

Full Length Article

Evaluation of lipid extractability after flash hydrolysis of algae

Ali Teymouri^a, Kameron J. Adams^a, Tao Dong^b, Sandeep Kumar^{a,*}^a Department of Civil & Environmental Engineering, Old Dominion University, Norfolk, VA 23529, USA^b National Bioenergy Center, National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401, USA

ARTICLE INFO

Keywords:

Microalgae
Flash hydrolysis
Lipid analysis
Oil extraction
Kinetics study
Hydrothermal liquefaction

ABSTRACT

Microalgae is identified as a promising feedstock for producing renewable liquid transportation fuels; however, lipids extraction from microalgae for downstream processing to biofuels is one of the important challenges for algal based biorefineries. This work aims at evaluating the potential of applying flash hydrolysis (FH) as a chemical-free technique to increase the lipids extractability of algal biomass as well as its integration with the hydrothermal liquefaction (HTL) of microalgae to enhance the biocrude yields and characteristics for fuel production. To this aim, the FH process was performed on three different algal species (*Scenedesmus* sp., *Nannochloropsis* sp., and *Chlorella vulgaris*) at 280 °C and 10 s of residence time. Following FH, in addition to the nutrients rich hydrolysate, approximately, 40 wt% of solids containing almost all (> 90 wt%) the lipids termed as biofuels intermediates (BI), were recovered. Kinetics study on lipids extractability from the BI and their lipid profile analyses were conducted for each algal species. The results showed that the FH process had significantly enhanced the lipids extractability. For all three algae species, lipid yields from BI were higher than that of the raw algae. Lipid yields of *Chlorella vulgaris* in the first 15 min were more than five times higher (52.3 ± 0.8 vs. 10.7 ± 0.9 wt%) than that of raw algae during n-hexane based solvent extraction. The kinetics of lipids extractability followed a zero-order reaction rate for all wet raw microalgae and the BI of *Scenedesmus* sp., while the BI recovered from the other two algal species were determined as a second-order reaction. Comparison of fatty acids profiles indicated the contribution of the FH process in saturating fatty acids. Subsequent to lipids extraction, a conventional hydrothermal liquefaction was performed at 350 °C and 1 h to compare the biocrude yields from raw versus BI of *Chlorella vulgaris* microalgae. The results showed that the biocrude yields from the BI and its quality was significantly enhanced post FH than that of raw algae. The FH process was proven to be a viable option for lipid extraction by increasing the extent of recovery and decreasing the extraction time. Its integration with HTL notably impact the biocrude yields and characteristics for fuel production.

1. Introduction

Efforts to reduce fossil fuel consumption have been attempted around the world with the aims of mitigating negative environmental harms such as air and water pollution, establishing energy independence, and inspiring innovation in alternative fuels development. The dependence on fossil fuels is heavily ingrained into society, while alternative energy only accounts for less than 10% of the global energy supply according to the United States Department of Energy [1]. Outstanding biological photosynthetic carbon assimilation potential is one of the main reasons that algal biomass is being considered as a clean fuel and bioproducts source [2]. There are over thousands of species of algae, but their basic composition mainly consist of proteins, lipids, and carbohydrates [3]. In particular, microalgae can accumulate lipids up to 20–50 wt%, which have high interest for a variety of bioproducts in food, cosmetics, and pharmaceutical industries [4] in addition to its

biofuels potential. The algae to biofuels production process has had much success from pilot to large scale operation, but equally as many obstacles that prevent it from becoming competitive with conventional fossil fuels. One of the scientific challenges of algal biofuels commercialization includes lipid extractability from algae cells [5]. Lipid extraction methods are key to the biofuels/bioproducts quality and yield from algae. The conventional oil extraction steps include breaking the algae cell walls, freeing the oil, and separating the oil out of the oil cake [6,7]. There are multiple technologies for lipid extraction from microalgae that are categorized under solvent extractions (Folch, Bligh and Dyer method), mechanical approaches (expeller press, bead beating, ultrasonic-assisted, microwave), and solvent-free methods (osmotic pressure, isotonic, enzyme-assisted) [8]. Choice of oil extraction typically depends on moisture content, quantity to be treated, quality of end-product, extraction efficiency, safety aspects, and cost economics [7]. Three methods including expeller, supercritical CO₂ fluid

* Corresponding author.

E-mail addresses: ateym001@odu.edu (A. Teymouri), kadam006@odu.edu (K.J. Adams), tao.dong@nrel.gov (T. Dong), skumar@odu.edu (S. Kumar).

extraction, and hexane extraction seem to be the most viable for industrial scale [5,7]. Among the three, hexane has been used in most applications of oil extraction [9]. It has high stability, low greasy residual effects, and low corrosiveness [9,10]. It has less toxicity compared to chloroform and methanol [6]. In addition, it is apolar (water immiscible) with low latent heat of boiling that makes it possible to be separated through low energy separation recovery methods [6,11]; however, it has a poor extractability efficiency compared to chlorinated solvents (i.e. chloroform) [12]. Techno-economic analyses (TEA) has shown that costs involved for lipid extraction with hexane is the second largest operational cost [13]; therefore, there is persistently strong demands for novel pretreatment methods of feedstock resulting in the overall improvement of the hexane extraction process. Alternative to the lipid extraction approach, hydrothermal liquefaction (HTL) of microalgae feedstock can directly convert the lipids into a biocrude oil, which is then subjected to further upgradation to fuel [14]. Many efforts have been made to optimize the process in terms of enhancing the biocrude yields [15–17]; however, high nitrogen content in HTL biocrude causes catalyst poisoning during downstream processing for liquid hydrocarbons/transportation fuels [18]. Production of high amounts of NO_x emissions in the downstream processing originating from nitrogenous compounds in proteins and chlorophyll content of microalgae is another serious challenge that this process needs to overcome [14,18].

Flash hydrolysis is a chemical-free subcritical water-based continuous process that fractionates microalgae components in a short residence time of 10 s. Our previous studies have shown multiple advantages of using the FH process for microalgae in terms of nutrient management either in forms of recycling or bioproducts formation [19–27] while protecting the lipids in solids. It was reported that 24–52 wt% (depending upon algal species) of the solid residue, known as biofuels intermediates (BI) are recovered after FH with diminished ash and nitrogen content [19]. We have also demonstrated that more than 90 wt% of total lipids available in the raw microalgae has been retained in the biofuels intermediates (BI) after the FH process [19,21]. The previous SEM images [19] of BI have shown its globular condensed appearance after the FH treatment. It has indicated that the process affected the physical dimensions of the particles to a smaller size; however, it is not clear if the FH process adversely affected the lipids extractability from the BI due to reduced solvent accessibility or entrapping oil after the recondensation process [6].

The current study investigates the lipid extraction efficiency of these BIs recovered after the FH process. In addition, a novel integrated FH-HTL process has been proposed to improve the biocrude yield and characteristics for fuel conversion. The main objectives of this study are to (i) conduct a kinetics study on the lipids extractability of the raw and BI from three common algal species (*Scenedesmus* sp., *Nannochloropsis* sp., and *Chlorella vulgaris*), (ii) analyze the fatty acids profile of lipids and compare it with that of lipids from untreated algae, (iii) produce biocrude from the BI of *Chlorella vulgaris* using HTL and compare the biocrude yield and quality with biocrude produced via direct HTL (no FH) of the same microalga. Fig. 1 shows the overall process including objectives and the products analyses among this study.

