energies

MDPI

Article

Experimental Investigations of Physical and Chemical Properties for Microalgae HTL Bio-Crude Using a Large Batch Reactor

Farhad M. Hossain [1,2,*], Jana Kosinkova [1,2], Richard J. Brown [1,2], Zoran Ristovski [1,2], Ben Hankamer [3], Evan Stephens [3] and Thomas J. Rainey [1,2,*]

[1] Biofuel Engine Research Facility, Queensland University of Technology (QUT), Brisbane, QLD 4001, Australia; jana.adamovska@hdr.qut.edu.au (J.K.); richard.brown@qut.edu.au (R.J.B.); z.ristovski@qut.edu.au (Z.R.)

[2] International Laboratory for Air Quality and Health, Queensland University of Technology (QUT), Brisbane, QLD 4001, Australia

[3] The Institute for Molecular Bioscience, University of Queensland, 306 Carmody Road, St. Lucia, QLD 4072, Australia; b.hankamer@imb.uq.edu.au (B.H.); e.stephens@imb.uq.edu.au (E.S.)

* Correspondence: mfarhad03@yahoo.com (F.M.H.); t.rainey@qut.edu.au (T.J.R.); Tel.: +61-3138-1977 (T.J.R.)

Academic Editor: Thomas E. Amidon

Received: 21 December 2016; Accepted: 28 March 2017; Published: 5 April 2017

Abstract: As a biofuel feedstock, microalgae has good scalability and potential to supply a significant proportion of world energy compared to most types of biofuel feedstock. Hydrothermal liquefaction (HTL) is well-suited to wet biomass (such as microalgae) as it greatly reduces the energy requirements associated with dewatering and drying. This article presents experimental analyses of chemical and physical properties of bio-crude oil produced via HTL using a high growth-rate microalga *Scenedesmus* sp. in a large batch reactor. The overarching goal was to investigate the suitability of microalgae HTL bio-crude produced in a large batch reactor for direct application in marine diesel engines. To this end we characterized the chemical and physical properties of the bio-crudes produced. HTL literature mostly reports work using very small batch reactors which are preferred by researchers, so there are few experimental and parametric measurements for bio-crude physical properties, such as viscosity and density. In the course of this study, a difference between traditionally calculated values and measured values was noted. In the parametric study, the bio-crude viscosity was significantly closer to regular diesel and biodiesel standards than transesterified (FAME) microalgae biodiesel. Under optimised conditions, HTL bio-crude's high density (0.97–1.04 $kg \cdot L^{-1}$) and its high viscosity (70.77–73.89 $mm^2 \cdot s^{-1}$) had enough similarity to marine heavy fuels. although the measured higher heating value, HHV, was lower (29.8 $MJ \cdot kg^{-1}$). The reaction temperature was explored in the range 280–350 °C and bio-crude oil yield and HHV reached their maxima at the highest temperature. Slurry concentration was explored between 15% and 30% at this temperature and the best HHV, O:C, and N:C were found to occur at 25%. Two solvents (dichloromethane and *n*-hexane) were used to recover the bio-crude oil, affecting the yield and chemical composition of the bio-crude.

Keywords: microalgae; hydrothermal liquefaction (HTL); bio-crude; fatty acid methyl esters (FAME); fuel properties

1. Introduction

Microalgae are of considerable interest for the production of next-generation biofuels [1,2] that are indistinguishable from petroleum fuels based on their properties [3]. This is because using microalgae for biofuel production would have fewer adverse effects on food supply and other agriculture [4,5] as they can be cultivated on non-arable land using fresh, waste, or saline water sources and enable more efficient nutrient recycling and achieve higher productivities [6,7].

A range of conversion techniques are under development to generate biofuels from microalgae. These include solvent extraction followed by trans-esterification to produce fatty acid methyl esters (FAME), fermentation to alcohols, as well as thermochemical conversion pathways, such as pyrolysis and hydrothermal liquefaction (HTL) [8,9], to produce bio-crude oils. HTL efficiently converts wet microalgae biomass feedstock into bio-crude oil [10–12] as it eliminates the need for expensive pre-drying of the raw material. Compared to trans-esterifying lipids obtained from microalgae by solvent extraction, HTL has the potential to require less energy for the conversion process which would improve production costs [13]. The high solvent losses associated with FAME production means its financial viability is directly related to the lipid content (high lipid content being better) whereas HTL can make use of more highly productive microalgae species (i.e., more tonnes per hectare) which have higher carbohydrate content, lower lipid content and the potential for additional co-products [14]. These advantages of HTL make it a competitive route for the conversion of raw microalgae biomass to fuels [10–12].

HTL converts microalgae biomass into bio-crude oil, as well as aqueous, gas, and solid phase products at elevated pressures (5–24 MPa) and moderate temperatures (250–400 °C) [15,16]. A wide range of microalgae biomass feedstocks has been explored using HTL, including laboratory and commercially grown strains *Botryococcus braunii* [17], *Arthrospira* (*Spirulina*), *Scenedesmus* sp. [18], and *Tetraselmis* sp. [2]. Among the green microalgae the most common *Scenedesmus* sp. has high productivity although the lipid yield can be optimised to reach over 60% [19], which makes this strain attractive for biofuel production. In general, the variation in the biochemical composition, particularly the carbon chain length and the degree of saturation, affects the conversion rate and the chemical-physical properties of the HTL bio-crude [20,21].

The effect of operating conditions such as residence time, temperature, slurry concentration, and catalyst, on physical and chemical properties of microalgae HTL bio-crude have been investigated previously in limited studies [22–25]. In this study, three temperatures and slurry concentrations were investigated in the range 280–350 °C and 15–30%, respectively; temperature and concentration, as well as reaction time of 60 min was based loosely on the literature [10,23]. Higher temperatures were not explored so as to remain below water's supercritical point. Jena et al. [23] demonstrated that among the various process parameters the temperature had the strongest influence on the higher heating value (HHV), viscosity and chemical composition. HTL produces bio-crude oil that, by weight, can have 6–8 times higher oxygen and nitrogen content than heavy fuel oil (HFO) [26]. The inorganic salts in many types of HTL bio-crude oil require modifications to be compatible with a traditional refining process [2,10,27,28]. Bio-crude oil may also need subsequent upgrading to improve quality and reduce these undesired components.

Most experimental research in HTL continues to use small batch reactors (less than 1000 mL), while scale-up reactors are usually continuous. The kinetics of small size reactors or autoclaves may differ from large ones, both batch and continuous, which lead to different chemical reactions. Therefore, the results from these reactors are not fully reliable in terms of scale-up. Batch reactors are commonly used to validate the process conditions for further commercial scale. Although industry tends to prefer continuous reactors to batch reactors because of higher energy efficiency, the steady production rate and uniformity of product, large batch reactors still have several key advantages [29]:

- Pumping the biomass slurry into a continuous reactor at high pressure and temperature remains a technological challenge at the industrial scale;
- Ability to easily switch between feedstocks; and
- For a factory, individual reactors can be taken out-of-service for maintenance.

Due to the lack of bio-crude for testing, most groups only reported values at optimised parameters. The aim of this study was to conduct more comprehensive experimental analyses with three key objectives: (i) to empirically investigate the relationship between the reaction parameters and experimentally determined values physical properties of bio-crude; (ii) to evaluate how the chemical composition varies with operating conditions; and (iii) to identify the best possible fuel physical and chemical properties of microalgae HTL bio-crude. Both physical and chemical properties are needed to meet legislated diesel and FAME biodiesel standards. In further analysis, the physical properties of the best bio-crude were compared with other fuel standards to determine the similarities and reveal areas for improvement.

