical signals in the ^1H NMR spectra of biocrude products have been described in our previous work [57]. A series of overlapping multiplets at 0.89, 1.26, 1.55, and 1.62 ppm can be assigned to methyl and methylene groups in aliphatic fragments of compounds present in biocrude products. As shown in Figure 5b, the percentage of total protons in this region (0.5–1.5 ppm) increased slightly with IPA and ethanol inclusions, compared to the baseline. This may be due, in part, to the dissolution of unreacted alcohols in the biocrude product. The peaks near 1.22 ppm and 1.25 ppm were ascribed to protons in methyl groups in ethanol and IPA, respectively [62]. The spectral region from 1.5–3.0 ppm included protons bonded to carbon that was adjacent to nitrogen or oxygen. Only the glycerol co-solvent case showed an increase in this region, compared to baseline, which may be due to the presence of trace levels of glycerol in the biocrude. A similar analysis of the other ^1H NMR spectral regions in Figure 5 show small differences among the baseline and co-solvent cases. Of note, was the large fraction of protons in the region of 6.0–8.5 ppm, which indicated the presence of cyclic nitrogen- and oxygen-containing compounds. Overall, the results shown in Figure 5 indicated that the use of co-solvents in the HTL process had minimal impact on biocrude composition.

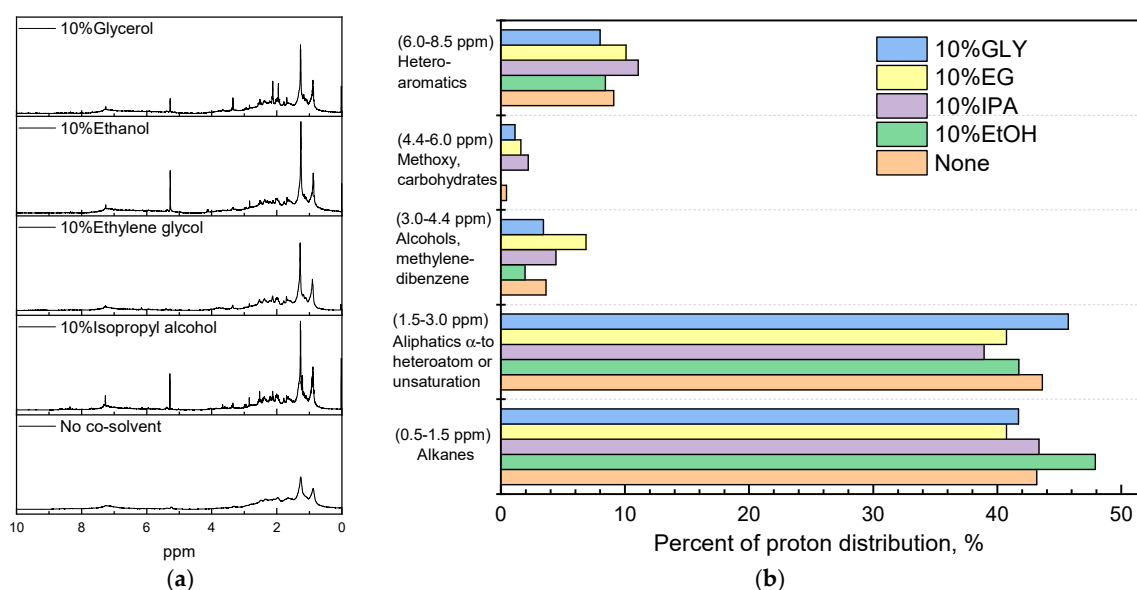


Figure 5. ^1H NMR spectra (a) and hydrogen percentage (b) of biocrude produced from hydrothermal liquefaction (HTL) treatment of *Tetraselmis* sp. at 350 °C when including different co-solvents.

Consistent with the ^1H NMR results, no marked differences were observed in the FTIR spectra of biocrude produced with different co-solvents. Because all the FTIR spectra were very similar, only that of the biocrude from glycerol inclusion is given as an example in Figure 6.