2. Materials and methods

2.1. Microalgae characterization

Three microalgae species including *Scenedesmus* sp., *Nannochloropsis* sp., and *Chlorella vulgaris* (*Chlorella* v.) was selected for this study. These species are known as the most promising candidates for biofuels and bioproducts production due to their lipid productivity and growth rate [2,6]. *Chlorella* v. was purchased from Arizona Center for Algae Technology and Innovation (AzCATI), *Nannochloropsis* sp. microalgae was received from Sandia National Laboratory (SNL), and *Scenedesmus* sp. was cultivated in a raceway open pond near Spring Grove, Virginia

[23]. All samples were freeze dried (FD) and stored at -20°C until application. In order to collect an adequate amount of BI for subsequent lipids extraction and HTL experiments, 10 FH tests were performed on each microalgae species at 280°C and 10 s of residence time using the method explained in our previous studies [21,25]. Briefly, solids (0.9–1.2 dry wt%) are loaded in the reactor at specified conditions; based on the reactor set up, deionized water is pumped until the desired temperature is reached, and the second pump delivers the algae slurry into the reactor. Followed by FH, products were centrifuged (Fisher Scientific accuSpin™ 400) and vacuum filtered (1.5 μm , Whatman 47 mm glass microfiber filters) to separate the solids (i.e. lipid rich BI) from the hydrolysate. The recovered BI from each algal species was freeze dried and stored at -20°C until application. All microalgae samples and their respective BIs were subjected to ash analysis using the dry oxidation method at $575 \pm 25^{\circ}\text{C}$ for 24 ± 6 h as described by the National Renewable Energy Laboratory (NREL) analytical procedure [28] followed by elemental analysis. Thermo Finnigan Flash EA 1112 elemental analyzer (ThermoFisher Scientific, Waltham, MA) with 2,5-Bis(5-tert-butylbenzoxazol-2-yl) thiophene (BBOT) standard (certified no. 202147-10/03/2015, ThermoFisher Scientific, Cambridge, UK) were used to characterize the elemental composition of algal biomass [19].

2.2. Experimental setup and procedure

2.2.1. Total lipid yields and FAME composition

To evaluate the lipid extraction performance of the microalgae feedstock, two critical factors including lipid yields and fatty acid methyl ester (FAME) profile needed to be considered [29]. First, the total extractable lipid was quantified from the respective BIs to evaluate if the FH process had positive impact on improving lipids yield. For quantifying the total extractable lipids, 0.35 g of dry biomass (BI or untreated algae) was fed into a glass tube and 4 ml of deionized (DI) water was added to the tube to fully soak the dry biomass at 4°C overnight. To assist the oil extraction, specifically free fatty acids (FFA) [6,30], 0.5 wt% of sulfuric acid was added to reduce the pH. A magnetic stir bar was added to stir the biomass on a multi-position magnetic plate and 3 ml of hexane was added to the tube. The extraction was carried out for 2 h on the magnetic plate. Tubes were vortexed for 30 s, every 30 min to improve the extraction. After the extraction, the tubes were centrifuged at 2000g for 10 min for phase separation. Then, the upper phase was moved to a preweighted glass tube. The solvent was evaporated in a vacuum oven at 40°C overnight [31]. The experiment was carried out in triplicate. The extractable lipid yields were calculated using the following Eq. (1) on moisture-free basis:

$$\text{Total lipid yields (wt\%)} = \frac{\text{extracted lipids (g)}}{\text{starting biomass (g)}} \times 100\% \quad (1)$$

Fatty acid content in the biomass was measured as total FAME content after an in situ transesterification procedure [32]. A total of 7 to 10 mg of lyophilized biomass was transesterified with 0.3 ml of HCl/methanol (5%, v/v) at the presence of 0.2 ml of chloroform/methanol (2:1, v/v) for 1 h at 85°C with a known amount of tridecanoic acid (C13) methyl ester as an internal standard. FAMES were extracted with hexane (1 ml) at room temperature for 1 h and analyzed by gas chromatography–flame ionization detection (GC–FID) on an Agilent 7890 N; DBWax-MS column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thickness). The individual FAME concentrations were normalized against the internal standard tridecanoic acid methyl ester.

2.2.2. Lipid extraction kinetics

To better understand the lipid extraction process, a lipid kinetic study was conducted. Lipid yields represent extraction efficiency and was measured through the Eq. (1). To perform the lipid extraction experiments, 1 g of raw algae or BI were added to 20 ml of Milli-Q water (EMD Millipore, Milli-Q Direct 16 water purification system) to make a

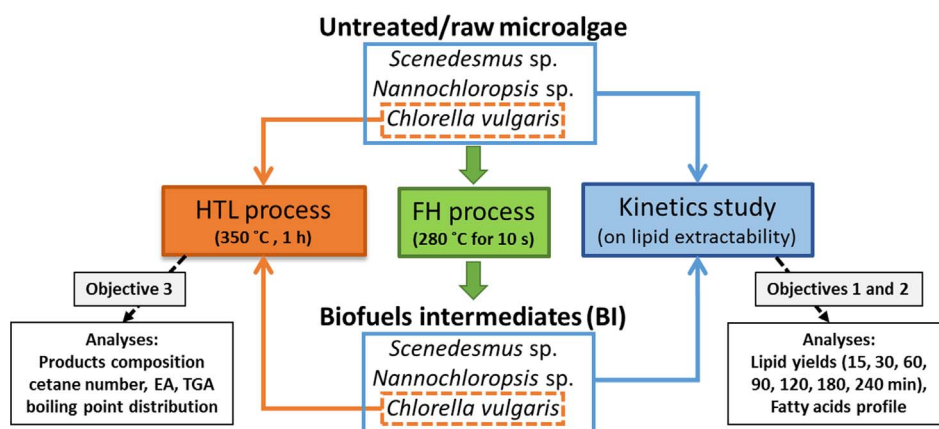
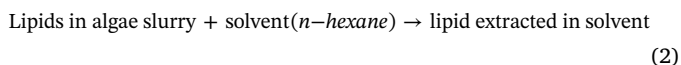


Fig. 1. Schematic diagram of the overall process including objectives and products analyses. Note: all analyses were performed in duplicate unless otherwise stated.

homogenous slurry in a 250 ml Erlenmeyer flask sealed with a rubber stopper to avoid evaporation. This was done to imitate the algae slurry after harvesting or FH process. The use of wet slurry for extraction is important to avoid the energy intensive drying stage and represent a feasible engineering method [9]. Twenty milliliters of *n*-hexane 85% (Fisher Chemical, high-resolution gas chromatography) was added to the same flask and placed on magnetic stir plates (Fisher Scientific Isotemp) for mixing with magnetic stir rods at 350 rpm at room temperature of approximately 25 °C. The lipids extraction experiments for kinetics study were conducted in duplicate for 15, 30, 60, 90, 120, 180, and 240 min of extraction time for both raw algae and the respective BIs. After mixing for the designated time, the resulting mixture was transferred into 50 ml centrifuge tubes. Each individual flask was rinsed 3 times with 1 ml aliquots of the same *n*-hexane solvent to recover all lipids to the tube and was centrifuged for 10 min at 2000g. The supernatant was removed after filtration using 0.2 µm nylon syringe filters (Fisherbrand, cat no. 09-719-006) and placed in pre-weighed glass vials. Solvent was removed by placing glass vials in oven at 55 °C for 48 h and then, weighed for recovered lipids. For long term lipids storage, glass vials were covered with Parafilm and transferred to a freezer at −20 °C for further analysis.

