HYDROTHERMAL LIQUEFACTION OF MICROALGAE: INFLUENCE OF VARYING CELL COMPOSITIONS ON BIOCRUDE YIELD AND QUALITY

BY

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THESIS

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ABSTRACT

There is strong interest in microalgae-derived biofuel as a sustainable replacement for fossil fuels due to the numerous advantages of microalgae as a feedstock. Hydrothermal Liquefaction (HTL) is a thermochemical process that uses water as the reaction medium to convert biomass into biocrude oil under elevated temperatures and pressures (200–350°C, 5–20 MPa). While there is extensive literature on the separate processes of microalgae cultivation and HTL conversion, relationships between different biochemical compositions of a single species and HTL which contribute to the understanding of the overall system remain unexplored. This study examines the influence of varying microalgae cell composition on HTL biocrude yield and chemical composition. *Nannochloropsis oculata* was cultivated under depleting nitrogen levels to obtain biomass with variable cell compositions (17–59 %dw lipids; 45–17 %dw proteins; 11–22 %dw carbohydrates). HTL of harvested biomass was conducted at commonly used process conditions (80 wt% moisture, 300°C, 30 min reaction time), and conversion products were characterized to evaluate the energy yield and chemical characteristic of the biocrude phase. Results suggest for the case of lipid-accumulating biomass that biocrude yield is strongly determined by the increasing fatty acid methyl ester (FAMEs) content and not gross lipid content as previously thought. A model linking biomass composition to HTL biocrude yield is proposed based on the contributions from a baseline (non-FAMEs) fraction and FAMEs fraction of the biomass. Biocrude yields and higher heating values (HHV, MJ/kg) both increase with increasing FAMEs content, leading to higher energy recovery within the biocrude product phase. Increasing FAMEs content also leads to biocrude products with an increasing fraction of compounds with boiling points between 300–400°C, decreasing nitrogen content, and decreasing average
molecular weight distribution. Results from this study contribute to the understanding of the interrelationships between biomass feedstock composition and the conversion products resulting from HTL. This information can be further linked to separate models for controlling biomass composition during upstream cultivation to enable more integrated analysis of the overall algal-HTL biofuel pathway.
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# TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION ................................................................................................................................. 1
  1.1 Motivation ....................................................................................................................................................... 1
  1.2 Background and Challenges ......................................................................................................................... 2
  1.3 Research Scope and Objectives .................................................................................................................. 4

CHAPTER 2: MATERIALS AND METHODS ............................................................................................................. 6
  2.1 Cultivation of *Nannochloropsis* Biomass ..................................................................................................... 6
  2.2 Biomass Composition Analysis ..................................................................................................................... 7
  2.3 HTL of Biomass ............................................................................................................................................. 9
  2.4 Product Analysis and Energy Balance ......................................................................................................... 11

CHAPTER 3: RESULTS AND DISCUSSION .............................................................................................................. 13
  3.1 Biomass Composition .................................................................................................................................. 13
  3.2 HTL of Replicate Batches .......................................................................................................................... 15
  3.3 HTL of Biomass with Varying Compositions ............................................................................................... 16
  3.4 Bulk Properties of HTL Product Fractions ................................................................................................. 17
  3.5 Biocrude Properties ..................................................................................................................................... 17
  3.6 Figures and Tables ........................................................................................................................................ 19

CHAPTER 4: DISCUSSION ....................................................................................................................................... 28
  4.1 Discussion on Results ................................................................................................................................... 28
  4.2 Broader Implications and Future Work ......................................................................................................... 30

CHAPTER 5: SUMMARY AND CONCLUSIONS ....................................................................................................... 33

REFERENCES ....................................................................................................................................................... 35

APPENDIX A: SUPPLEMENTARY INFORMATION ................................................................................................. 39
CHAPTER 1
INTRODUCTION