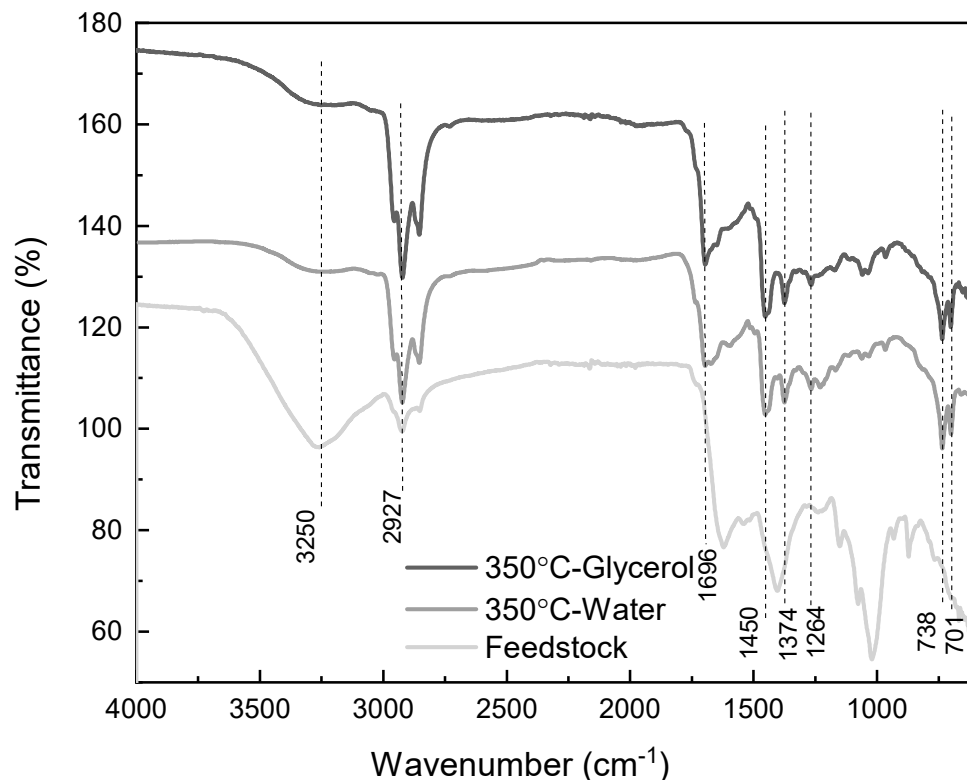


Figure 6. FTIR spectra of algal feedstock and biocrude products from HTL treatment of *Tetraselmis* sp. at 350 °C—with and without the inclusion of 10% glycerol as co-solvent.

The intense and broad band around 3300 cm^{-1} in the spectra of the *Tetraselmis* sp. feedstock was due to amino- and hydroxyl-functional groups within the protein, cellulose, and polysaccharide compounds. The reduction of this absorption band in biocrude products indicated the destruction of these biochemical components by HTL treatment. Biocrude spectra showed prominent bands caused by C–H stretching ($2860\text{--}2960\text{ cm}^{-1}$) and C–C bending (1374 cm^{-1} , 1450 cm^{-1}). This was consistent with the ^1H NMR characterization in which over 40% of the H distribution was in the region of 0.5 to 1.5 ppm, indicating the high aliphatic content of the biocrude. Numerous absorbance bands at $1150\text{--}1680\text{ cm}^{-1}$ were seen in the biocrude spectra. These indicated the presence of heteroatom (N and O) functionality, which was consistent with the observation of abundant nitrogen-containing compounds from ^1H NMR analysis. The band near 1700 cm^{-1} was suggestive of unsaturated ketones, unsaturated aldehydes, and esters groups [63]. Since no significant aldehyde functionality was observed in the ^1H NMR spectra (9.5–10 ppm), this band was likely due to other carbonyl groups such as ketones, carboxylic acids, and carboxylic acid esters [64]. Other studies using pure alcohol solvents as microalgae liquefaction media have reported the presence of an aliphatic ester band at $1740\text{--}1750\text{ cm}^{-1}$ [20,25]. The absence of such a band in this study was not surprising, given the low lipid content of the feedstock and the low alcohol concentration in the co-solvent mixture. This was consistent with the co-solvent liquefaction treatment of microalgae by Caporgno et al. [33], which showed by GC-MS analysis that esters present in the biocrude product did not depend upon the amount of ethanol used as a co-solvent.