2. Materials and Methods

2.1. Hydrothermal Liquefaction (HTL)

The experiments were performed in a 1.8 L batch reactor system (Parr Instruments Co., Moline, IL, USA) and data were collected across three physical variables: temperature, slurry concentration, and solvents for recovering the bio-crude from the liquid mixture. The experimental temperatures used were 280 °C, 300 °C, and 350 °C, and solid concentrations in the slurry were 15%, 25%, and 30% by weight. When loading the reactor, the headspace was purged thoroughly using nitrogen to remove oxygen, and pre-pressurised to 2 bar with nitrogen gas. The reactor was heated to the desired temperature (heating rate ~3.3 °C/min) and held constant for one hour which is consistent with the literature [23,30–34]. At the end of the reaction time, the reactor was cooled by passing water through an internal cooling tube until room temperature was reached. Experiments were performed in duplicate and the average yield of two runs is reported.

After the gas was vented from the reactor, the vessel was opened and the mixture was separated (following the steps in Figure 1). The walls of the reactor were washed thoroughly with solvent (dichloromethane or *n*-hexane) and mixed with the liquid phase. The amount of solvent added was determined as the volume of the solvent per mass dry weight of algae. The solid phase was removed from the liquid mixture by vacuum filtration before being washed with the remaining solvent. The solids were then oven-dried at 105 °C overnight.

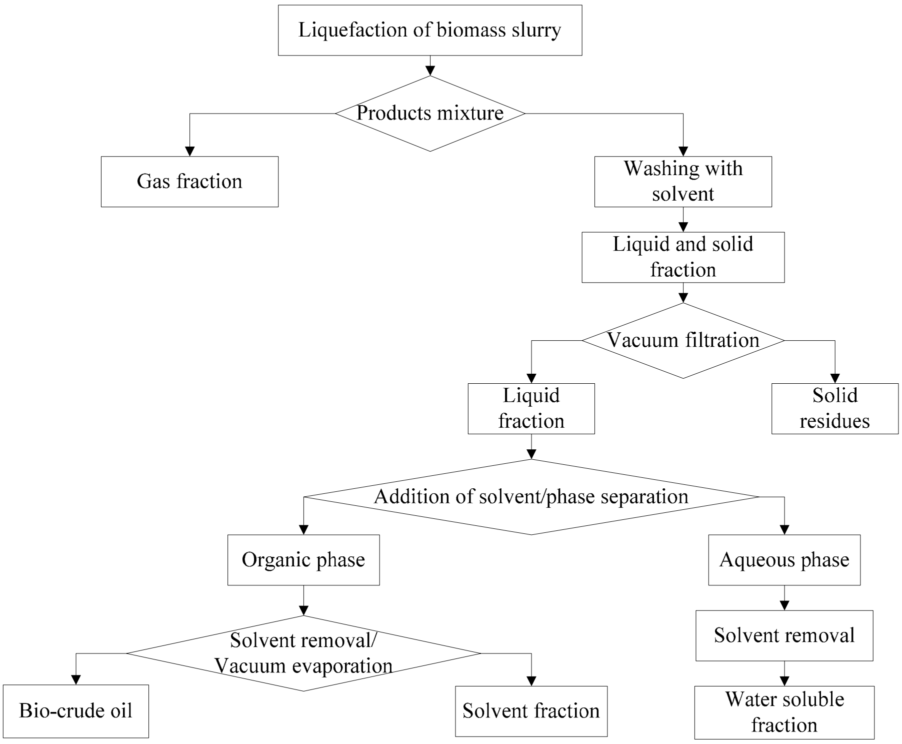

Figure 1. Hydrothermal liquefaction (HTL) product recovery workflow.

The liquid phase mixture was poured into a separation funnel and the water-insoluble components separated (organic phase) were separated from the water soluble (aqueous) phase. The solvent was evaporated from the bio-crude by a rotary vacuum evaporator. Due to the volatile nature of dichloromethane (DCM) we have used 40 °C (760 Tor) and placed an additional trap between the vacuum source and the condenser unit, whereas for *n*-hexane the temperature was set at 68 °C (760 Tor). The evaporation was continued until no further yield was obtained. The time for the DCM was shorter than that for *n*-hexane.

2.2. Raw Materials

A robust and fast growing green freshwater microalga *Scenedesmus* sp. which had been isolated as part of previous work [35] was used for these experiments. The *Scenedesmus* sp. bulk biomass was produced at the University of Queensland's Solar Biofuels Research Centre facility at Pinjarra Hills near Brisbane, Australia. High density slurry was frozen and stored at −20 °C, directly after harvest to prevent degradation during the course of the HTL experiments. Proximate analyses were performed according to American Society for Testing and Materials (ASTM) standards and using a thermo-gravimetric analyzer (TGA–NETZSCH thermal analyser, NETZSCH Australia Pty Ltd., New South Wales, Australia). Ultimate analyses were carried out using a LECO TruSpec Micro CHNS elemental analyser (LECO Corporation, Saint Joseph, MI, USA) (Table 1). The HHV of the dried microalgae sample was calculated according to the formula used by Demirbas [36] and Friedl [37], which were 18.07 MJ·kg^{-1} and 19.5 MJ·kg^{-1}, respectively, for *Scenedesmus* species microalgae. The lipid content for the samples used in this study was typically 15–20%.

Table 1. Microalgae proximate and ultimate analyses data.

Proximate Analyses		Ultimate Analyses	
Composition	**Percentage of Weight**	**Element**	**Percentage of Weight**
Fixed carbon	24.8	C	46.3
Volatile matter	67.3	H	6.9
Ash	3.2	O	32.3
Moisture	85	N	7.3
-	-	S	2.3

2.3. Analytical Methods

The conversion and product yields were defined as the mass fraction of the respective product (i.e., bio-crude and solid residue) as a function of the initial mass of biomass. For approximation, the total yield of gas + aqueous products were determined by difference according to the approach commonly used in the literature (e.g., [38]).

$$\text{Solid residues (wt.\%)} = \left(\frac{\text{mass of solid residues}}{\text{mass of raw material}}\right) \times 100\% \tag{1}$$

$$\text{Bio} - \text{crude yield (wt.\%)} = \left(\frac{\text{mass of bio} - \text{crude}}{\text{mass of raw material}}\right) \times 100\% \tag{2}$$

2.4. Bio-Crude Properties Measurements

The chemical and physical bio-crude properties such as HHV, viscosity, density, and chemical composition were measured experimentally. HHV of the bio-crude was measured with a Parr 6200 compensated jacket calorimeter (Moline, IL, USA). For comparative purposes, the correlations which are used for fossil fuels (liquid, gas and coal) form the basis of calculation applied in other research articles. Boie's and Dulong's formulae are shown Equations (3) and (4), respectively [36,39].

$$\text{HHV (MJ/kg)} = 35.16\,\text{C} + 116.225\,\text{H} - 10.09\,\text{O} + 6.28\,\text{N} + 10.465\,\text{S} \tag{3}$$

$$\text{HHV (MJ/kg)} = 0.3383\,\text{C} + 1.422\,(\text{H} - \text{O}/8) \tag{4}$$

A Brookfield DV-III ultra-programmable rheometer (Brookfield engineering laboratories, Inc., Middleboro, MA, USA) was used to measure the viscosity of the bio-crude at constant temperature. The accuracy of the rheometer for viscosity measurements is ±1.0% of full scale range for a specific spindle running at a specific speed. The density of the bio-crude was measured using German industrial standard (DIN) 1306.