1.1 Motivation

Rapidly depleting hydrocarbon fuel resources in the face of the world’s increasing energy demands has necessitated the development of renewable energy technologies that cause minimal impact to the environment.\textsuperscript{1–3} Microalgae is a potential feedstock for renewable energy with advantages such as rapid growth rates and high energy contents as compared to lignocellulosic feedstocks, relatively low nutritional requirements, and non-competition with food crops.\textsuperscript{4–6} Hydrothermal Liquefaction (HTL) is a thermochemical process that produces biocrude oil as the main product from wet biomass using water at elevated temperatures (200$^\circ$C–350$^\circ$C) and pressure (5–15 MPa) as the reaction medium,\textsuperscript{7–9} conveniently eliminating energy intensive drying steps when combined with microalgae harvested as a wet slurry.\textsuperscript{8,10} Reaction by-products include aqueous, gas and solid phases that can be utilized for nutrient (N and P) recovery.\textsuperscript{11–13} Recent interest in harnessing microalgae as a feedstock for renewable biofuels has led to numerous studies on the manipulation of biochemical composition for downstream biofuel conversion,\textsuperscript{5,14} while the HTL of different species of microalgae with variable compositions has observed variances in the yields and chemical properties of conversion products.\textsuperscript{15,16}

Despite these research efforts in HTL for downstream processing of microalgae feedstocks, quantitative links between feedstock cell composition and the products of HTL conversions processes have yet to be fully established. Previous attempts to address the influences of biomass composition on HTL products provided basic information that served as fundamental starting points for current research, but these observations were made with different
algae species each with a single fixed cell composition. Lack of variations in composition within a single species and characterization of biomass properties beyond proximate analysis diminish the impact of observed trends. The synergy between accumulating energy-rich product fractions in microalgae and net benefits in terms of HTL biocrude yield and quality also remains unexplored. In order to advance the field toward unified models for biofuels and bioproducts production that consider both upstream cultivation and downstream conversion steps in an overall system, the HTL of a single algae species with systematically varied biochemical composition has to be studied to derive predictive relationships for the conversion process.

1.2 Background and Challenges

A number of studies have established that microalgae cell composition can be controlled during cultivation.\textsuperscript{5,17} In particular, research efforts have focused on selecting for the accumulation of energy rich lipids, either through mutants that have starch production deficiencies\textsuperscript{18,19} or through growth conditions that select for lipids during carbon storage.\textsuperscript{20–23} Rodolfi et al. demonstrated for the marine microalgae \textit{Nannochloropsis} that lipid content could be varied within 30–60 wt\% through nitrogen depletion over a 9 day growth cycle.\textsuperscript{5} The distribution of various fatty acids can also shift during growth cycles.\textsuperscript{24}

Key determinants in downstream HTL outcomes such as the yield and chemical properties of biocrude,\textsuperscript{16,25} and gravimetric, carbon and nitrogen distributions between the different HTL product fractions,\textsuperscript{15,25–27} are intrinsically tied to all or some portion of the biomass composition (i.e., lipids, proteins, and carbohydrates).\textsuperscript{15} The neutral and polar lipid fractions and fatty acid profiles are also key determinants of the chemical properties of the biocrude product, such as elemental composition, higher heating values (HHV), functional group chemistry and
molecular weight distribution. Biller and Ross have attempted to provide a simple estimation of biocrude yield through the linear superposition of yields obtained for individual model compound conversions. However, model predictions matched observed yields for only two of the four microalgae species investigated, leading them to suggest that the complexity of the relationship extends beyond the summation of independent yields for individual biomass fractions estimated from model compounds.

The above-mentioned studies involved different algae species including marine and freshwater microalgae and cyanobacteria all with fixed cell compositions, which gives rise to a number of pertinent issues. The results obtained from a small number of biomass samples or model compounds without any form of systematically varying and well analyzed cell compositions cannot provide meaningful predictive trends. Cellular physiological differences between species, such as cell wall thickness, might also affect HTL yield and products and obscure the true relationships to composition. In addition, proximate biomass analysis cannot provide detailed information on the potential effects attributable to specific fatty acid profiles or neutral versus polar lipid distributions. Finally, the extent to which lipid accumulation in microalgae will result in net benefits to HTL has not yet been clarified. The combination of biochemical compositions with HTL is an inevitable future; the tailoring of biomass to optimize the overall system and leverage on the numerous benefits is essential moving forward. These challenges stated herein must therefore be addressed for microalgae-derived HTL biofuels to serve as a sustainable, economical, and renewable energy source.
1.3 Research Scope and Objectives