Based on comparisons with other reported interpretations of biocrude spectra, the following tentative assignments can be made. The weak absorbance at 1373 cm^{-1} was consistent with aromatic products remaining after the breakdown of carbohydrates. The band around 1260 cm^{-1} can be

attributed to the methoxyl groups, while those between 1071 cm^{-1} and 1285 cm^{-1} were mainly due to C–O–C and C–OH groups, arising from ether and alcohol functionalities, respectively [65]. The bands at 1040 and 1080 cm^{-1} were characteristic of hydroxyl groups and can be assigned to C–O stretching vibrations in the alcohol co-solvents, and their derivatives. The bands between 701 cm^{-1} and 738 cm^{-1} indicated substituted aromatic functional groups. Consistent with the ^1H NMR analyses, these FTIR spectra indicated the presence of abundant aliphatic and cyclic alkyl functional groups in biocrude products.

3.3. Effect of Varying Co-Solvent Concentration on Product Yield

A series of liquefaction experiments were conducted to examine the effects of varying co-solvent concentration. The feedstock, *Tetraselmis* sp., was treated at $350\text{ }^\circ\text{C}$ for 30 min using IPA and EG as co-solvents at 0%, 10%, 20%, and 30%. As shown in Figure 7b, the reaction pressure increased with increasing IPA concentration but decreased with increasing EG concentration.

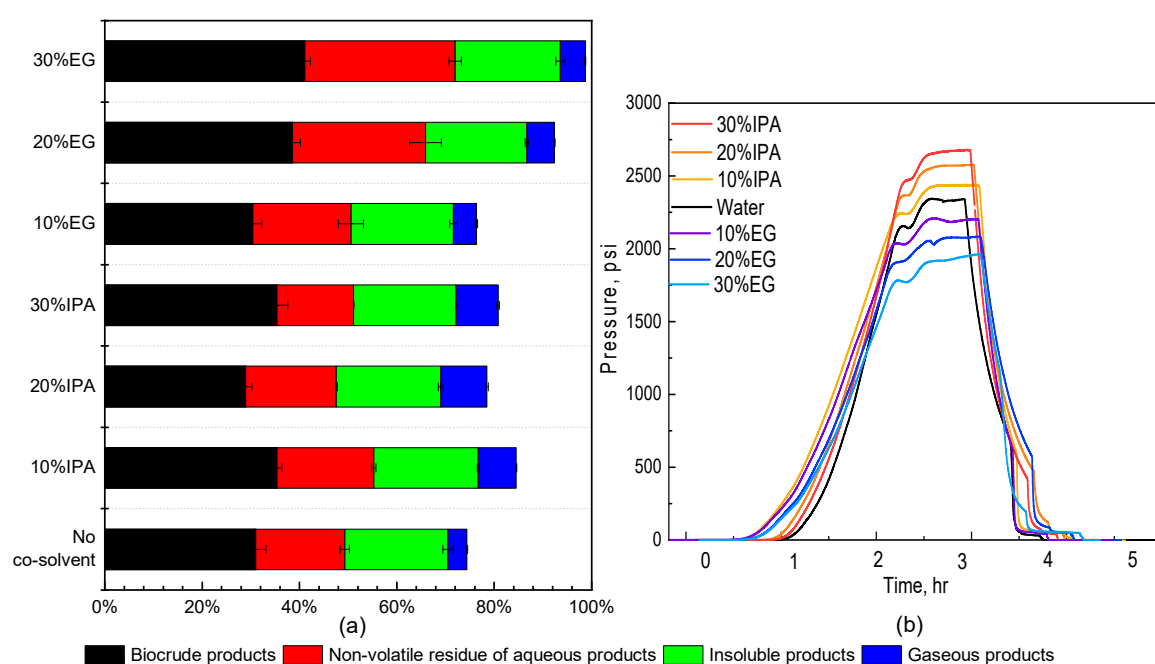


Figure 7. Effects of co-solvent concentration on product yields (a) and reaction pressures (b) during liquefaction of *Tetraselmis* sp. at $350\text{ }^\circ\text{C}$. (IPA: Isopropyl alcohol; EG: Ethylene glycol). Error bars represent standard deviation from duplicate experiments.