Gas chromatography with mass spectroscopy (GC-MS) was used to identify the chemical compositions in the bio-crude samples. GC-MS analyses were performed using a Thermoscientific, Trace 1310 system (Thermo Scientific™, Milan, Italy), equipped with a single quadrupole mass selective detector (ISQ). Each sample was dissolved and diluted in dichloromethane. The injector was set to 250 °C and a Thermo TG-5MS (30 m long, 0.25 mm ID, 0.25 mm film) column was used. The oven was programmed at an initial temperature at 50 °C (held 1/min) then heated at a constant rate of 10 $^\circ\text{C}\cdot\text{min}^{-1}$ until a temperature of 250 °C was reached, and then held for 9 min with a split ratio of 1:25 and a column flow of 1.4 $\text{mL}\cdot\text{min}^{-1}$. The MS detector scanned from 40 to 400 m/z with a solvent cut time of 1.8 min and the ion source and transfer line temps were both set at 250 °C. The carrier mode was set to constant flow.

3. Results and Discussion

To investigate the influence of reaction conditions on product yields, HTL was conducted using a range of reaction temperatures, solid concentrations, and two types of solvents (dichloromethane and *n*-hexane) to recover the bio-crude.

3.1. Influence of Solvents in Product Separation

After completion of HTL, the bio-crude must be separated from the aqueous and solid phases. The most common solvents reported in the literature, with a focus on maximising bio-crude yield are the organic solvents dichloromethane, chloroform, acetone, and *n*-hexane [34,40]. However, there was insufficient information about the effect of these polar and non-polar solvents on the bio-crude quality. The data presented in Figure 2 was obtained from experimental runs with 25% slurry concentration at 350 °C for 60 min in a nitrogen atmosphere.

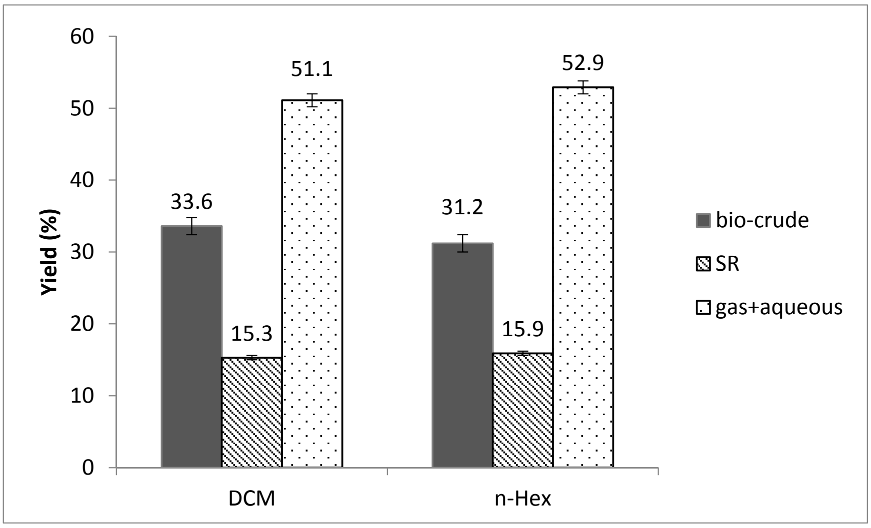

Figure 2. Effect of solvent on the recovery of bio-crude, solids and gas + aqueous components after HTL treatment (350 °C, 60 min, 25% slurry concentration).

Suitable nonpolar (*n*-hexane) and more polar (dichloromethane; DCM) solvents were selected based on previous studies and the product yields and chemical composition were compared (see Section 3.4). For the same algae species, and under identical reaction conditions, bio-crude yield varied from 31.2% (wt) with *n*-hexane to 33.6% (wt) with DCM. This differed from the results of Valdez et al. [34] who obtained the highest bio-crude yield (39%) with nonpolar solvents (hexadecane and decane) and the lowest value with 30% DCM for *Nannochloropsis* sp. at 350 °C for 60 min. This supports the view that both biomass properties and the solvent type used for separating products after HTL has an influence on bio-crude recovery. However, as *n*-hexane has a higher boiling point than DCM, this could lead to higher losses of volatile compounds during the evaporation process and this could possibly account for the small difference (2.4%). Despite this, DCM was used as a solvent of choice for product separation in all subsequent experiments.

3.2. Effect of Reaction Temperature on Yield and HHV

The effect of three different temperatures (280 °C, 300 °C, 350 °C) was investigated using a 60 min reaction time and a nitrogen atmosphere at an initial pressure of 2 bar (Figure 3). Bio-crude yield increased with temperature from 24.5% at 280 °C to 32.5% at 300 °C, and reached a maximum of 33.6% at 350 °C, in the range analysed. Similar results for temperature's influence on bio-crude yield have been reported recently [23,32,41]. The HHV was characterized for bio-crude generated at temperatures of 300 °C and 350 °C only, due to an insufficient amount of bio-crude at 280 °C. The HHV followed the trend of bio-crude yield; it increased from 26.1 MJ·kg^{-1} to 29.8 MJ·kg^{-1}, which was approximately 50% higher than in the original microalgae biomass. The solid residue yield decreased gradually from 20.1% to 15.3% with rising temperature suggesting organic conversion mostly into the aqueous and gas phases. These outcomes generally support processing at the higher temperature range where bio-crude yield was maximized. Higher temperatures than these were not investigated because of potential corrosion issues related to supercritical fluids. To this end, 350 °C was used for future experiments.

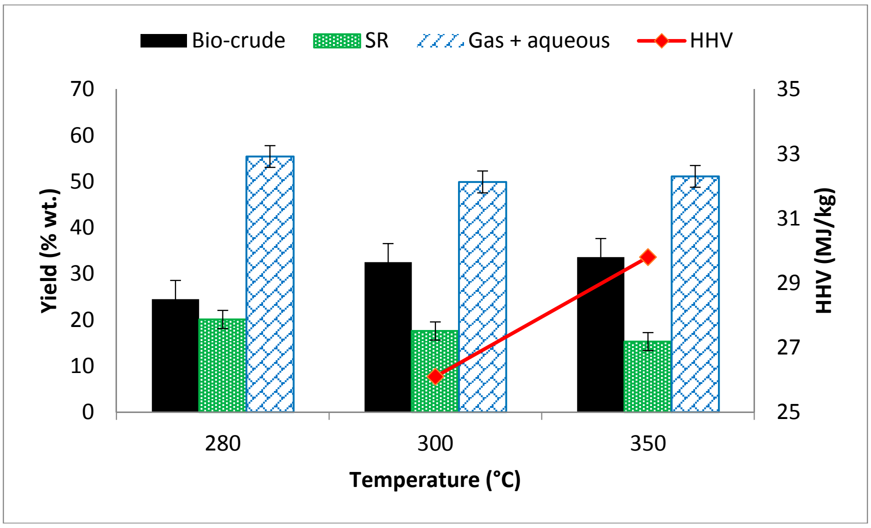

Figure 3. Influence of the reaction temperature on products yields at 25% solids concentration and 60 min reaction time in nitrogen atmosphere (2 bar at commencement). Values are for yield data.

3.3. Effects of Solid Concentration on Yield and HHV

Liquefaction of *Scenedesmus* sp. was conducted at 350 °C for 60 min in a nitrogen atmosphere using a range of algae slurry concentrations between 15% and 30% by weight (Figure 4). These results show that the solid concentration of algae slurry may have had a minor effect upon the bio-crude yield (ranging between ~28.9% and 33.6%) and HHV (26.5–29.8 MJ·kg^{-1}) with the highest yields and HHV observed at 25% biomass concentration. However, the slurry concentration did have a significant effect upon chemical speciation (see Section 3.4). A similar trend for bio-crude and the water soluble fraction was reported by Jena et al. [23]. The fact that bio-crude yield peaked at the 25% slurry concentration suggests that the substrate was limiting at lower concentrations and that H^+ and OH^- ions responsible for liquefaction become limiting at higher concentrations [23]. The presence of active hydrogen formed from the water under HTL conditions stabilizes the biomass liquefaction intermediates. This prevents formation of more new compounds that do not decompose easily, producing a higher yield of bio-crude [42]. The yield of the gas + aqueous fraction ranged between 51.1% and 60.6% over the slurry solid concentration range tested and the lowest yield 51.1% was observed at 25% solid concentration which corresponded with the highest bio-crude yields. Solid residues increased from 10.5% to 17.1% with slurry concentration due to the increase of solid mass fraction in the slurry. This corresponded with the report of Jena et al. [23] where solid residues had a small increase (5.4–7%) with solids concentration in range from 10% to 50% [23].