The objective of this research was to evaluate the influence of microalgae cell composition on the yields and characteristics of HTL biocrudes. To accomplish this, the marine microalgae *Nannochloropsis oculata* was cultivated and subjected to various nitrogen-depleted conditions to obtain biomass with variable cell composition that was processed by HTL. *Nannochloropsis* was selected as a model microalgae species given its wide ranging lipid contents under variable cultivation conditions,\(^5,21,24\) and commercially produced biomass with a single composition has been extensively studied as a feedstock for HTL conversion.\(^15,29–31\)

Harvested biomass was analyzed for crude lipid, protein and carbohydrate fractions, fatty acid profiles, and neutral and polar lipid distributions.\(^15,16,28\) Mass yields of the HTL biocrude products were compared for different harvested *Nannochloropsis* batches and biocrude properties were extensively characterized for elemental composition, HHV, molecular weight distribution, and boiling point fractions.\(^16,32\) Distributions of initial biomass carbon among the product fractions were also analyzed.\(^11,12,26\)

To the best of our knowledge, this is the first study in which microalgae biomass has been cultivated specifically to examine the influence of cell composition on HTL biocrudes, providing new insights into chemical-level processes in the hydrothermal environment that contribute to biomass conversion to liquid fuels and supporting the development of pseudo-mechanistic models for HTL conversion of microalgae feedstocks. Experimental methods are described in detail in Chapter 2. Results are then presented in Chapter 3 and discussed as a whole in Chapter 4. Results were examined for significant relationships between components in cultivated biomass and HTL biocrude yield and quality, and also used to propose cell
composition as a unifying factor between upstream cultivation and downstream conversion in the overall algae biomass to biofuel process.
CHAPTER 2
MATERIALS AND METHODS

2.1 Cultivation of *Nannochloropsis* Biomass

A flat-panel, acrylic photobioreactor (PBR) with a working volume of 3.5 L and 1 in. light path was constructed as described by Guest and co-workers.\(^20\) The PBR was UV-sterilized prior to adding 3.5 L of autoclaved modified f2 growth media following the recipe of Guillard and Ryther\(^{33}\) with silica omitted and additional phosphate and nitrate added (as 0.03 g/L \(\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}\) and 0.5 g/L \(\text{NaNO}_3\), respectively). Two 15 mL starter cultures of *Nannochloropsis oculata* (strain CCMP525) in L1-Si media were obtained from the National Center for Marine Algae and Microbiota (East Boothbay, ME) and used to inoculate the PBRs immediately upon arrival. Interspaced blue and red LED lights mounted on a wall parallel to the reactor provided continuous illumination of 250 \(\mu\text{E/m}^2\cdot\text{s}\) at the surface of the reactor. Temperature was maintained at 16°C with a circulatory water bath. A diffuser at the bottom of the reactor provided 2 L/min of ambient air filtered through a HEPA filter for aeration and mixing. The culture was maintained at pH 7.8–8.2 with a solenoid valve and pH controller which delivered pure CO\(_2\) through the diffuser as necessary to reduce pH.

*Nannochloropsis* is an Eustigmatophyceae known to shift from a green-colored culture to brown as nutrients are depleted in the growth medium, particularly nitrogen as nitrate.\(^24,34\) This visual cue was employed to determine when a batch was ready to harvest (by draining the PBR through a valve attached to the side), after which an equivalent volume of replete media was added to initiate the next batch growth cycle. This technique was utilized to harvest seven batches of varying intensities of green and brown. The drained media was centrifuged at 6,300 \(\times\)
for 15 min (Sorvall™ RC 6+). The supernatant was decanted and the solids rinsed with deionized (DI) water (>18 MΩ-cm) to remove residual salt from the media. The biomass was centrifuged again at 6,300 × g (Eppendorf 5810R) for 15 min before removing the supernatant and collecting the biomass pellet. To preserve the biochemical composition during storage prior to analysis and HTL processing, the wet biomass was lyophilized (Model 77500 Freeze Dry System, FreeZone), and the resulting solid was ground and homogenized with a mortar and pestle and stored in a desiccator at 4°C.