As shown in Figure 7a, inconsistent biocrude yields were obtained when increasing the IPA concentration: Yields of 35.4% were obtained with 10% and 30% IPA, but only 28.9% with 20% IPA. A similar biocrude yield pattern with IPA concentration was found when the liquefaction was conducted under milder reaction conditions (Figure S3). This variation in biocrude yield was likely due to a change in the product workup methodology when using higher concentrations (20% and 30%) of IPA. When using only 10% IPA as a co-solvent, biocrude was recovered from the DCM extracted filtrate in a rotary evaporator at $60\text{ }^\circ\text{C}$ under reduced pressure for 30 min. With higher concentrations of IPA, however, longer rotovap times (60–90 min) were required to remove the IPA from the biocrude completely. With this prolonged evaporation, more volatile products were likely stripped from the heavier biocrude product. This could also explain why the biocrude products from 20% and 30% IPA experiments had higher HHV and viscosity compared to biocrude from the 10% IPA experiment (see Table 3). These results illustrated that rotary evaporation parameters, such as pressure, water bath temperature, and evaporation duration, can affect the yields and properties of the recovered biocrude.

Table 3. Effect of varying co-solvent concentrations on the yields (wt%, dry basis) and properties of different product streams from HTL treatment of *Tetraselmis* sp. at 350 °C.

Co-Solvent	Biocrude Product Yield, %	Insoluble Product Yield, %	Yield of NVR of Aqueous Product, %	Gaseous Product Yield, %	Total Yield, %	pH of Aqueous Products	HHV of Biocrude, (MJ/kg)	HHV of Insolubles, (MJ/kg)	Viscosity of Biocrude ¹ , (mm ² /s @40 °C)
None	31.0 ± 2.1	21.2 ± 1.1	18.3 ± 0.9	3.9 ± 0.1	74.4	8.5 ± 0.1	33.3 ± 2.1	5.7 ± 0.9	14.7
10% IPA	35.4 ± 1.1	21.4 ± 0.2	19.8 ± 0.5	7.8 ± 0.1	84.5	8.5 ± 0.3	32.9 ± 2.3	4.6 ± 1.1	5.3 ± 0.6
20% IPA	28.9 ± 1.3	21.4 ± 0.5	18.7 ± 0.2	9.5 ± 0.3	78.5	8.6 ± 0.0	36.1 ± 0.3	2.1 ± 0.0	102.4 ± 6.9
30% IPA	35.4 ± 2.2	21.1 ± 0.0	15.8 ± 0.0	8.6 ± 0.2	80.8	8.6 ± 0.0	35.4 ± 0.4	1.1 ± 0.2	40.0 ± 15.8
10% EG	30.4 ± 2.3	21.1 ± 0.8	20.2 ± 2.6	4.7 ± 0.3	76.3	8.3 ± 0.2	34.4 ± 4.1	5.6 ± 0.3	26.1
20% EG	38.6 ± 1.6	20.8 ± 0.3	27.3 ± 3.3	5.7 ± 0.1	92.4	7.9 ± 0.0	32.7 ± 0.6	5.8 ± 0.2	10.6 ± 0.9
30% EG	41.1 ± 1.1	21.6 ± 0.9	30.9 ± 1.3	5.1 ± 0.1	98.7	7.6 ± 0.0	32.8 ± 0.2	4.8 ± 0.4	16.5 ± 1.2

Table notes: NVR—Non-volatile residue; HHV—Higher heating value; IPA—Isopropyl alcohol; EG—Ethylene glycol. ¹ Viscosity was measured by a Zeitfuchs cross-arm flow viscometer at 40 °C.