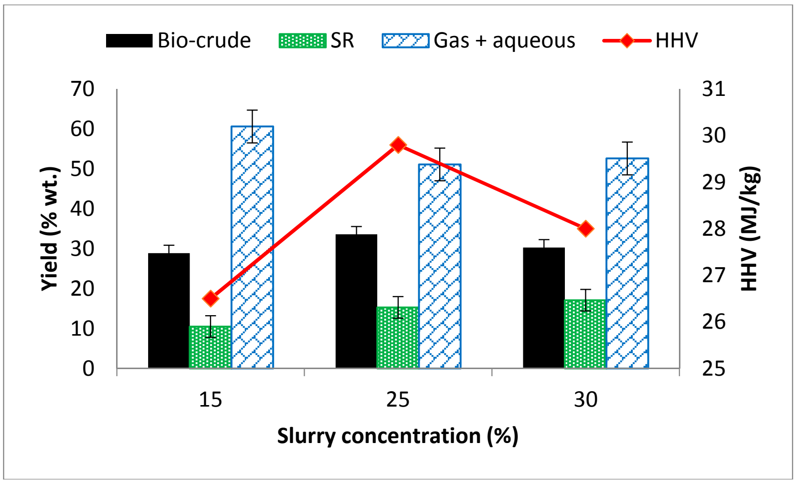

Figure 4. Effect of slurry concentration on the recovery of bio-crude, solids and gas + aqueous components after HTL treatment (350 °C, 60 min). Standard deviations are based on two replicates; values provided are for yield data.

3.4. Chemical Characterisation of Bio-Crude Oil

The chemical composition of each bio-crude oil sample was characterized by GC-MS. Due to the complex composition of the bio-crude oil only abundant compounds were evaluated based on the peak areas (defined by the percentage of the chromatographic area of the compound out if the total area). GC-MS revealed distinct amounts of chemical compounds which consisted of more than 2% of the total area within the retention time range of 3–34 min. Around 64 compounds were identified which accounted for approximately 80–90% of the total peak area from all bio-oil samples and 28 of them are listed in Tables 2 and 3. The majority of the compounds obtained were cyclic nitrogenates (e.g., pyrolle, pyrazine, piperidine) and cyclic and aromatic oxygenates (e.g., phenols, ketones) which are similar to previous studies [18,23,31]. These compounds are formed from carbohydrates and proteins obtained from the feedstock which undergo depolymerisations, decompositions, and reformation [43]. The minor compounds were mostly hydrocarbons and esters which may be derived from lipid content. Lipids can produce stable hydrocarbons via decarboxylation and decarbonylation reactions [43,44]. The tables show a comparison of the identified chemical compounds in the bio-crude oil under different reaction conditions.

Table 2 presents only the key compounds that were identified through GC-MS for both solvents. It shows the effectiveness of the *n*-hexane and dichloromethane in terms of the extraction of bio-crude oil from the water soluble fraction, independently. Dichloromethane and *n*-hexane extracted 57.1% and 40.2% of heterocyclic and aromatic compounds (mostly nitrogenated) from the water, respectively. Ketones were the most abundant oxygenated compounds. Neither solvent had a strong influence on aliphatic recovery rates, although a slight increase was observed with dichloromethane. Valdez et al. [34] also reported that the total yields of aliphatic compounds did not vary significantly between the polar to nonpolar solvents. They also found that dichloromethane extracted more light low molecular products such as aromatics, nitrogen-, oxygen-, and sulphur-obtaining compounds, than *n*-hexane [34].

Table 2. Major compounds of recovered bio-oils obtained from HTL (350 °C, 60 min, 2 bar nitrogen atmosphere) using two different extraction solvents (DCM and *n*-hexane).

RT (Retention Times, min)	Name of Compound	Area %	
		DCM	*n*-Hexane
4.5	4-hydroxy-4-methyl-2-pentanone	5.03	-
4.82	Ethylbenzene	8.86	3.38
4.98	1,4-dimethyl benzene	11.99	6.34
5.44	1,3-dimethyl benzene	3.44	5.12
5.82	2,5-dimethyl pyrazine	4.09	-
6.91	2,6-dimethyl-4-heptanone	-	4.74
7.54	Trimethyl pyrazine	2.76	4.3
7.79	2,3,5-trimethyl-1H-pyrrole	-	3.06
8.18	2,3-dimethyl-2-cyclopenten-1-one	5.32	4.36
8.49	3,7-dimethyl undecane	-	2.82
8.77	4-methyl phenol	3.53	4.45
9.06	1-acetate-1,2,3-propanetriol	2.99	7.11
9.21	Undecane	-	3.91
9.32	1-ethyl-2-pyrrolidinone	10.38	-
10.08	2,2,5,5-tetramethyl-3-cyclopenten-1-one	-	5.15
11.42	1-butyl-2-pyrrolidinone	4.25	2.72
12.93	1-pentyl piperidine	3.78	4.84
13.49	2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester, propanoic acid	-	5.11
24.91	Di(2-propylpentyl) ester, phthalic acid	10.78	-
Total	-	77.2	67.41

-, chemical compounds either not detected or peak area less than 2%.

Table 3 presents the variation of key chemical compounds from the bio-crude oil samples produced with a microalgae mass fraction in the feed ranging from 15–30% under various temperatures (280–350 °C). The main compounds include: nitrogenated compounds (pyrolle, pyrazine, piperidine); oxygenated compounds (phenols, ketones, esters); aliphatics (alkanes, alkenes); and aromatics (benzene).

Table 3. Major compounds of recovered bio-oils obtained from HTL under various solid concentrations and temperature using (DCM).

RT (min)	Name of Compound	Area (%)					
		Slurry Concentration at Fixed 350 °C Temperature			Temperature at Fixed 25% Slurry Concentration		
		15%	25%	30%	280 °C	300 °C	350 °C
4.01	2-methyl pyrimidine	3.92	-	4.05	4.94	8.18	-
4.41	4-hydroxy-4-methyl-2-pentanone	-	5.03	-	23.8	4.15	4.03
4.82	Ethylbenzene	0.68	8.86	-	1.34	1.75	8.86
4.97	1,4-dimethyl benzene	-	11.9	-	2.48	2.77	11.3
5.67	2-methyl-2-cyclopenten-1-one	4.71	-	2.72	9.19	10.0	-
5.79	2,5-dimethyl pyrazine	4.84	4.09	3.53	12.1	12.4	4.09
6.8	3-methyl-2-cyclopenten-1-one	6.66	4.86	2.31	2.7	6.0	4.5
7.1	Phenol	3.24	3.33	2.16	-	2.43	3.32
8.15	2,3-dimethyl-2-cyclopenten-1-one	12.8	12.0	5.32	2.29	5.73	6.05
9.03	1-acetate-1,2,3-Propanetriol	4.72	5.09	5.63	2.58	4.22	5.19
9.27	Undecane	9.20	11.1	12.6	2.49	5.9	8.99
9.94	1,3-diethyl-3-methyl-2,5-pyrrolidinedione	2.27	2.5	2.72	-	2.48	2.51
10.69	1-propyl-2-pyrrolidinone	3.04	2.32	3.48	-	-	2.30
11.39	1-butyl-2-pyrrolidinone	3.3	4.25	3.16	-	-	4.20
24.91	Di(2-propylpentyl) ester, phthalic acid	-	10.7	8.25	-	-	10.7

-, chemical compounds either not detected or peak area less than 2%.