2.2 Biomass Composition Analysis

Moisture content was determined gravimetrically by drying triplicate samples at 105°C for 1 h, and ash content was determined by igniting the dried biomass at 550°C for 30 min. Total C, H and N content (wt% of biomass) was measured at the University of Illinois Microanalysis Laboratory (Urbana, IL) using an Exeter Analytical CE-440 Elemental Analyzer. Oxygen content of the volatile solids was estimated by difference (%O = 100% − %C − %H − %N − %Ash) and assuming sulfur content is insignificant (≤0.5 wt%).35 HHV was estimated from the elemental composition via the method of Dulong.15,30 Crude protein was estimated by multiplying %N by 6.25.35,36 Although this factor has been argued to substantially overestimate the protein content in Nannochloropsis and other microalgae in general, suggested alternative multipliers (e.g., 4.78)37,38 were found to be highly dependent on growth conditions and did not provide the intended mass closure in other studies.39 Biomass nutrient profiling of Nannochloropsis has previously shown ≤2 wt% of nucleic acids in the biomass,35 and were thus not analyzed with the reasonable assumption that most of the measured %N could be attributed to proteins.
Crude lipid was analyzed gravimetrically according to the Folch method using a 2:1 chloroform-methanol mix, modified only such that the biomass was briefly sonicated prior to solvent extraction to enhance cell rupture and promote lipid dissolution.\textsuperscript{40-42} The crude lipid extract was dissolved in 5 mL of 86:14 v/v chloroform-methanol and stored at $-20^\circ$C for lipid fractionation analysis. Crude carbohydrate content was estimated using the colorimetric Dubois method.\textsuperscript{43} Literature on summative mass closures of algae biomass routinely perform proximate analysis according to similar methods, therefore near complete closure through the summation of the ash, moisture, crude lipid, crude carbohydrate, and crude protein fractions was expected.\textsuperscript{35,39,44}

The crude lipid extract was further characterized to provide more detailed chemical speciation information. Neutral lipid (NL) and polar lipid (PL) fractions were separated by solid phase extraction (SPE; Waters Sep-Pak\textsuperscript{®} cyanopropyl vac cartridges with 1 g sorbent) and determined gravimetrically after evaporation of eluents.\textsuperscript{45-47} The SPE cartridge was first conditioned with 10 mL of n-hexane and then loaded with approximately 30 mg crude lipids dissolved in 2 mL n-hexane. Elution with 8 mL of 9:1 v/v hexane-diethyl ether provided the NLs, which appeared as a yellowish oil-like substance after solvent removal. Subsequent elution with 8 mL of 2:1 v/v chloroform-methanol followed by 4 mL of methanol yielded a deep-green substance indicating the presence of pigments and dyes, such as chlorophyll. NLs are defined as compounds with polarity less than or similar to triacylglycerides (TAGs),\textsuperscript{48-50} and is expected to contain compounds such as sterols, waxes, and carotenoids in addition to TAGs. The PL fraction is then expected to include phospholipids, sphingolipids, and glyrophospholipids.\textsuperscript{45,51}

Fatty acid profiles of the lipid extracts were obtained following the direct in-situ transesterification FAMEs analysis method described by Laurens et al,\textsuperscript{52} and methyl tricosanoate
(C23:0) internal standards was used in place of methyl tridecanoate (C13:0). FAMEs were prepared accordingly and analyzed with a HP 5890 Series II gas chromatograph with a flame ionization detector (GC-FID), equipped with a Restek Stabilwax-DA column (30 m × 0.25 mm × 0.25 μm). Helium (2.5 mL/min) served as the carrier gas and injector split flow was set at 50 mL/min. Oven temperature was held at 210°C for 5 min then increased to 250°C at a rate of 20°C/min and held for 12 min. The injector and detector were set to 250°C. A 0.5 μL injection volume was used, and two injections were made for each sample. GC-FID response peaks were calibrated and quantified with F.A.M.E. mix analytical standards (Sigma-Aldrich #18919). Concentration was normalized to the recovery of the C23:0 internal standard and reported as percent dry weight of biomass (%dw).