The quantified amount of extracted lipids of each feedstock provided the data to model the mass transfer of lipid molecules from wet algal slurry to the solvent medium. Following the reaction (Eq. (2)) was used to perform the kinetics study:



The detail mass transfer of lipids through water and solvent layers were discussed elsewhere [6,33]. The reaction order and reaction rate constant (*k*) were calculated by fitting the experimental data obtained from lipid extraction experiments as explained in our prior study [21]. Briefly, we plotted C_t (for zeroth order), $\ln C_t$ (first order), $1/C_t$ (second order) versus extraction time, where C_t was the grams of lipids available in the algae at time *t*. The reaction rate constant was obtained from the slope of the plotted graph whose linearity was the best fit.

2.2.3. Hydrothermal liquefaction

For the HTL process, 6.1 g of each feedstock: freeze dried raw and BI of the *Chlorella v.* microalgae, were added to 60 ml of Milli-Q water resulting in 9.4 ± 0.0 ($\pm$ standard deviation) and 9.5 ± 0.0 wt% solids content, respectively. The preference for *Chlorella v.* as the HTL feedstock over the *Nannochloropsis sp.* and *Scenedesmus sp.* was due to its higher lipid content and the fact that lipids have the most contribution to the biocrude yield. The experiments were carried out in duplicate for the BIs at 350 °C and 1 h of residence time as the most common conditions for the HTL experiments [14,34]. A 31.5 ml stainless steel cylindrical reactor (High Pressure Equipment Co.) equipped

with pressure gauge (Omega Engineering, Inc.) and thermocouple (P/N: TJ36-CAXL-116G-6, Omega Engineering, Inc.) was used to monitor the pressure and temperature throughout the experiment. A fluidized sand bath (SBS-4, Techne) was used as a heating source equipped with a temperature and flow controller (TC-9D, Techne). After the completion of the reaction, the reactor was quenched in cold water for 5 min and left at room temperature for ~60 min to equilibrate. Products were then transferred to a 50 ml centrifuge tube. The reactor was rinsed with 20 ml of dichloromethane (in 2 ml portions) and transferred to the same centrifuge tube. The mixture was then vortexed (3000 rpm for 1 min) and centrifuged (2000g for 1 min) which resulted in separation of aqueous fractions (on top) from the mixture of solids and organics (on the bottom). The aqueous phase was removed, filtered using 1.5 µm glass microfiber filters (Whatman), and transferred to preweighed vials. The solids and organic fraction were separated with 0.45 µm glass microfiber filters (Whatman) using vacuum filtration. The solid phase was oven dried at 65 °C for 24 h. The organic phase was transferred to preweighed vials and the dichloromethane was evaporated to dryness by flowing ultrapure nitrogen gas over the tubes for approximately 8 h to calculate biocrude yield. The liquid products including biocrude, aqueous phase, and solid residue were analysed for gravimetric yield and elemental composition.

Algal biomass (raw and BI), solid residue (char) formed after HTL process, and biocrude were subjected to EA as explained in Section 2.1, while total organic carbon/total nitrogen (TOC/TN) analyser (TOC-VCSN, Shimadzu) equipped with an ASI-V auto sampler were used for the recovered aqueous phase. The following equations were used to calculate the yield and elemental distribution on dry basis:

$$\text{Yield(wt\%)} = \frac{\text{mass of product fraction(g)}}{\text{mass of alga(g)}} \times 100\% \quad (3)$$

$$\text{Elemental distribution(\%)} = \frac{(\text{mass of element in product fraction})}{(\text{mass of element in alga})} \times 100\% \quad (4)$$

Higher heating value (HHV) of the biocrudes were estimated based on Dulong's empirical formula (Eq. (5)):

$$\text{HHV(MJ/kg)} = 0.3383 C + 1.422(H - O/8) \quad (5)$$

where, C, H, and O are the elemental percentages of the biocrude that were measured through elemental analysis [35]. Thermogravimetric analysis (TGA) of crude oil was performed using TGA-50H (Shimadzu Corporation) from 25 °C to 900 °C in 50 ml/min nitrogen gas flow at 10 °C/min to estimate the boiling point range [36,37].

Cetane number (CN) is a measurement of the quality of diesel fuel that considers ignition delay time and combustion quality [38]. CN for this study was estimated using models from the work of Stansell et al. [38], who used a combination of models from the work of Lapuerta et al. [39] and Tong et al. [40]. CNs were calculated using the following equations where *n* is carbon number and *db* is double bond number:

For saturated fatty acids:

$$CN = -107.71 + 31.126n - 2.042n^2 + 0.499n^3 \quad (6)$$

For mono-unsaturated fatty acids:

$$CN = 109 - 9.292n + 0.354n^2 \quad (7)$$

For poly-unsaturated fatty acids:

$$CN = -21.157 + (7.965 - 1.785db + 0.235db^2)n - 0.099n^2 \quad (8)$$

CN for the specific biodiesel/FAME is given by the following equation where CN_i is cetane number of each class and m_i is mass percentage of each FAME in the biodiesel:

$$CN = 1.068 \sum (CN_i m_i) - 6.747 \quad (9)$$

3. Results and discussion

3.1. Elemental composition

The elemental composition of all three microalgae species were presented in the Table 1. As demonstrated, the FH process resulted in biofuels intermediates with higher percentages of carbon and hydrogen and reduced nitrogen content. BI of *Chlorella v.* showed approximately, 80 wt% less ash compared to the original untreated microalgae. This was similar to our prior study on *Nannochloropsis gaditana* [25], which denotes the potential of FH as a treatment process for high ash feed-stocks.

3.2. Lipid extraction

Data from the non-polar lipid (fatty acids) yield (Table 2) revealed that total extracted amounts were significantly higher after the FH process. It increased about 2 and 3 times for *Scenedesmus* sp. and *Nannochloropsis* sp., respectively; however, this number was much higher (32 times) for the lipid-rich *Chlorella v.* microalgae. The cell wall composition of *Chlorella v.* includes hemicellulose and saccharides that impede the release of intercellular lipids. Thus, the derived lipid concentration is highly dependent on the effectiveness of the disruption process [41]. This confirms the promising effect of FH as a treatment method on the algal cell disruption for lipid recovery.

In order to model the mass transfer in the lipid extraction process from the wet algal biomass, lipid yields from raw and BI of each algae species were compared in the range of 15–240 min of extraction time (Fig. 2).

Fig. 2 shows, for all microalgae species throughout the extraction times, BI of each microalgae produced higher lipid yields compared to raw/untreated algae. The yield was proportionally higher for the algal biomass with high lipids content. In other words, the FH process had substantial effect on lipid extractability of high lipid content microalgae such as *Chlorella v.* The lipid yields of BI of *Chlorella v.* after 15 min extraction is more than 5 times than that of raw algae after 4 h of extraction time (52.3 ± 0.8 vs. 10.7 ± 0.9 wt%). This is approximately a 16-fold reduction in the extraction time in addition to the higher lipid yields. Table S1 (supplementary information) presents the average lipid

Table 2

Non-polar lipid yield of raw and BI microalgae selected for this study.