As shown in Table 3, cyclic oxygenated compounds (mostly ketones such as 2,3-dimethyl-2-cyclopenten-1-one), are slightly decreasing with increasing solids concentration. This was in contrast to the aliphatic components, such as undecane, which increased with the solid concentration. Cyclic nitrogenated and oxygenated compounds including 1-butyl-2-pyrrolidinone, 1,3-diethyl-3-methyl-2,5-pyrrolidinedione, and 1-propyl-2-pyrrolidinone were the most abundant in each sample. In the low temperature reaction at 280 °C, bio-crude oil had the highest amount of oxygenated compounds mostly aliphatic, prevalent ketones, including 4-hydroxy-4-methyl-2-pentanone, 2-methyl-2-cyclopenten-1-one, and 2,3-dimethyl-2-cyclopenten-1-one. Abundance of hydrocarbons and aromatics were the lowest for the bio-crude oil at 280 °C but had the highest percentages at 350 °C. The number of identified nitrogenated compounds such as pyrazine, 2-methyl-2,5-dimethyl, pyrimidine, trimethyl and pyrazine, initially, increased with temperature and reached the maximum at 300 °C after which it decreased. Jena et al. obtained similar results from bio-crude oil comparing five different temperatures [23]. Esters such as Di(2-propylpentyl) ester, phthalic acid, 15-methyl ethyl ester, and heptadecanoic acid, were observed only at the highest temperature. The detailed analysis shows the strong dependency of chemical compounds on process condition.

3.5. Effect of Temperature and Concentration on Chemical and Physical Properties

Both the chemical and physical properties of biofuels are important to define fuel quality in terms of combustibility, density, energy content, and lubricity. These fuel properties vary with chemical composition and influence engine performance and emission results [20,45–49]. It was observed that the chemical components and compositions of the bio-crude varied with the process conditions (Tables 2 and 3) which subsequently affected the fuel's physical properties. Table 4 shows the effect of temperature and concentration on ultimate analysis and higher heating value (HHV) of the microalgae bio-crude. The chemical and physical properties of microalgae HTL bio-crude are shown in Table 5 and compared with FAME microalgae biofuel and mineral diesel.

Table 4. Ultimate analysis and higher heating value (HHV) of the microalgae bio-crude.

Component (wt. %)	**25% Slurry Concentration at Different Temperature**			**350 °C Temperature at Slurry Concentration**		
	280 °C	**300 °C**	**350 °C**	**15%**	**25%**	**30%**
C	68.1	70.4	75.6	74.1	75.6	73.7
H	9.3	8.9	10.1	9.7	10.1	9.8
O	15.7	12.1	10.3	11.2	10.3	10.8
N	6.9	8.6	4.0	5.0	4.0	5.7
H:C	0.14	0.13	0.13	0.13	0.13	0.13
O:C	0.23	0.17	0.14	0.15	0.14	0.15
N:C	0.10	0.12	0.05	0.07	0.05	0.08
HHV (Cal.), MJ·kg^{-1}	33.5	34.3	37.4	36.4	37.5	36.5
HHV(Meas.), MJ·kg^{-1}	–	26.1	29.8	26.5	29.8	28.0

3.5.1. Bio-Crude Chemical Composition

The extracted bio-crude contained a range of complex hydrocarbon groups including aliphatics, aromatics, as well as nitrogenated and oxygenated compounds. Hydrocarbons mostly contained either oxygen or nitrogen in the C–H chain. Table 4 shows the elemental composition of the bio-crude obtained at different temperatures with 25% slurry concentrations and for different slurry concentration at 350 °C. The elemental analysis showed that the mass percentage of oxygen decreased from 16% to 10% with increasing temperature of the experiment for 25% slurry. In contrast, the hydrogen percentage was relatively stable: within 8.93–10.14% over the experimental range. The differences in chemical composition and molecular structure (e.g., C, H, and O composition, straight chain, cyclic, and heterocyclic compounds) affect the physical properties of the bio-crude oil including higher heating value, density, viscosity, cetane number, and surface tension; important parameters for internal combustion (IC) engines. The slow heat-up and cooling down time can potentially lead to polymerisation and secondary reactions. It is expected that the engine performance and exhaust emissions would be different compared to FAME biodiesel. For instance, the oxygen creates a permanent dipole moment which results in stronger hydrogen bonding and oxygenated fuels with increased molecular affinity. Consequently, compressibility is decreased because the free space between the molecules is similar. A follow-on effect is an increase in NO_x [45]. Nabi et al. [50] has also shown NO_x emissions and adiabatic flame temperature present a linear decrease with increasing oxygen content.

O:C, N:C, and H:C significantly affect fuel quality and emissions, and so the temperature variation and slurry concentration effect on bio-crude were compared with diesel and FAME biodiesel standards in the Van Krevelen diagram (Figure 5) [51,52]. It is important to note that the lowest N:C and O:C were found at the same conditions which gave maximum bio-crude yield and HHV (i.e., 350 °C and 25% slurry concentration; for this range, the author's viewed lower O:C to be more beneficial). Figure 5 shows that O:C changes with respect to N:C ratio, and H:C is almost constant; thus, N:C and O:C varied more than H:C. The HHV is the highest at 350 °C due to the low O:C and relatively good H:C. N:C also sharply reduced at high temperature. The bio-crude oils were more similar to FAME biodiesel than fossil fuel diesel in terms of H:C while the nitrogen concentration was much higher than diesel and biodiesel standards due to the high amount of proteins in the raw feedstock. We note that if *n*-hexane had been used for the extraction rather than DCM the polarity of the bio-crude would fall and, consequently, the point would shift to the left on Figure 5 due to the lower oxygen content.

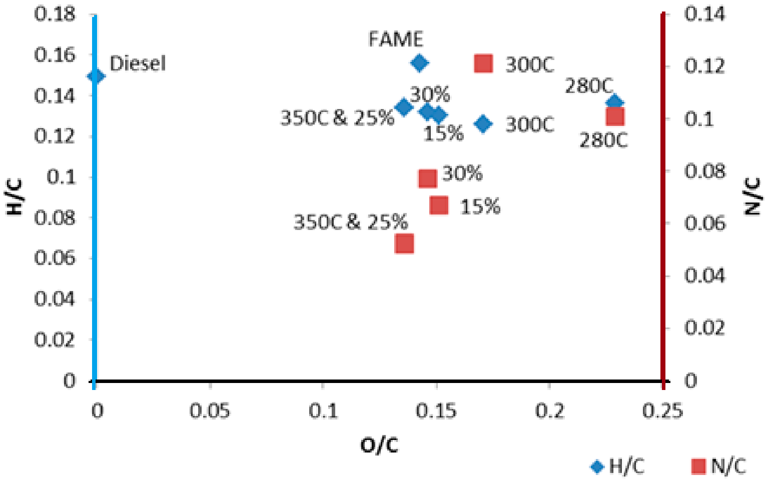

Figure 5. Van Krevelen diagram of bio-crudes gained for different temperatures (280 °C, 300 °C, and 350 °C) and slurry concentration (15%, 20% and 30%) in comparison with diesel and FAME biodiesel standards [53].

The separated bio-crude oil samples had a dark colour, high viscosity, and an acrid smoky odour. Chemical and physical properties were analysed for the best bio-crude produced at 350 °C, and 25% initial solids concentration and compared with various types of fuel standards (Table 5).