2.3 HTL of Biomass

HTL of the harvested batches was conducted using 316-stainless steel tube batch reactors as previously described by others.29,30,53 HTL of each *Nannochloropsis* batch was carried out in duplicate. The method was adapted for 6 in. tubes (3/8 in. outer diameter, 0.049 in. wall thickness, 5.93 mL working volume) plugged with Swagelok® SS-316 port connectors on both ends. DI water was added to freeze-dried biomass to obtain an 80 wt% moisture slurry, about 4 g of which was loaded into the tube reactor under ambient air. Reactors were placed in a muffle furnace (Type 30400, Thermolyne), preheated to 300°C, for 30 min with an additional 5 min of reactor heat-up time.30 This single HTL condition was selected based on optimal conditions in terms of yield or net energy efficiency reported to be within 300–350°C and 30–60 min for various biomass.6,10,29,54,55 After reaction, the tubes were removed and quenched in a cold water bath, then transferred to a glass desiccator for 1 h at room temperature to allow equilibration.
prior to product recovery. The tube reactors were weighed to ensure that no mass was lost during the HTL reaction, and carefully opened to vent gas phase products generated from the reaction. Gas product yield was then determined by re-weighing the reactor. No analysis of the gas products were performed here and the composition was assumed to be ~100% CO$_2$ when estimating C mass balances. This assumption is based on reports by Brown et al. and Valdez et al. that the gas phase is predominantly CO$_2$ (91.5 mol% at 300°C for 1 h, and >93 mol% under alternative HTL conditions in the respective studies) during HTL of *Nannochloropsis*.$^{29,30}$

Reactor contents were poured out into a glass beaker, and 30 mL of dichloromethane (DCM) was added to completely extract any DCM-soluble products, which is classified as the biocrude phase. The reactor was then rinsed with 30 mL of DI water to recover any residual aqueous phase product. Both DCM-dissolved biocrude and DI water were added to the beaker. An equivalent amount of DCM and DI water ensured there were no experimental artifacts from the artificial partitioning of products with different volumes of aqueous and organic solvents. Finally, the reactors were scraped with glass Pasteur pipets to recover any solids stuck on the sidewalls. The tube reactor was dried at 65°C for 1 h and weighed after cooling to room temperature to ensure minimal product remaining in the reactor (<2.5 %dw of loaded biomass observed for all experiments). The collected product mixture was filtered into a separatory funnel through 0.45 μm Teflon filter cartridges (Whatman) to isolate the DCM/DI water insoluble solid phase product. Cartridges were dried in a desiccator overnight and weighed to obtain the solid phase yield. The separatory funnel was shaken to thoroughly mix the biocrude and aqueous phases which were then allowed to self-separate. The DCM phase containing the biocrude was then isolated and DCM removed under a stream of N$_2$ at 50°C for 2 h before weighing to obtain the biocrude yield. The aqueous phase was diluted to 50 mL DI water, and two separate 10 mL
aliquots of the diluted sample were dried at 65°C for 16 h and then weighed to determine the aqueous phase yield. Gravimetric mass yields of the four product phases are reported as %dw.

In order to demonstrate that the composition of a harvested batch and its subsequent HTL conversion can be replicated, two batches were cultivated and harvested under identical conditions as the cultivated batch with lowest lipid (Batch 2) and designated 2(b) and 2(c). For comparisons to results in literature, *Nannochloropsis* biomass slurry (>70 wt% moisture) was purchased from Reed Mariculture, Inc. (Campbell, CA) similar to previous HTL studies and was subjected to the above described biomass processing method for consistency and designated Batch 1. Supplier documentation indicates this biomass as *Nannochloropsis oculata* strain CCMP525 which is identical to the one used for cultivation in this study.

### 2.4 Product Analysis and Energy Balance

The biocrude product was analyzed for elemental composition and HHV as described for the biomass samples, with the ash content of the biocrude assumed to be negligible (\(\%C + \%H + \%N + \%O = 100\) wt%). Size exclusion chromatography (SEC) and simulated distillation (Sim-Dist) were also performed according to methods described previously. SEC provided the molecular weight distributions of the biocrude mixture, while Sim-Dist using an adapted ASTM-7169-05 method provided the approximate boiling point distributions. Pure fatty acids (≥99%, Sigma-Aldrich) were also analyzed via Sim-Dist to identify individual peaks observed in the biocrude boiling point profile.