Algae Species	Non-polar lipid yield (wt%)	
	Raw	BI
<i>Scenedesmus</i> sp.	1.4 ± 0.2	2.9 ± 0.1
<i>Nannochloropsis</i> sp.	3.0 ± 0.1	8.6 ± 0.1
<i>Chlorella v.</i>	1.7 ± 0.2	53.8 ± 1.4

yields values of performed experiments for all three algal species including standard deviations. Many other studies have also investigated the effect of different pretreatment methods on the extraction efficiency of biomass [42]. A parametric study on lipid extraction after dilute acid pretreatment was conducted by impact of biochemical composition on susceptibility of algal biomass to acid-catalyzed pretreatment for sugar and lipid recovery [31]. Cravotto et al. reported 50–500% increase in the lipid yield and up to 10-fold reduction in extraction time using combined effects of temperature, ultrasound, and microwave [43].

3.3. Lipid kinetics study

The rate constant (k) and reaction order of the lipid extractability of the three algal biomass are reported in the Table 3. All algal species followed the zero-order reaction rate. This is different from the first-order kinetics of lipid extraction from wet algal biomass that was previously reported by Halim et al. studies [29,33].

Conversely, we observed different behavior for the lipid extraction from BI of the three algal species. The susceptibility of biomass to pretreatment can be significantly affected by its biochemical composition [31]. As presented in the Table 3, lipid extraction from the BI of *Scenedesmus* sp. followed a zero-order reaction rate, while this changed to a second-order reaction rate for the BI of *Nannochloropsis* sp. and *Chlorella vulgaris*. For the raw biomass, the extraction constant increased as the lipid content increased; however, there are multiple factors affecting the k value such as agitation/mixing of the algal biomass, ratio of organic solvent to dried microalgae, and the extraction temperature [33].

3.4. FAME analysis

In order to verify whether the FH process might have affected the quality of lipids, the FAME profiles of the lipids from raw and BI of each microalga were compared in the Table 4.

The BI showed 40.7, 18.3, and 121.9 wt% higher FAME content than the untreated microalgae for *Scenedesmus* sp., *Nannochloropsis* sp., and *Chlorella v.*, respectively. This was expected due to the cell wall hydrolysis capability of subcritical water [6]. FFA is easier to be extracted in an aqueous environment than bipolar phospholipids and FFA is a preferred biofuel precursor as well [44]; moreover, phospholipids are known to be hydrolyzed to FFA under the FH condition [45]. Another observation is that the lipids extracted from the BIs had higher saturated fatty acids compared to untreated microalgae. Among the three species, *Nannochloropsis* sp. showed the highest change from 19.1

Table 1

Microalgae characterization (raw/untreated and biofuels intermediates) used for this study. All values are wt% ($\pm$ standard deviation).

Algae Species	Treatment	Carbon	Nitrogen	Hydrogen	Ash
<i>Scenedesmus</i> sp.	Raw	49.1 ± 0.3	7.3 ± 0.1	7.1 ± 0.1	4.5 ± 0.7
	BI	55.5 ± 0.3	6.7 ± 0.3	7.6 ± 0.1	n/a
<i>Nannochloropsis</i> sp.	Raw	46.8 ± 0.2	7.9 ± 0.0	7.4 ± 0.1	11.0 ± 0.0
	BI	61.8 ± 0.4	7.0 ± 0.3	8.4 ± 0.0	n/a
<i>Chlorella v.</i>	Raw	52.9 ± 0.7	2.8 ± 0.9	7.9 ± 0.7	2.5 ± 0.6
	BI	68.3 ± 0.2	2.6 ± 0.1	10.2 ± 0.0	0.5 ± 0.0

n/a: Not analyzed.

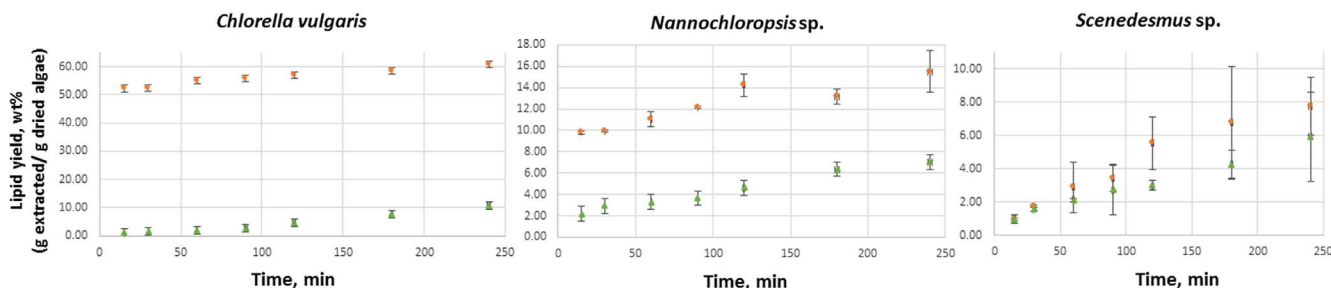


Fig. 2. Lipid yields comparison of raw (green triangles) and biofuels intermediates/BI (orange dots) of *Chlorella v.* (left) *Nannochloropsis sp.* (center), and *Scenedesmus sp.* (right), over time. Error bars are the standard deviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Reaction orders and constants (k) of the three algal species.

Algae species	Reaction order	Reaction constant, k	Correlation factor, r^2
<i>Scenedesmus sp.</i>	Raw	0	0.022
	BI	0	0.0325
<i>Nannochloropsis sp.</i>	Raw	0	0.0256
	BI	2	0.0027
<i>Chlorella v.</i>	Raw	0	0.043
	BI	2	0.0021

to 36.0 wt% and *Chlorella v.* contained the lowest change from 25.8 to 27.2 wt% of total fatty acids identified. These saturated fatty acids are the most suitable for biofuels production [46]. Poly unsaturated fatty acids (PUFA) such as C16:4, C18:3, C20:3-5 decreased in the extracted lipids from the BI compared to the raw algae feedstock. This is desirable since PUFA are known to be responsible for poor volatility, low oxidation stability, and direction towards gum formation in some oilseed-derived biodiesel [33].

3.5. Cetane number

Cetane number for diesel fuel in the United States is regulated at ≥ 40 and much higher in the European Union (EU) at ≥ 51 [39]. All algae species used in this study confirms the regulations with respect to CN for the US and some for the EU. It is important to note that in the cases of all algae species, *Nannochloropsis sp.*, *Scenedesmus sp.*, and *Chlorella v.*, the biofuels intermediate have a higher CN number with an 18%, 16% and 2% CN increase from raw algae to BI, respectively (Table 5). For BIs, the species with the smallest percentage of

Table 4

Fatty acid compositions of lipids in raw and biofuels intermediates (BI) of *Scenedesmus sp.*, *Nannochloropsis sp.*, and *Chlorella v.* microalgae. Values are weight percentage (wt%) of each fatty acid with respect to the total FAME identified.

	<i>Scenedesmus sp.</i>		<i>Nannochloropsis sp.</i>		<i>Chlorella v.</i>	
	raw	BI	raw	BI	Raw	BI
Total FAME (w/w)	5.4 $\pm$ 0.1	7.6 $\pm$ 0.1	10.9 $\pm$ 0.2	12.9 $\pm$ 0.5	28.3 $\pm$ 0.1	62.8 $\pm$ 0.0
C14:0	2.9	4.2	3.1	5.6	0.0	0.0
C16:0	24.4	38.3	15.8	29.9	24.4	25.7
C16:1	13.1	15.0	27.2	42.1	4.4	4.5
C16:2	3.0	1.9	0.5	0.4	2.4	2.3
C16:3	10.2	5.4	0.3	0.7	6.0	5.4
C16:4	1.5	0.6	0.0	0.0	2.0	1.7
C18:0	2.2	3.6	0.2	0.5	1.4	1.5
C18:1	10.6	14.2	3.6	5.9	23.6	23.8
C18:2	7.9	3.9	2.3	1.8	20.0	19.8
C18:3	22.0	6.8	0.1	0.1	14.0	12.4
C20:0	0.6	1.1	0.0	0.0	0.0	0.0
C20:3	0.0	0.0	1.4	0.7	0.0	0.0
C20:4	0.0	0.0	6.1	1.9	0.0	0.0
C20:5	0.0	0.0	38.1	9.1	0.0	0.0

Note: FAME with content less than 0.5% was not shown.