Table 5. Comparison of chemical and physical properties of bio-crude produced at 350 °C and 25% solids with trans-esterified microalgae biodiesel, diesel, biodiesel, and marine fuels standards. *: at 20° C.

Name of the Properties	HTL *Scenedesmus* sp. Biocrude Oil	FAME *Crypthecodinium cohnii* Biodiesel [52]	Biodiesel Standards EN 14214	Petroleum Diesel	Marine Fuels ISO 8217
Kinematic viscosity@40 °C ($mm^2 \cdot s^{-1}$)	70.7–73.8	5.06	3.5–5	2.64	1.4–11.0
Density @15 °C ($kg \cdot L^{-1}$)	0.97 *	0.91	0.86–0.9	0.84	0.96–0.99
HHV ($MJ \cdot kg^{-1}$)	29.7	39.8	-	44	44–45
Oxygen content (wt. %)	10.3	10.4	-	0	-
Hydrogen Content (wt. %)	10.1	11.1	-	13.8	-
Carbon Content (wt. %)	75.5	78.4	-	86.1	-
Nitrogen Content (wt. %)	3.97	0	-	0	-

3.5.2. Viscosity

Under the best conditions, the viscosity of the HTL microalgae bio-fuel was closer to conventional diesel and biodiesel than FAME from microalgae and varied with chemical composition, possibly due to the varying degree of chemical saturation [54]. The variation of bio-crude compositions influenced the intermolecular forces. The variation in viscosity can potentially affect injection timing, spray, atomization, and combustion compared to microalgae FAME [28]. There is a possibility to use bio-crude in a heavy-duty diesel engine or a marine ship engine with minimal upgrading.

3.5.3. Density

The fuel density affects the mass of fuel injected because fuel injection systems in modern diesel engines measure the fuel on a volume basis [45]. At best conditions, the density of the HTL microalgae bio-crude was 13% higher than conventional diesel fuel and not comparable with any fuel standards in Table 5. The increased density of the HTL microalgae bio-crude might be due to it containing many aromatic hydrocarbons and cyclic chemicals as well as an amount of high molecular weight compounds that are beyond detection in GC-MS. However, the density value (0.97 $kg \cdot L^{-1}$) is comparable to the marine residual fuel standard ISO 8217:2012 (0.96–0.99 $kg \cdot m^{-3}$).

3.5.4. Higher Heating Value

Among the fuels, the HHV of microalgae bio-crude had the lowest value 29.8 $MJ \cdot kg^{-1}$ (best H:C) [55]. Enrichment in double bonds generally results in lower than expected levels of heats of combustion due to strong intra-molecular bonding [56]. The vapour heat capacity and thermal conductivity relates to the heating value of the fuel. These affect droplet-surrounding heat transfer, temperature distribution and the mass air fuel ratio that will reduce combustion performance [57].

3.6. Comparison with Previous Studies

The findings of this study are compared with previous workers in Table 6 where most researchers worked with very small batch reactors (<100 mL) although there has been a few studies with larger continuous reactors (typically ~1000 mL). The vast majority of HTL studies report physical properties, HHV in particular, derived from correlations which use elemental analysis results for gaseous, liquid, coal and biomass materials [36,39]. Our calculated values for HHV are similar to that reported elsewhere, but we also report a much lower measured value which was also reported by Li [58], although despite the different oxygen content.

The density and viscosity of the bio-crude are not widely reported (Table 6). The bio-crude viscosity was significantly closer to regular diesel and biodiesel standards than transesterified (FAME) microalgae biodiesel. Under optimised conditions, HTL bio-crude's high density (0.97–1.04 $kg \cdot L^{-1}$) and its high viscosity (70.7–73.8 $mm^2 \cdot s^{-1}$) had enough similarity to marine heavy fuels that it could be immediately used without further processing, although the measured higher heating value, HHV, was lower (29.8 $MJ \cdot kg^{-1}$).

Table 6. Comparison of results from this study with literature.

HTL Bio-Crude	Reactor Type	Reactor Volume (mL)	Operating Conditions		Yield wt. %	Physicochemical Properties								References
			Temperature (°C)	Time (min)		Chemical Composition %				HHV (MJ/kg)		Kinematic Viscosity (mm^2/s)	Density (kg/L)	
						C	H	N	O	Calculated	Measured			
Scenedesmus sp.	Batch reactor	1800	350	60	33.6	75.6	10.1	3.97	10.3	37.4	29.8	70.7–73.8	0.97	Current study
		500	300	30	45	72.6	9.0	6.5	10.5	35.5	-	-	-	[59]
Enteromorpha prolifera		25	300	30	23	64.5	7.7	5.4	22.4	30.8	-	-	-	[60]
Lemna sp.		25	350	30	17.5	72.1	7.8	4.6	15.5	32.8	-	-	-	[61]
Laminaria saccharina		75	350	15	19.3	82.0	7.1	4.9	6.0	37.4	-	-	-	[27]
Chlorella pyrenoidosa		17.2	350	60	41	75.1	9.9	7.3	7.7	38.1	-	-	-	[62]
Nannochloropsis sp.		35	350	60	43	76.0	10.3	3.9	9.0	39	-	-	-	[32]
Enteromorpha prolifera		25	370	40	31.7	77.9	9.6	5.6	6.9	39.4	-	-	-	[10]
Sargassum patens C. Agardh		1000	340	10	32.1	64.6	7.4	2.5	25.5	-	27.1	-	-	[58]
Saccharina sp.	Continuous-flow reactor	1000	340	87	58.8	79.4	8.0	4.1	8.5	37.5	-	-	-	[63]
NB238		1000	350	-	38	78.6	10.4	4.2	5.3	-	-	-	-	[64]

4. Conclusions

This paper studied the relationship of HTL operating conditions (reaction temperature and slurry concentration) on the chemical pomposities and physical and chemical properties of the microalgae bio-crude. Physical properties were able to be measured experimentally because of the larger scale of the reactor rather than being derived using correlations based on elemental analysis. We note a significant difference in the values for calculated and measured values, which is consistent with the literature. The highest bio-crude oil yield (33.6%) was produced at 350 °C and at 25% solids concentration. The aliphatic, aromatic, nitrogen containing hetero-cyclic, oxygenated compounds were the major group of components of HTL microalgae bio-crude. These conditions also produced the lowest O:C and N:C. The bio-crude had higher density and lower HHV than diesel and biodiesel and was closer in character to heavy fuel oil where it could be used directly. Future work will focus on further improving chemical and physical properties, such as HHV, density, and decreasing N and O percentages, via catalytic upgrading, or via reactions in situ, followed by engine testing.

Acknowledgments: This work was financially supported by the QUT Early Career Academic Recruitment and Development program and by a Ph.D. scholarship from the QUT School of Chemistry, Physics, and Mechanical Engineering. The authors thank to the QUT Central Analytical Research Facility for their assistance.