The product separation and isolation procedures were not modified to accommodate the high concentrations of EG, due to the immiscibility of this co-solvent with DCM. Because of this, nearly all added EG, regardless of the amount added, was fully dissolved in the water layer, and thus was readily separated from the DCM layer containing the biocrude. As shown in Figure 7, the biocrude yield increased with an increasing amount of EG, despite the corresponding reduction in reaction pressure. Another benefit of increased EG concentration was a reduction in the amount of emulsion layer between the DCM and water layers. It was not known whether this was related to the observed increase in biocrude yield. Although higher EG concentration increased the biocrude yield, it failed to improve the calorific value of the oil and had little effect on viscosity (see Table 3).

The relatively high pH values (8.5–8.6) of the aqueous products observed in experiments with varying IPA concentrations may result from the presence of alkali and alkaline earth metals in the raw feedstock. The pH values from the EG co-solvent experiments were noticeably lower, at 8.3, 7.9, and 7.6 for 10%, 20%, and 30% EG concentrations, respectively. To understand the effect on the pH from adding EG, a series of control experiments were conducted in which EG/water solutions (without algae feedstock) were subjected to the same HTL conditions at 350 °C. The pH value of the aqueous product stream was observed to decrease from 7.2 to 5.4 as the EG loading increased from 10% to 30%. This suggested that under severe HTL conditions, EG degrades to produce acidic species, probably acetic acid.

From all the IPA and EG co-solvent experiments shown in Table 3, the insoluble product yields remained nearly constant at 21%. These products were obtained as brown colored solids, with the color lightening as the co-solvent concentration increased (either IPA or EG). The HHVs of the insoluble products from the EG experiments were not significantly different from the baseline value (without co-solvent). With the IPA experiments, however, the HHV values of insoluble products steadily decreased with the amount of IPA addition. This suggested that while the insoluble products were primarily ash constituents from the starting algal feedstock, they also contained other materials, whose conversion or solubility was affected by the presence of the co-solvents.

As shown in Figure 3, the inclusion of 10% IPA co-solvent dramatically increased the production of propene. However, increasing the concentration of IPA to 20% and 30% did not result in a corresponding increase in propene (Figure 8). Slight decreases in CO₂ and CO formation were also observed as the IPA concentration increased. The reasons for this are unclear but may be related to greater absorption of CO₂ in the aqueous phase due to changes in pH values. Inclusion of EG as a co-solvent resulted in the higher formation of C₂H₄, CO, CO₂, and H₂, likely resulting from the dehydration and dehydrogenation of EG. Overall, the effects of added co-solvents on gaseous product yield and composition have large uncertainty and need further investigation.

3.4. Effect of Varying Co-Solvent Concentration on Biocrude Properties

The FTIR spectra of biocrude produced when using varying concentrations of IPA or EG as co-solvent are shown in Figure 9. These spectra were similar to that of the biocrude produced from the water only treatment. The intensified bands at 816 cm⁻¹ and 960 cm⁻¹, along with the bands from 1104–1159 cm⁻¹ (especially with 30 wt% IPA), were attributed mainly to the dissolution of IPA in the biocrude product. The presence of IPA in the biocrude was also supported by the observation of sharp peaks around 1.2 ppm and 4.0 ppm in the ¹H NMR spectra from the IPA co-solvent experiments (Figure S6). These peaks were attributed to hydrogen atoms within IPA itself. The broad IR band around 3300 cm⁻¹ in the spectrum from the 30% IPA experiment also indicated the presence of the OH functional group, likely due to a small amount of unreacted IPA left in the biocrude.