Table 5

Cetane numbers of raw and biofuels intermediates (BI) of three algal species used in this study.

Algae species	Treatment	CN
<i>Nannochloropsis sp.</i>	Raw	47.2
<i>Nannochloropsis sp.</i>	BI	56.9
<i>Scenedesmus sp.</i>	Raw	48.2
<i>Scenedesmus sp.</i>	BI	56.1
<i>Chlorella v.</i>	Raw	49.1
<i>Chlorella v.</i>	BI	49.9

unsaturated FA, *Scenedesmus sp.* yielded the higher CN although CN values were in the same range. Stansell et al. [38] reported CN of algae species by class which can be compared to species used in this study. Eustigmatophyceae, Cryptophyceae, and Trebauxiophyceae the classes of *Nannochloropsis sp.*, *Scenedesmus sp.*, and *Chlorella v.* had cetane numbers of 52.3, 45.6 and 46.3, respectively [38]. CNs calculated from biodiesel produced from this experiment suggest that biodiesel produced from biofuels intermediates result in a higher quality fuel and that all biodiesel produced (from raw algae/biofuel intermediates) exceeds the US regulations, making these species a promising candidate for biodiesel usage.

3.6. HTL of *Chlorella v.* microalgae (raw vs. BI)

As stated earlier, in order to further confirm the enhanced lipid extractability and quality of biocrude from BIs, a typical HTL experiment was performed on the BI of *Chlorella v.* (Fig. 3). As demonstrated, product yields includes 68.9 ± 3.2 wt% of biocrude and a very low amount of char (2.1 ± 0.3). Nevertheless, no similar studies on the

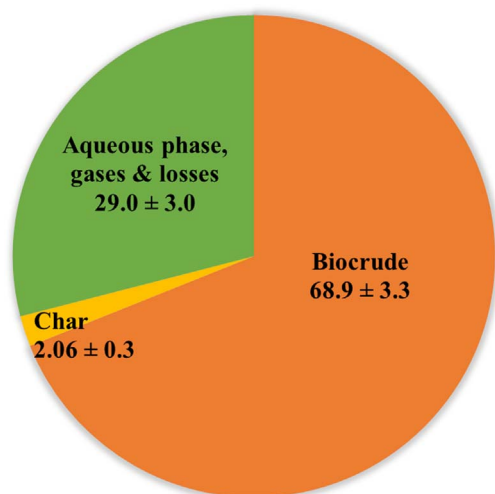


Fig. 3. HTL product yields at 350 °C and 1 h reaction time for BI of *Chlorella v.* microalgae.

biocrude yields from the HTL process on the post-algae hydrolysis residue were found as a means of comparison.

Reported results are the average values of duplicate experiments $\pm$ standard deviation.

We collected the HTL product yields in literature that were performed at the same conditions of 350 °C and 1 h reaction time and also conducted a control HTL experiment with the untreated *Chlorella v.*

microalgae (Fig. 4) to compare the biocrude yields. As illustrated in the Fig. 4, the biocrude yields from all collected data is in the range of 20–50 wt%. The biocrude yields using BI is significantly higher than control and literature data shown in Fig. 4. It is expected that microalgae with various biochemical compositions result in different biocrude yields [17] and the lipid fraction has the most contribution to biocrude formation [47].

Perhaps, this might be the reason for the higher HTL biocrude yields from the BIs compared to the untreated microalgae. The biocrude yields and composition also depends on the loading concentration, temperature, residence time, and the use of catalyst [55]. In addition, the quality of the recovered biocrude was always a matter of concern due to high nitrogen content. The results from elemental analysis were shown in Table 6. As indicated in Table 6, the H/C atomic ratio of biocrude improved from 1.55 to 1.74. The N/C atomic ratio also decreased by about 50% to a lower value of 0.016. The oxygen content of the biocrude had a marginal decrease of 0.2 wt%, which is probably the reason for the higher heating value of biocrude yields from the BI feedstock (Table 6) [35]. This HHV value is higher than prior studies which ranged between 33 and 39 MJ/kg [37,56].

3.6.1. TGA of HTL biocrude

In order to evaluate the boiling point distribution of the biocrudes, TG analysis was performed. Results from TGA were plotted in the Fig. 5. This process can be interpreted as a miniature distillation [36].

The detailed comparison of biocrudes boiling point distribution is reported in Table 7. Biocrude yield from the HTL experiment of BI showed higher weight loss as compared to that of raw algae in the overall process under an inert environment (96.4 wt% compared to

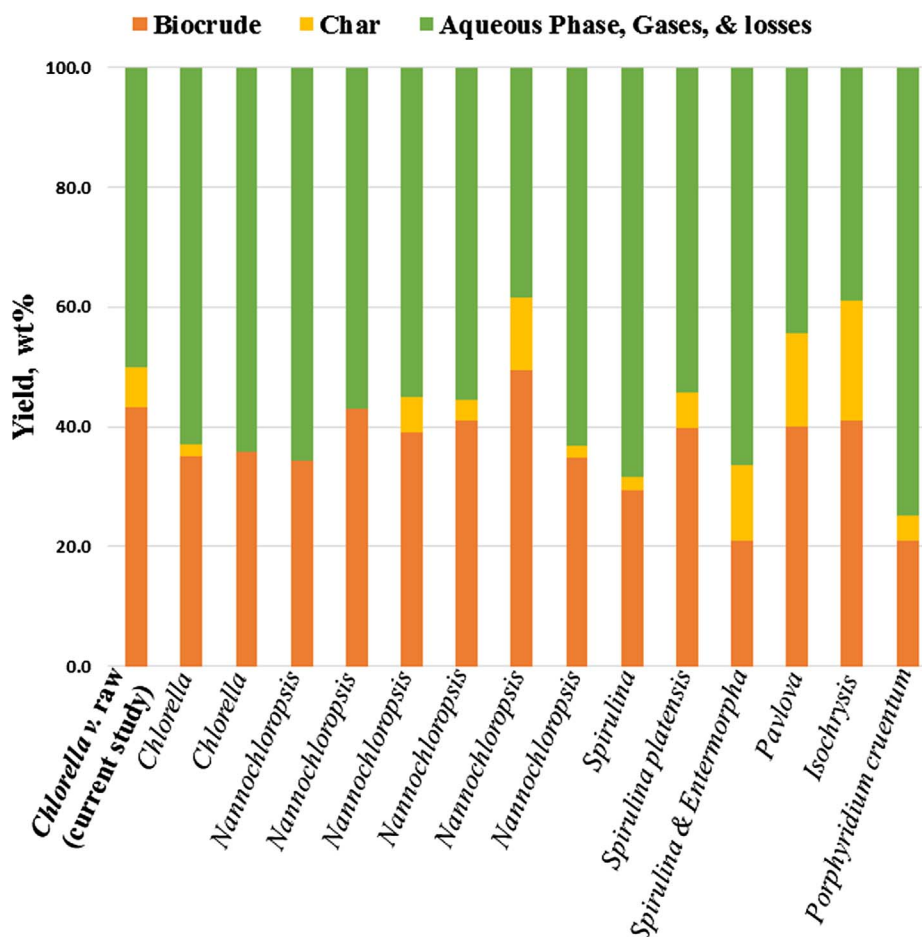


Fig. 4. Comparison of HTL product yields at 350 °C and 1 h reaction time for the untreated *Chlorella v.* microalgae as the control experiment (first bar from left) with data provided in the literature for the same experimental conditions [37,48–54].