Author Contributions: All authors contributed to this publication and have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Islam, M.; Magnusson, M.; Brown, R.; Ayoko, G.; Nabi, M.; Heimann, K. Microalgal species selection for biodiesel production based on fuel properties derived from fatty acid profiles. *Energies* **2013**, *6*, 5676–5702. [CrossRef]
2. Eboibi, B.E.-O.; Lewis, D.M.; Ashman, P.J.; Chinnasamy, S. Hydrothermal liquefaction of microalgae for biocrude production: Improving the biocrude properties with vacuum distillation. *Bioresour. Technol.* **2014**, *174*, 212–221. [CrossRef] [PubMed]
3. Azad, A.K.; Rasul, M.G.; Khan, M.M.K.; Sharma, S.C.; Hazrat, M.A. Prospect of biofuels as an alternative transport fuel in Australia. *Renew. Sustain. Energy Rev.* **2015**, *43*, 331–351. [CrossRef]
4. Oncel, S.S. Microalgae for a macroenergy world. *Renew. Sustain. Energy Rev.* **2013**, *26*, 241–264. [CrossRef]
5. D'Ippolito, G.; Sardo, A.; Paris, D.; Vella, F.M.; Adelfi, M.G.; Botte, P.; Gallo, C.; Fontana, A. Potential of lipid metabolism in marine diatoms for biofuel production. *Biotechnol. Biofuels* **2015**, *8*, 28. [CrossRef] [PubMed]
6. Kosinkova, J.; Doshi, A.; Maire, J.; Ristovski, Z.; Brown, R.; Rainey, T.J. Measuring the regional availability of biomass for biofuels and the potential for microalgae. *Renew. Sustain. Energy Rev.* **2015**, *49*, 1271–1285. [CrossRef]
7. Subramanian, S.; Barry, A.N.; Pieris, S.; Sayre, R.T. Comparative energetics and kinetics of autotrophic lipid and starch metabolism in chlorophytic microalgae: Implications for biomass and biofuel production. *Biotechnol. Biofuels* **2013**, *6*. [CrossRef] [PubMed]
8. Guccione, A.; Biondi, N.; Sampietro, G.; Rodolfi, L.; Bassi, N.; Tredici, M.R. Chlorella for protein and biofuels: From strain selection to outdoor cultivation in a Green Wall Panel photobioreactor. *Biotechnol. Biofuels* **2014**, *7*, 84. [CrossRef] [PubMed]
9. Naveena, B.; Armshaw, P.; Tony Pembroke, J. Ultrasonic intensification as a tool for enhanced microbial biofuel yields. *Biotechnol. Biofuels* **2015**, *8*. [CrossRef] [PubMed]
10. Xu, Y.-P.; Duan, P.-G.; Wang, F. Hydrothermal processing of macroalgae for producing crude bio-oil. *Fuel Process. Technol.* **2015**, *130*, 268–274. [CrossRef]
11. Bennion, E.P.; Ginosar, D.M.; Moses, J.; Agblevor, F.; Quinn, J.C. Lifecycle assessment of microalgae to biofuel: Comparison of thermochemical processing pathways. *Appl. Energy* **2015**, *154*, 1062–1071. [CrossRef]
12. López Barreiro, D.; Prins, W.; Ronsse, F.; Brilman, W. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects. *Biomass Bioenergy* **2013**, *53*, 113–127. [CrossRef]

13. Peng, X.; Ma, X.; Lin, Y.; Wang, X.; Zhang, X.; Yang, C. Effect of process parameters on solvolysis liquefaction of Chlorella pyrenoidosa in ethanol–water system and energy evaluation. *Energy Convers. Manag.* **2016**, *117*, 43–63. [CrossRef]
14. Amin, S. Review on biofuel oil and gas production processes from microalgae. *Energy Convers. Manag.* **2009**, *50*, 1834–1840. [CrossRef]
15. Srirangan, K.; Akawi, L.; Moo-Young, M.; Chou, C.P. Towards sustainable production of clean energy carriers from biomass resources. *Appl. Energy* **2012**, *100*, 172–186. [CrossRef]
16. Zhang, L.; Xu, C.; Champagne, P. Overview of recent advances in thermo-chemical conversion of biomass. *Energy Convers. Manag.* **2010**, *51*, 969–982. [CrossRef]
17. Dote, Y.; Sawayama, S.; Inoue, S.; Minowa, T.; Yokoyama, S.-Y. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. *Fuel* **1994**, *73*, 1855–1857. [CrossRef]
18. Vardon, D.R.; Sharma, B.K.; Blazina, G.V.; Rajagopalan, K.; Strathmann, T.J. Thermochemical conversion of raw and defatted algal biomass via hydrothermal liquefaction and slow pyrolysis. *Bioresour. Technol.* **2012**, *109*, 178–187. [CrossRef] [PubMed]
19. Mandal, S.; Mallick, N. Microalga Scenedesmus obliquus as a potential source for biodiesel production. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 281–291. [CrossRef] [PubMed]
20. Rahman, M.M.; Pourkhesalian, A.M.; Jahirul, M.I.; Stevanovic, S.; Pham, P.X.; Wang, H.; Masri, A.R.; Brown, R.J.; Ristovski, Z.D. Particle emissions from biodiesels with different physical properties and chemical composition. *Fuel* **2014**, *134*, 201–208. [CrossRef]
21. Demirbas, A. Progress and recent trends in biodiesel fuels. *Energy Convers. Manag.* **2009**, *50*, 14–34. [CrossRef]
22. Hossain, F.M.; Rainey, T.J.; Ristovski, Z.; Brown, R.J. Performance and exhaust emissions of diesel engines using microalgae FAME and the prospects for microalgae HTL biocrude. *Renew. Sustain. Energy Rev.* **2017**, submitted.
23. Jena, U.; Das, K.C.; Kastner, J.R. Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*. *Bioresour. Technol.* **2011**, *102*, 6221–6229. [CrossRef] [PubMed]
24. Xu, D.; Savage, P.E. Characterization of biocrudes recovered with and without solvent after hydrothermal liquefaction of algae. *Algal Res.* **2014**, *6*, 1–7. [CrossRef]
25. Eboibi, B.E.; Lewis, D.M.; Ashman, P.J.; Chinnasamy, S. Effect of operating conditions on yield and quality of biocrude during hydrothermal liquefaction of halophytic microalga *Tetraselmis* sp. *Bioresour. Technol.* **2014**, *170*, 20–29. [CrossRef] [PubMed]
26. Zheng, Z.; Tang, X.; Asa-Awuku, A.; Jung, H.S. Characterization of a method for aerosol generation from heavy fuel oil (HFO) as an alternative to emissions from ship diesel engines. *J. Aerosol Sci.* **2010**, *41*, 1143–1151. [CrossRef]
27. Anastasakis, K.; Ross, A.B. Hydrothermal liquefaction of the brown macro-alga Laminaria Saccharina: Effect of reaction conditions on product distribution and composition. *Bioresour. Technol.* **2011**, *102*, 4876–4883. [CrossRef] [PubMed]
28. Ramirez, J.A.; Brown, R.J.; Rainey, T.J. A review of hydrothermal liquefaction bio-crude properties and prospects for upgrading to transportation fuels. *Energies* **2015**, *8*, 6765–6794. [CrossRef]
29. Smook, G. *Handbook for Pulp and Paper Technologists*; TAPPI Press: Quebec, QC, Canada, 1982; Chapter 8; p. 76.
30. Biller, P.; Riley, R.; Ross, A. Catalytic hydrothermal processing of microalgae: Decomposition and upgrading of lipids. *Bioresour. Technol.* **2011**, *102*, 4841–4848. [CrossRef] [PubMed]
31. Biller, P.; Ross, A. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresour. Technol.* **2011**, *102*, 215–225. [CrossRef] [PubMed]
32. Brown, T.M.; Duan, P.; Savage, P.E. Hydrothermal liquefaction and gasification of *Nannochloropsis* sp. *Energy Fuels* **2010**, *24*, 3639–3646. [CrossRef]
33. Matsui, T.-O.; Nishihara, A.; Ueda, C.; Ohtsuki, M.; Ikenaga, N.-O.; Suzuki, T. Liquefaction of micro-algae with iron catalyst. *Fuel* **1997**, *76*, 1043–1048. [CrossRef]
34. Valdez, P.J.; Dickinson, J.G.; Savage, P.E. Characterization of product fractions from hydrothermal liquefaction of *Nannochloropsis* sp. and the influence of solvents. *Energy Fuels* **2011**, *25*, 3235–3243. [CrossRef]
35. Jakob, G.; Wolf, J.; Bui, T.V.; Posten, C.; Kruse, O.; Stephens, E.; Ross, I.; Hankamer, B. Surveying a diverse pool of microalgae as a bioresource for future biotechnological applications. *J. Phylogenet. Evol. Biol.* **2013**, *4*, 1000153.1–1000153.8. [CrossRef]