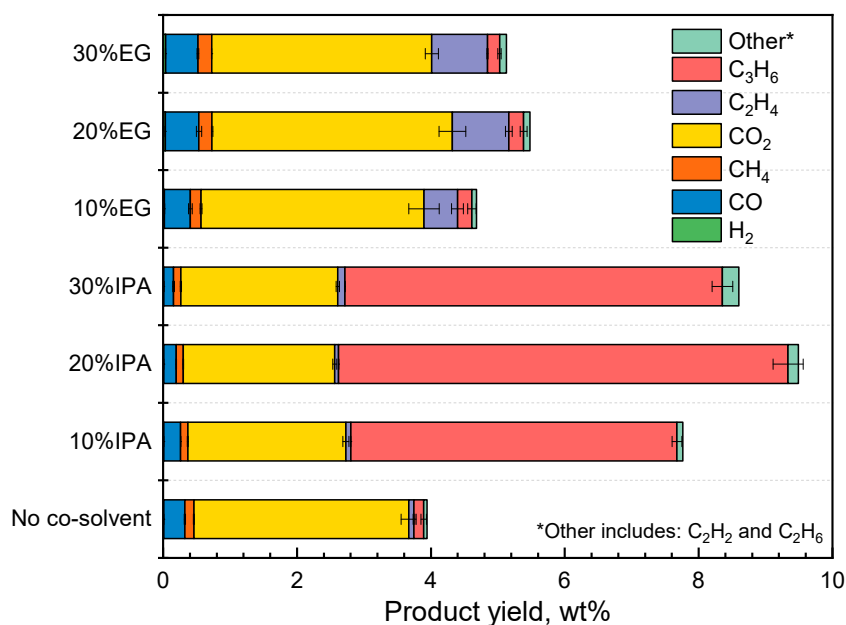


Figure 8. Gaseous products compositions and yields from HTL treatment of *Tetraselmis* sp. at 350 °C for 30 min, with varying co-solvent concentration (IPA: Isopropyl alcohol; EG: Ethylene glycol). Error bars represent standard deviations from duplicate experiments.

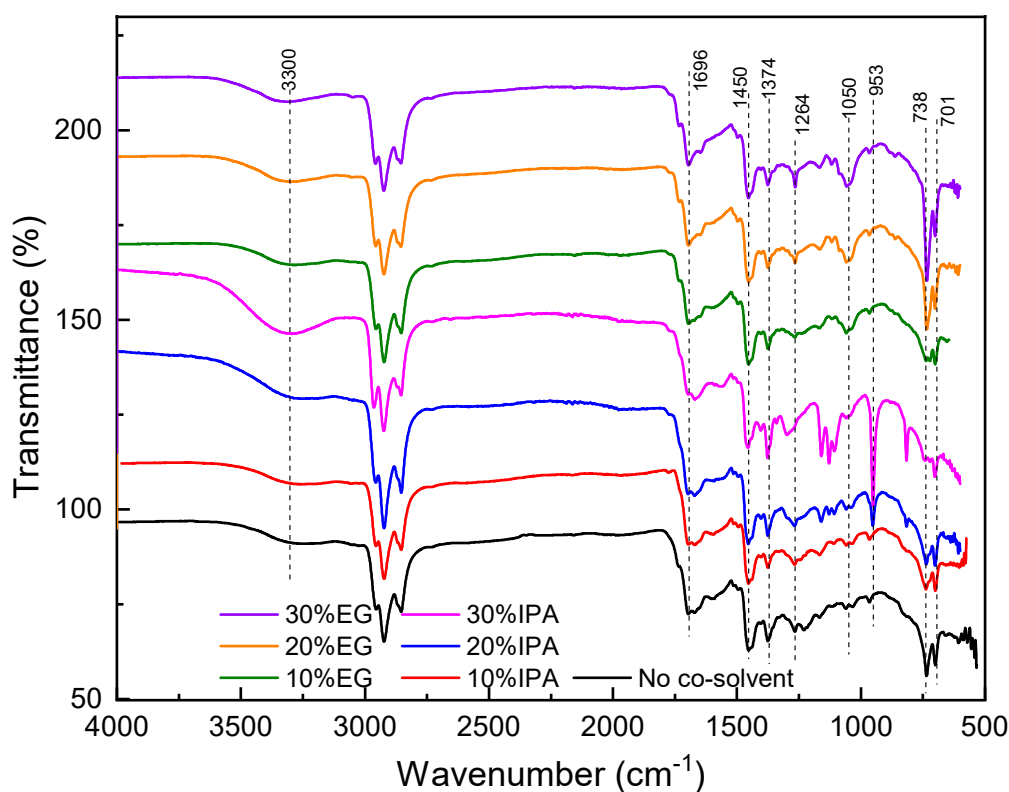


Figure 9. FTIR spectra of biocrude products from HTL of *Tetraselmis* sp. at 350 °C with the inclusion of varying amounts of co-solvent. (IPA: Isopropyl alcohol; EG: Ethylene glycol).