Table 6

Elemental composition of the biocrude obtained through hydrothermal liquefaction of *Chlorella v.* microalgae. All values are weight percentage $\pm$ standard deviation. The total values are slightly (< 1 wt%) above 100, since all elements are the averages of measured values.

Biocrude sample	C (wt%)	N (wt%)	H (wt%)	O (wt%)	H/C (mol)	N/C (mol)	HHV (MJ/kg)
Biocrude from raw <i>Chlorella v.</i>	76.6 $\pm$ 0.3	2.8 $\pm$ 0.0	9.9 $\pm$ 0.1	10.9 $\pm$ 0.4	1.55	0.031	38.1
Biocrude from the BI of <i>Chlorella v.</i>	76.9 $\pm$ 0.4	1.4 $\pm$ 0.0	11.2 $\pm$ 0.1	10.7 $\pm$ 0.0	1.74	0.016	40.0

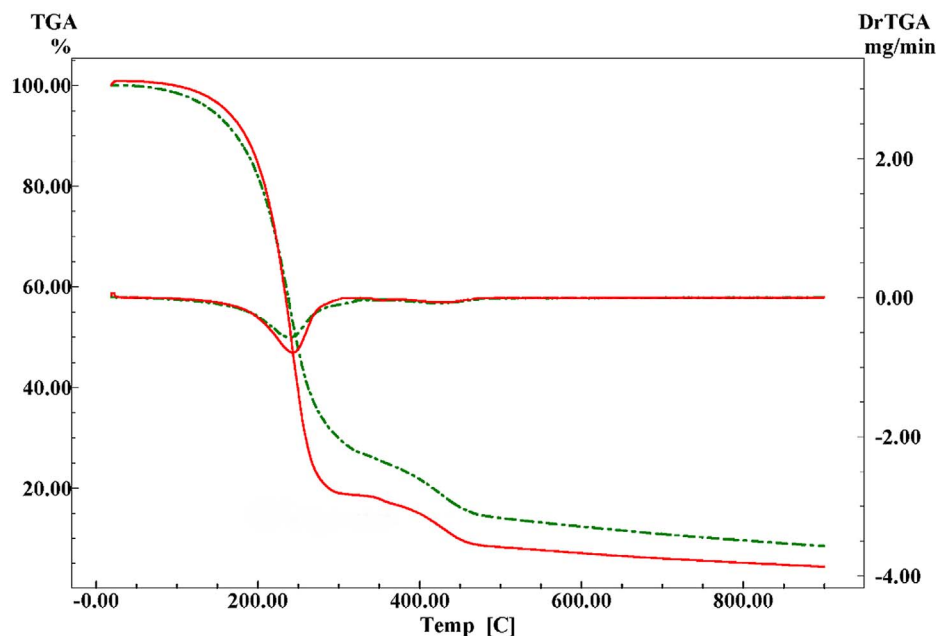


Fig. 5. TGA comparison of biocrude recovered after hydrothermal liquefaction of raw (green lines and dots) and biofuels intermediates, BI, (solid red line) of *Chlorella v.* The peaks in derivative graph for raw and BI biocrude happened at 239.5 and 243.3 °C, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 7

Boiling point distribution of the biocrudes (wt%).

Distillate range, °C	Typical application of the coke oil [36]	Raw algae biocrude	BI biocrude
20–110	Bottle gas and chemicals	2.1	1.2
110–200	Gasoline	15.8	14.9
200–300	Jet fuel, fuel for stoves, and diesel oil	52.2	65.7
300–400	Lubricating oil for engines, fuel for ships, and machines	8.1	3.9
400–550	Lubricants and candles, fuel for ships	8.6	7.3
550–700	Fuel for ships, factories, and central heating	2.3	1.6
700–800	Asphalt and roofing	1.2	0.8
800–900	Residues	1.1	0.8

91.5 wt%). This indicates the higher presence of volatiles and less amount of residues in the biocrude. Losses below 120 °C were due to drying of water and any remaining solvent. The HTL experiment on the BI resulted in a lighter biocrude. A higher fraction of biocrude (65.7 wt % compared to 52.2 wt%) is suitable for jet fuel and diesel oil production.

3.6.2. Material balance

Another way to compare biocrude obtained from raw *Chlorella v.* and its BI, is the C, N, and H elemental balance alongside the process (Fig. 6). As illustrated, solid residues are much less when BI is subjected to the HTL process. This is partially due to the FH contribution to the ash diminution in the BI [25]. Another interesting merit of the FH process prior to the regular HTL is the capability to extract nutrients in the forms of amino acids and soluble peptides in the hydrolysate [21].

This integration (FH + HTL) significantly reduced the nitrogen content in the biocrude. Comparing elemental balance in the Fig. 6, biocrude obtained from the raw algae (section a) carried up to 42.6 wt% of the total nitrogen content of the original microalgae, versus only 11.3 wt% of that in the biocrude obtained from BI (section b). Nitrogen content reduction in the biocrude is desirable to minimize the amount of NO_x formation during combustion and to move forward with the catalytic upgradation process [56,57].

Although, the overall biocrude yield in the HTL of the untreated algae is higher, it is worth considering the higher quality of biocrude obtained from the HTL of BI in addition to all other potential high value bioproducts such as polyurethane foams, peptides, arginine, dittmerite, and hydroxyapatite that could be recovered from the algal hydrolysate [19–21,24,27]. A comprehensive life cycle assessment (LCA) and techno-economic analysis (TEA) was performed in order to quantify environmental impacts and assess economic profitability of this study [58]. The integrated FH-HTL process with the coproducts conversion technology (hydrothermal mineralization/HTM) indicated a 4% higher profitability index (PI) compared with the standalone HTL model [58].

4. Conclusions

This study investigated the effect of the flash hydrolysis (FH) process on the lipid extractability of three microalgae species including *Scenedesmus sp.*, *Nannochloropsis sp.*, and *Chlorella vulgaris*.