36. Demirbas, A. Calculation of higher heating values of biomass fuels. *Fuel* **1997**, *76*, 431–434. [CrossRef]
37. Friedl, A.; Padouvas, E.; Rotter, H.; Varmuza, K. Prediction of heating values of biomass fuel from elemental composition. *Anal. Chim. Acta* **2005**, *544*, 191–198. [CrossRef]
38. Yu, G.; Zhang, Y.; Schideman, L.; Funk, T.L.; Wang, Z. Hydrothermal liquefaction of low lipid content microalgae into bio-crude oil. *Am. Soc. Agric. Eng.* **2011**, *54*, 239–246.
39. Boie, W. Fuel technology calculations. *Energietechnik* **1953**, *3*, 309–316.
40. Xu, C.; Etcheverry, T. Hydro-liquefaction of woody biomass in sub-and super-critical ethanol with iron-based catalysts. *Fuel* **2008**, *87*, 335–345. [CrossRef]
41. Garcia Alba, L.; Torri, C.; Samorì, C.; van der Spek, J.; Fabbri, D.; Kersten, S.R.; Brilman, D.W. Hydrothermal treatment (HTT) of microalgae: Evaluation of the process as conversion method in an algae biorefinery concept. *Energy Fuels* **2011**, *26*, 642–657. [CrossRef]
42. Huang, H.; Yuan, X.; Zeng, G.; Wang, J.; Li, H.; Zhou, C.; Pei, X.; You, Q.; Chen, L. Thermochemical liquefaction characteristics of microalgae in sub-and supercritical ethanol. *Fuel Process. Technol.* **2011**, *92*, 147–153. [CrossRef]
43. Toor, S.S.; Rosendahl, L.; Rudolf, A. Hydrothermal liquefaction of biomass: A review of subcritical water technologies. *Energy* **2011**, *36*, 2328–2342. [CrossRef]
44. Peterson, A.A.; Vogel, F.; Lachance, R.P.; Fröling, M.; Antal, M.J., Jr.; Tester, J.W. Thermochemical biofuel production in hydrothermal media: A review of sub-and supercritical water technologies. *Energy Environ. Sci.* **2008**, *1*, 32–65. [CrossRef]
45. Varatharajan, K.; Cheralathan, M. Influence of fuel properties and composition on NO_x emissions from biodiesel powered diesel engines: A review. *Renew. Sustain. Energy Rev.* **2012**, *16*, 3702–3710. [CrossRef]
46. Giakoumis, E.G. A statistical investigation of biodiesel physical and chemical properties, and their correlation with the degree of unsaturation. *Renew. Energy* **2013**, *50*, 858–878. [CrossRef]
47. Pandey, R.K.; Rehman, A.; Sarviya, R.M. Impact of alternative fuel properties on fuel spray behavior and atomization. *Renew. Sustain. Energy Rev.* **2012**, *16*, 1762–1778. [CrossRef]
48. Kegl, B. Effects of biodiesel on emissions of a bus diesel engine. *Bioresour Technol.* **2008**, *99*, 863–873. [CrossRef] [PubMed]
49. Vajda, B.; Lešnik, L.; Bombek, G.; Biluš, I.; Žunič, Z.; Škerget, L.; Hočevar, M.; Širok, B.; Kegl, B. The numerical simulation of biofuels spray. *Fuel* **2015**, *144*, 71–79. [CrossRef]
50. Nabi, M.N. Theoretical investigation of engine thermal efficiency, adiabatic flame temperature, NOx emission and combustion-related parameters for different oxygenated fuels. *Appl. Therm. Eng.* **2010**, *30*, 839–844. [CrossRef]
51. Senneca, O. Kinetics of pyrolysis, combustion and gasification of three biomass fuels. *Fuel Process. Technol.* **2007**, *88*, 87–97. [CrossRef]
52. Yuliansyah, A.T.; Hirajima, T. *Efficacy of Hydrothermal Treatment for Production of Solid Fuel from Oil Palm Wastes*; INTECH: Rijeka, Croatia, 2012.
53. Tyson, K.S.; McCormick, R.L. *Biodiesel Handling and Use Guidelines*; DIANE Publishing: Collingdale, PA, USA, 2006.
54. Boelhouwer, J.; Nederbragt, G.; Verberg, G. Viscosity data of organic liquids. *Appl. Sci. Res.* **1951**, *2*, 249–268. [CrossRef]
55. Vihar, R.; Seljak, T.; Rodman Oprešnik, S.; Katrašnik, T. Combustion characteristics of tire pyrolysis oil in turbo charged compression ignition engine. *Fuel* **2015**, *150*, 226–235. [CrossRef]
56. Lang, X.; Dalai, A.K.; Bakhshi, N.N.; Reaney, M.J.; Hertz, P. Preparation and characterization of bio-diesels from various bio-oils. *Bioresour. Technol.* **2001**, *80*, 53–62. [CrossRef]
57. Abramzon, B.; Sazhin, S. Convective vaporization of a fuel droplet with thermal radiation absorption. *Fuel* **2006**, *85*, 32–46. [CrossRef]
58. Li, D.; Chen, L.; Xu, D.; Zhang, X.; Ye, N.; Chen, F.; Chen, S. Preparation and characteristics of bio-oil from the marine brown alga Sargassum patens C. Agardh. *Bioresour. Technol.* **2012**, *104*, 737–742. [CrossRef] [PubMed]
59. Vardon, D.R. Hydrothermal Liquefaction for Energy Recovery from High-Moisture Waste Biomass. Master's Thesis, University of Illinois at Urbana Champaign, Champaign, IL, USA, 2012.
60. Zhou, D.; Zhang, L.; Zhang, S.; Fu, H.; Chen, J. Hydrothermal liquefaction of macroalgae enteromorpha prolifera to bio-oil. *Energy Fuels* **2010**, *24*, 4054–4061. [CrossRef]

61. Duan, P.; Chang, Z.; Xu, Y.; Bai, X.; Wang, F.; Zhang, L. Hydrothermal processing of duckweed: Effect of reaction conditions on product distribution and composition. *Bioresour. Technol.* **2013**, *135*, 710–719. [CrossRef] [PubMed]
62. Duan, P.; Bai, X.; Xu, Y.; Zhang, A.; Wang, F.; Zhang, L.; Miao, J. Catalytic upgrading of crude algal oil using platinum/gamma alumina in supercritical water. *Fuel* **2013**, *109*, 225–233. [CrossRef]
63. Elliott, D.C.; Hart, T.R.; Neuenschwander, G.G.; Rotness, L.J.; Roesijadi, G.; Zacher, A.H.; Magnuson, J.K. Hydrothermal processing of macroalgal feedstocks in continuous-flow reactors. *ACS Sustain. Chem. Eng.* **2013**, *2*, 207–215. [CrossRef]
64. Elliott, D.C.; Hart, T.R.; Schmidt, A.J.; Neuenschwander, G.G.; Rotness, L.J.; Olarte, M.V.; Zacher, A.H.; Albrecht, K.O.; Hallen, R.T.; Holladay, J.E. Process development for hydrothermal liquefaction of algae feedstocks in a continuous-flow reactor. *Algal Res.* **2013**, *2*, 445–454. [CrossRef]