Little difference was seen in the IR spectra of biocrude produced using varying amounts of EG as co-solvent, except for the intensified bands from 699 cm^{-1} to 732 cm^{-1} , which were indicative of aromatic functionality. This was further supported by the IR band around 1050 cm^{-1} , which was

enhanced when using EG. These observations suggested that additional chemical processes may occur when using EG as a co-solvent.

4. Discussion

In general, co-solvents used in HTL processes should be chosen such that they readily dissolve and/or react with algal degradation products. An example that satisfies these requirements for liquefaction of lignin-containing woody biomass is phenol and its derivatives. In the present study, simple alcohols and polyalcohols were mixed with water and used as liquefaction media for *Tetraselmis* sp., marine water microalgae. It appears that physical properties of the added alcohols—such as boiling point, solubility in water, and solubility in organic solvents—have stronger effects on the liquefaction process and product distributions than do their chemical properties, such as hydrogen donor ability. This is supported by analysis of the gaseous products, which shows that the added simple alcohols primarily undergo dehydration reactions, not dehydrogenation. Furthermore, the H/C molar ratio remained nearly constant, regardless of co-solvent addition, indicating that very little hydrogen transfer from co-solvent to biocrude occurred.

To initiate hydrogenation reactions, a heterogeneous catalyst is generally required. For example, in a study where IPA was used as a hydrogen source for the conversion of furfural to methyl furan, the authors pointed out that the IPA protons do not play an active role in hydrogenation in the absence of the Ru/C catalyst [11]. In another catalytic transfer hydrogenation study using glycerol as a hydrogen donor solvent, Wolfson et al. found that dehydrogenation of glycerol in the presence of a Pd/C catalyst resulted in the formation of dihydroxyacetone [49]. In a direct catalytic liquefaction experiment using a mixture of feedstocks (straw, wood, and grass) in pure tetralin, Beauchet et al. [66] showed that the Raney nickel catalyst enhanced biocrude quality by increasing hydrogen transfer between the organic solvent and hydrotreated biocrude. However, the use of a heterogeneous catalyst is not practical in liquefaction processes involving marine algae due to the presence of high ash content, which makes recovery of the catalyst difficult.

The use of alcohols as co-solvents also poses technical and cost challenges for wastewater treatment needed to clean up the aqueous product stream from the HTL process. With IPA, for example, approximately one-half of the added alcohol is dissolved in the DCM extraction solvent, along with the biocrude product. This IPA can be recovered along with DCM through rotary evaporation, but the rest of the IPA remains in the aqueous phase. Recovery of this IPA by distillation is possible, but expensive. Alternatively, the aqueous phase IPA could be removed through conventional wastewater treatment processes. Another alternative is to simply recycle the entire aqueous product stream from the HTL process. Because consumption of the alcohol co-solvent is very low, the aqueous product stream is rich in alcohols. However, it has been reported that such recycling can also enrich the nitrogen and oxygen content of the biocrude product [52,67]. Considering the difficulties of recovering and recycling alcohol co-solvents, a preferred approach may be only to use low concentrations of co-solvents ($\leq 10\%$) and not attempt recovery.

In the present study, added alcohols appeared to function primarily as stabilizers. Evidence of this is the reduced viscosity of biocrude produced with co-solvents—especially IPA—and a reduction in the rate of viscosity increase as the biocrudes age. It is hypothesized that IPA associates with algal decomposition intermediates from the feedstock in a manner that stabilizes these materials, and prevents them from secondary reactions, such as repolymerization, which would increase their viscosity. Minami et al. pointed out that a prolonged residence time of at least 30 min was needed for the supercritical liquefaction of biomass with various kinds of alcohol [68]. Thus, this benefit of reduced viscosity with the use of alcohol co-solvents may only apply to batch reactor systems and not to continuous reactors.