Results revealed that the FH process significantly improved the lipid extraction of wet algal biomass by both reducing the extraction time and increasing the yield. Kinetics studies' results indicated that wet extraction of untreated algal biomass followed a zero-order reaction rate; however, biofuels intermediates (BI) that recovered from FH at 280 °C and 10 s, followed a second-order rate (BI of *Scenedesmus sp.* exceptionally followed zeroth order). Fatty acid profile comparison of

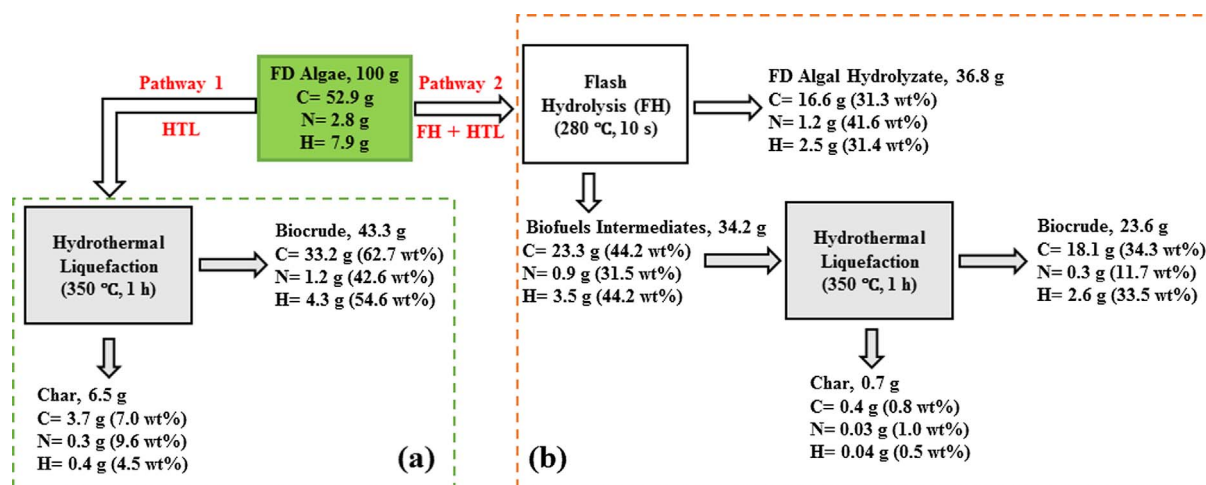


Fig. 6. CNH elemental balance and product yields after hydrothermal liquefaction of raw *Chlorella v. Microalgae* (a) and its BI (b). CNH weights in every stage is based on the total input weight of each element in the original freeze dried (FD) algae. Weight percentages (wt%) shown in the parenthesis calculated as: grams of the element recovered in that stage/grams of that element initially entered the system. CNH values regarding gas and aqueous phase is excluded.

raw and BI of each microalga showed the contribution by FH in increasing the percentage of saturated FAMES in the profile. Biocrude yield via hydrothermal liquefaction of *Chlorella v. BI* was higher (68.9 wt%) compared to the raw algae (43.3 wt%). It contained less amounts of nitrogen, oxygen, and had a higher H/C ratio. TGA results also implied that the wt% of jet fuel and diesel range distillate increased in the BI of *Chlorella vulgaris*.

Acknowledgements

The authors would like to acknowledge Dr. Ryan Davis at Sandia National Laboratories for providing the *Nannochloropsis sp.* biomass used for this study and the National Science Foundation (NSF) for the financial support of this work through the NSF CAREER Award# 1351413 and PFI: AIR TT Grant# 1640593.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fuel.2018.03.044>.

Reference

- [1] U.S. energy consumption by energy source. https://www.eia.gov/energyexplained/?page=us_energy_home.
- [2] Laurens LML, Markham J, Templeton DW, Christensen ED, Van Wychem S, Vadelius EW, et al. Development of algae biorefinery concepts for biofuels and bioproducts; a perspective on process-compatible products and their impact on cost-reduction. *Energy Environ Sci* 2017;10(8):1716–38.
- [3] Keymer P, Ruffell I, Pratt S, Lant P. High pressure thermal hydrolysis as pre-treatment to increase the methane yield during anaerobic digestion of microalgae. *Bioresour Technol* 2013;131(Supplement C):128–33.
- [4] Ren X, Zhao X, Turcotte F, Deschênes J-S, Tremblay R, Jolicoeur M. Current lipid extraction methods are significantly enhanced adding a water treatment step in *Chlorella protothecoides*. *Microb Cell Fact* 2017;16:26.
- [5] Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. Biofuels from algae: challenges and potential. *Biofuels* 2010;1(5):763–84.
- [6] Dong T, Knoshaug EP, Pienkos PT, Laurens LML. Lipid recovery from wet oleaginous microbial biomass for biofuel production: a critical review. *Appl Energy* 2016;177:879–95.
- [7] Ramesh D. Lipid identification and extraction techniques. *Biotechnological Applications of Microalgae: Biodiesel and Value-added Products*; 2013. p. 89–97.
- [8] Ranjith Kumar R, Hanumantha Rao P, Arumugam M. Lipid extraction methods from microalgae: a comprehensive review. *Front Energy Res* 2015;2(61).
- [9] Suganya T, Renganathan S. Optimization and kinetic studies on algal oil extraction from marine macroalgae *Ulva lactuca*. *Bioresour Technol* 2012;107(Supplement C):319–26.
- [10] Erickson DR. *Practical handbook of soybean processing and utilization*. Elsevier; 2015.
- [11] Chemat S. *Edible Oils: Extraction, Processing, and Applications*. CRC Press; 2017.

- [12] Balasubramanian RK, Yen Doan TT, Obbard JP. Factors affecting cellular lipid extraction from marine microalgae. *Chem Eng J* 2013;215(Supplement C):929–36.
- [13] Davis R, Fishman D, Frank ED, Wigmosta MS, Aden A, Coleman AM, et al. *Renewable diesel from algal lipids: an integrated baseline for cost, emissions, and resource potential from a harmonized model*. Golden, CO: National Renewable Energy Laboratory (NREL); 2012.
- [14] López Barreiro D, Prins W, Ronse F, Brilman W. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects. *Biomass Bioenergy* 2013;53(Supplement C):113–27.
- [15] Guo Y, Yeh T, Song W, Xu D, Wang S. A review of bio-oil production from hydrothermal liquefaction of algae. *Renew Sustain Energy Rev* 2015;48(Supplement C):776–90.
- [16] Chan YH, Quitain AT, Yusup S, Uemura Y, Sasaki M, Kida T. Optimization of hydrothermal liquefaction of palm kernel shell and consideration of supercritical carbon dioxide mediation effect. *J Supercrit Fluids* 2017.
- [17] Sheehan JD, Savage PE. Modeling the effects of microalga biochemical content on the kinetics and biocrude yields from hydrothermal liquefaction. *Bioresour Technol* 2017;239(Supplement C):144–50.
- [18] Duan P, Savage PE. Catalytic hydrothermal hydrodenitrogenation of pyridine. *Appl Catal B: Environ* 2011;108–109(Supplement C):54–60.
- [19] Garcia-Moscoso JL, Obeid W, Kumar S, Hatcher PG. Flash hydrolysis of microalgae (*Scenedesmus sp.*) for protein extraction and production of biofuels intermediates. *J Supercrit Fluids* 2013;82:183–90.
- [20] Kumar S, Hablot E, Moscoso JLG, Obeid W, Hatcher PG, Duquette BM, et al. Polyurethanes preparation using proteins obtained from microalgae. *J Mater Sci* 2014;49(22):7824–33.
- [21] Garcia-Moscoso JL, Teymouri A, Kumar S. Kinetics of peptides and arginine production from microalgae (*Scenedesmus sp.*) by flash hydrolysis. *Ind Eng Chem Res* 2015;54(7):2048–58.
- [22] Barbera E, Sforza E, Kumar S, Morosinotto T, Bertuccio A. Cultivation of *Scenedesmus obliquus* in liquid hydrolysate from flash hydrolysis for nutrient recycling. *Bioresour Technol* 2016;207:59–66.
- [23] Talbot C, Garcia-Moscoso J, Drake H, Stuart BJ, Kumar S. Cultivation of microalgae using flash hydrolysis nutrient recycle. *Algal Res* 2016;18:191–7.
- [24] Barbera E, Teymouri A, Bertuccio A, Stuart BJ, Kumar S. Recycling minerals in microalgae cultivation through a combined flash hydrolysis-precipitation process. *ACS Sustain Chem Eng* 2017;5(1):929–35.
- [25] Teymouri A, Kumar S, Barbera E, Sforza E, Bertuccio A, Morosinotto T. Integration of biofuels intermediates production and nutrients recycling in the processing of a marine algae. *AIChE J* 2017;63(5):1494–502.
- [26] Ali Teymouri BS, Sandeep Kumar. Kinetics of phosphate precipitation from the nutrients-rich algal hydrolysate. The 253rd ACS National Meeting, San Francisco, California 2017.
- [27] Teymouri A, Stuart BJ, Kumar S. Effect of reaction time on phosphate mineralization from microalgae hydrolysate. *ACS Sustain Chem Eng* 2017.
- [28] Van Wychem S, Laurens LML. Determination of Total Solids and Ash in Algal Biomass: Laboratory Analytical Procedure (LAP); NREL/TP-5100-60956 United States 10.2172/1118077 NREL English; National Renewable Energy Laboratory (NREL), Golden, CO.: 2013; p Medium: ED; Size: p. 11.
- [29] Halim R, Gladman B, Danquah MK, Webley PA. Oil extraction from microalgae for biodiesel production. *Bioresour Technol* 2011;102(1):178–85.
- [30] Laurens LML, Nagle N, Davis R, Sweeney N, Van Wychem S, Lowell A, et al. Acid-catalyzed algal biomass pretreatment for integrated lipid and carbohydrate-based biofuels production. *Green Chem* 2015;17(2):1145–58.
- [31] Dong T, Van Wychem S, Nagle N, Pienkos PT, Laurens LML. Impact of biochemical composition on susceptibility of algal biomass to acid-catalyzed pretreatment for sugar and lipid recovery. *Algal Res* 2016;18(Supplement C):69–77.
- [32] Laurens LML, Quinn M, Van Wychem S, Templeton DW, Wolfrum EJ. Accurate and