The Energy Consumption Ratio (ECR), defined as the ratio of energy required in heating the reactors for HTL processing to the energy available in the biocrude oil product through
combustion, was calculated with typical combustion and thermal energy recovery loss assumptions:\textsuperscript{15,25,56}

\[
ECR = \frac{\Delta T [W_i C_{pw} + C_{pb} (1-W_i) (1-R_h)]}{Y (1-W_i) (HHV_{b}) R_c}
\]  

(2.1)

where \(\Delta T\) is the temperature increase to reach reaction conditions (275 K for a 300°C reaction, assuming 25°C ambient temperature), \(W_i\) is the initial moisture content (0.8), \(C_{pw}\) is the specific heat of water (4.18 kJ/kg.K), \(C_{pb}\) is the specific heat of biomass (1.25 kJ/kg.K)\textsuperscript{25,56}, \(R_h\) is the heat recovery efficiency (assumed 0.5), \(R_c\) is the combustion energy efficiency (assumed 0.7), \(Y\) is the biocrude yield, and \(HHV_{b}\) the higher heating value of the biocrude oil in kJ/kg. Energy Recovery Percentage (ER\%), defined as the fraction of energy in the dry biomass feedstock recovered as energy in the biocrude oil,\textsuperscript{15,29} was calculated by:

\[
ER\% = \frac{(HHV_{b})(Y)}{HHV_m} \times 100\%
\]  

(2.2)

where \(HHV_m\) is the higher heating value of biomass in kJ/kg.

The fraction of carbon in the aqueous phase was determined by analysis of total organic carbon (TOC; Shimadzu TOC-V CPN TOC analyzer). Only a very small mass of solid was generated during HTL reactions (<20 mg for most batches), so carbon distribution to the solid phase was estimated based on previous reports of HTL of \textit{Nannochloropsis}. The solid phase was assumed to be 10.75 wt% carbon, estimated from the average values previously reported for HTL at 300°C for 20 and 40 min (11.4 and 10.1 wt%, respectively).\textsuperscript{29} Carbon content in the gas and biocrude phases were determined as described above.
CHAPTER 3
RESULTS AND DISCUSSION

3.1 Biomass Composition

The results from the proximate analysis of all seven batches of Nannochloropsis, and two replicates of Batch 2, can be found in Appendix A. Summation of measured biomass components ranged from 102–107 wt% across the batches, indicating that mass closure has been established, albeit with a slight overestimation (>100 wt%). This is generally expected given that the crude proximate methods might count some components like glycoproteins (consists of protein and carbohydrate) within the biomass twice, though not necessarily with the same magnitude.\(^{39}\) To make meaningful comparisons between the varying compositions, summation of each batch was corrected to 100 wt% by applying a multiplicative factor to the 5 components equally. The results for the seven batches are presented in Figure 1 as %dw, and for the two replicate batches inset in Figure 2. Elemental analysis, HHV, and lipid fractionation results are listed in Table 1. All tables and figures reporting properties related to the seven batches and their corresponding HTL products are arranged unless otherwise specified in ascending lipid content, which also coincides with increasing cultivation time required before harvesting.

The proximate analysis of commercially obtained Nannochloropsis, Batch 1, falls within the range of reported compositions,\(^{15,29,30}\) with the minor differences attributable to differences such as growth conditions, analytical methods, and product quality control. Batches 2 to 7 display an expected increasing trend in terms of lipid content (30–58 %dw, Figure 1) since they were cultivated in increasingly nitrogen deplete media, with a correspondingly decreasing protein content (52–17 %dw) and smaller variations in carbohydrate content (12–22 %dw).
Elemental composition shows that %C and %H increases and %N decreases with increasing lipid fraction in the batches, whereas %O remains fairly constant across the batches (Table 1). Increased %C and %H corresponds to greater HHV_m, which should be expected given the higher fraction of C- and H-rich lipids and lower fraction of N-rich proteins.