5. Conclusions

This study provided a literature review of co-solvent use in HTL processing of biomass and a comprehensive description of HTL treatment of marine microalgae based on our own experiments. The published literature includes reports of various feedstocks, reaction conditions, product separation procedures, and reaction systems—making it difficult to compare the experimental results, although there is some evidence of increased biocrude yield with the addition of alcohol co-solvents. However, most reported studies focused on HTL treatment of freshwater microalgae using a single alcohol co-solvent mixture. This motivated our study focused on HTL processing of a specific marine microalgae, *Tetraselmis* sp., using a variety of water-alcohol mixtures. The investigated alcohols included ethanol, isopropanol, ethylene glycol, and glycerol. A small set of experiments was also carried out to investigate the effects of varying alcohol concentrations on HTL product yields and distributions.

The experimental work revealed that each co-solvent mixture affected reaction pressure and product distribution in a different way. It is likely that the physical properties of the added alcohols, such as boiling point, viscosity, and solubility, play more important roles in affecting the HTL process than do their chemical properties. Hydrogen donor ability of the alcohol co-solvents did not appear to be a significant factor under the experimental conditions used, as biocrude yields and compositions changed only slightly with the use of these co-solvents. Rather, the co-solvents, especially IPA and ethanol, appeared to function as stabilizing materials that reduced the viscosity of the biocrude product and the aging rate of the biocrude.

Variations in co-solvent type and amount affect the ability to isolate and quantify the HTL products. For example, recovered biocrude yield was affected by varying the concentration of IPA co-solvent, due to changes in rotary evaporation conditions necessary to remove the IPA from the biocrude. Similarly, higher NVR contents of the aqueous products were obtained when using EG as a co-solvent, due to the difficulty in evaporating this high boiling material. These methodological impacts create challenges in accurately determining the true effects of co-solvent addition upon HTL product yields and compositions.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1996-1073/13/1/124/s1>, Figure S1: Effects of co-solvents on product yields (a) and reaction pressures (b) during liquefaction of *Tetraselmis* sp. at 300 °C, Figure S2: Gaseous products compositions, and yields from HTL treatment of *Tetraselmis* sp. at 300 °C with different co-solvent inclusion, Figure S3: Effects of adding varying co-solvent concentration at 300 °C reaction temperature on product yields during liquefaction of *Tetraselmis* sp., Figure S4: Gaseous products compositions, and yields from HTL treatment of *Tetraselmis* sp. at 300 °C for 30 min with varying co-solvent concentration, Figure S5: FTIR spectra of biocrude products from HTL treatment of *Tetraselmis* sp. at 300 °C (a) and 350 °C (b)—with the inclusion of different co-solvents at 10% concentration, Figure S6: ¹H NMR spectra (a) and proton distribution (b) of biocrude produced from HTL treatment of *Tetraselmis* sp. at 350 °C with varying IPA concentrations, Table S1: Co-solvent effects on product yields (wt%, dry basis) and properties from HTL treatment of *Tetraselmis* sp. at 300 °C; Table S2: Effect of varying co-solvent concentrations on the yields (wt%, dry basis) and properties of different product streams from HTL treatment of *Tetraselmis* sp. at 300 °C.

Author Contributions: Conceptualization and methodology, Y.H. and K.H.; data curation, Y.H. and P.D.; writing—original draft preparation, Y.H.; writing—review and editing, K.H.; validation, K.H., U.J. and P.D.; funding acquisition, U.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received funding from the Qatar National Priorities Research Program (NPRP grant, grant number 8-646-4-727 from the Qatar National Research Fund).

Acknowledgments: The authors would like to thank Steve Kohl and Patrick Hurbain (DRI) for their assistance in laboratory analysis. Stephen Spain (University of Nevada, Reno) is also acknowledged for his support with FTIR and ¹H NMR analyses.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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