- reliable quantification of total microalgal fuel potential as fatty acid methyl esters by in situ transesterification. *Anal Bioanal Chem* 2012;403(1):167–78.
- [33] Halim R, Danquah MK, Webley PA. Extraction of oil from microalgae for biodiesel production: a review. *Biotechnol Adv* 2012;30(3):709–32.
- [34] Patel B, Guo M, Izadpanah A, Shah N, Hellgardt K. A review on hydrothermal pretreatment technologies and environmental profiles of algal biomass processing. *Bioresour Technol* 2016;199:288–99.
- [35] Wei R, Zhang L, Cang D, Li J, Li X, Xu CC. Current status and potential of biomass utilization in ferrous metallurgical industry. *Renew Sustain Energy Rev* 2017;68(Part 1):511–24.
- [36] Chen W-T, Zhang Y, Zhang J, Yu G, Schideman LC, Zhang P, Minarick M. Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil. *Bioresour Technol* 2014;152(Supplement C):130–9.
- [37] Biller P, Ross AB. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresour Technol* 2011;102(1):215–25.
- [38] Stansell GR, Gray VM, Sym SD. Microalgal fatty acid composition: implications for biodiesel quality. *J Appl Phycol* 2012;24(4):791–801.
- [39] Lapuerta M, Rodríguez-Fernández J, de Mora EF. Correlation for the estimation of the cetane number of biodiesel fuels and implications on the iodine number. *Energy Policy* 2009;37(11):4337–44.
- [40] Tong D, Hu C, Jiang K, Li Y. Cetane Number Prediction of Biodiesel from the Composition of the Fatty Acid Methyl Esters. *J Am Oil Chem Soc* 2011;88(3):415–23.
- [41] Zheng H, Yin J, Gao Z, Huang H, Ji X, Dou C. Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. *Appl Biochem Biotechnol* 2011;164(7):1215–24.
- [42] Lee SY, Cho JM, Chang YK, Oh Y-K. Cell disruption and lipid extraction for microalgal biorefineries: a review. *Bioresour Technol* 2017;244(Part 2):1317–28.
- [43] Cravotto G, Boffa L, Mantegna S, Perego P, Avogadro M, Cintas P. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrason Sonochem* 2008;15(5):898–902.
- [44] Dong T, Fei Q, Genelot M, Smith H, Laurens LML, Watson MJ, Pienkos PT. A novel integrated biorefinery process for diesel fuel blendstock production using lipids from the methanotroph, *Methylobacterium buryatense*. *Energy Convers Manage* 2017;140(Supplement C):62–70.
- [45] Levine RB, Pinnarat T, Savage PE. Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification. *Energy Fuels* 2010;24(9):5235–43.
- [46] Steriti A, Rossi R, Concas A, Cao G. A novel cell disruption technique to enhance lipid extraction from microalgae. *Bioresour Technol* 2014;164(Supplement C):70–7.
- [47] Ross AB, Biller P, Kubacki ML, Li H, Lea-Langton A, Jones JM. Hydrothermal processing of microalgae using alkali and organic acids. *Fuel* 2010;89(9):2234–43.
- [48] Shukla R, Whelen J, Adhikari S, Mahadevan R, Neupane S. Effect of temperature and Na₂CO₃ catalyst on hydrothermal liquefaction of algae. *Algal Res* 2015;12:80–90.
- [49] Valdez PJ, Nelson MC, Wang HY, Lin XXNN, Savage PE. Hydrothermal liquefaction of *Nannochloropsis* sp.: systematic study of process variables and analysis of the product fractions. *Biomass Bioenergy* 2012;46:317–31.
- [50] Valdez PJ, Dickinson JG, Savage PE. Characterization of product fractions from hydrothermal liquefaction of *nannochloropsis* sp. and the influence of solvents. *Energy Fuels* 2011;25(7):3235–43.
- [51] Brown TM, Duan P, Savage PE. Hydrothermal liquefaction and gasification of *nannochloropsis* sp. *Energy Fuels* 2010;24(6):3639–46.
- [52] Biller P, Riley R, Ross AB. Catalytic hydrothermal processing of microalgae: decomposition and upgrading of lipids. *Bioresour Technol* 2011;102(7):4841–8.
- [53] Jena U, Das KC, Kastner JR. Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina platensis*. *Bioresour Technol* 2011;102(10):6221–9.
- [54] Jin B, Duan P, Xu Y, Wang F, Fan Y. Co-liquefaction of micro- and macroalgae in subcritical water. *Bioresour Technol* 2013;149:103–10.
- [55] Chiaramonti D, Prussi M, Buffi M, Rizzo AM, Pari L. Review and experimental study on pyrolysis and hydrothermal liquefaction of microalgae for biofuel production. *Appl Energy* 2017;185(Part 2):963–72.
- [56] Anastakis K, Ross AB. Hydrothermal liquefaction of the brown macro-alga *Laminaria Saccharina*: effect of reaction conditions on product distribution and composition. *Bioresour Technol* 2011;102(7):4876–83.
- [57] Yu G, Zhang YH, Schideman L, Funk T, Wang ZC. Distributions of carbon and nitrogen in the products from hydrothermal liquefaction of low-lipid microalgae. *Energy Environ Sci* 2011;4(11):4587–95.
- [58] Bessette AP, Teymouri A, Martin MJ, Stuart BJ, Resurreccion EP, Kumar S. Life cycle impacts and techno-economic implications of flash hydrolysis in algae processing. *ACS Sustain Chem Eng* 2018.