Lipid fractionation analysis (Table 1) reveals that the lipid content increase is almost solely attributable to the accumulation of neutral lipids (NL, 3.7–50 %dw) while retaining a fairly constant polar lipid (PL) content as indicated by the PL/protein ratio (0.3–0.61). Although detailed characterization of NL speciation was not performed here, previous studies have shown that the increase in NL fraction can be attributed to the accumulation of TAGs.24,57 This likely occurs as the cell uptakes carbon during nutrient depletion and stores it as reduced compounds in the form of TAGs, while only slightly varying the functional structural lipid content that includes phospholipids and pigments.24

The total FAMEs content across the seven batches increased in a pattern similar to the NL content from 10.5–52.0 %dw (Table 2). FAMEs content for every batch was consistently higher than the corresponding NL content, likely due to a small fraction of FAMEs that was derived from certain components in the PLs that can be transesterified.45,58 Total polyunsaturated fatty acids (PUFAs) changed relatively little as compared to the saturated and mono-unsaturated FAMEs content, which corroborates with the accumulation of storage products while maintaining structural lipid components.59 Analysis of the individual FAMEs reveals that regardless of batch, palmitic (C16:0) and palmitoleic (C16:1) were the predominant fatty acids (FAs), along with comparatively smaller portions of myristic (C14:0), oleic (C18:1), eicosatrienoic (C20:3n3), and eicosapentenoic (C20:3n5) acids. These five FAs are commonly
reported as the dominant majority in *Nannochloropsis*, \textsuperscript{24,35,57,60} though their exact distribution can vary widely depending on cultivation methods.\textsuperscript{59,60}

### 3.2 HTL of Replicate Batches

Results from the comparison of HTL of varying *Nannochloropsis* compositions would not provide any meaningful contribution if the cell compositions if a given batch cannot be reasonably reproduced identical cultivation conditions. The same applies to HTL conversions; large variances in product yields from the HTL of very similar compositions would indicate that downstream conversion is governed less by cell composition and instead by other factors like reaction conditions. The results of the proximate analysis and subsequent HTL conversion are presented in Figure 2. In this section we ignore the exact product distributions as related to cell composition, which will be discussed in exhaustive detail in Section 3.3, and instead focus on the reproducibility of biomass composition and HTL results.

The proximate analysis of all 3 batches have <2 %dw differences for each of the three components. Likewise, the HTL converted biocrude oil and solid phase yields are almost identical at 53 and 3 %dw, respectively. There are slightly larger variances in the aqueous phase (about 6 %dw) and gas yields (about 4.5 %dw). These differences are not likely due to the small differences in feedstock composition, but rather to errors inherent to the relevant gravimetric methods. Moisture removal via heating at 16 h creates a larger room for error, as does gas release from the tube instead of more accurate quantification with internal inert gas standards.\textsuperscript{30} Still, the overall variances in Figure 2 can be considered small in view of the breadth and continuity of work involved in cultivation and conversion. The results obtained via HTL at identical
conditions are thus shown to be reproducible so long as the biochemical composition is identical, which is achievable by cultivating and harvesting under the same conditions for each batch.

3.3 HTL of Biomass with Varying Compositions

For each batch converted under HTL, total product recovery ranged from 98.1–99.7 %dw with the sole exception of Batch 1, commercially obtained *Nannochloropsis*, at 80.9 %dw. Total carbon recovery for Batch 1 is likewise comparatively lower (86.6 %C and >95 %C for all other batches, Table 3). The low product and carbon recovery could be due to the higher inorganic carbon content in the aqueous phase similarly observed by Valdez et al. (~14 %C for HTL at similar conditions)\(^29\), which would not be detected during gravimetric analysis of aqueous dissolved solids and TOC. Low recovery aside, similar product distributions for HTL of Batch 1 have been reported in other studies.\(^{15,53}\)

The biocrude oil yield increases from 30–68 %dw and aqueous phase yield decreases from 42–13 %dw as lipid content in the *Nannochloropsis* feedstocks increase (Figure 3), with relatively minor changes being observed in the solid (2–5 %dw) and gas yields (11–22 %dw). As previously mentioned, the gas phase has been reported to be largely CO\(_2\) (>90%) with only small amounts of CH\(_4\), C\(_2\)H\(_4\), H\(_2\)O, and has little value beyond serving as a potential CO\(_2\) source for upstream phototrophic algae cultivation within an integrated processing facility.\(^{11}\) The solids might have some utility as bio-char,\(^{13,61}\) though at such small yields the valorization of solid products is not likely to be meaningful. The biocrude oil and aqueous phase products are largely dominant regardless of cell composition, together accounting for >70 %dw of total product yield and the majority of carbon in the biomass (>85 %C, Table 3). Subsequent discussion will focus mostly on these two dominant product fractions.
3.4 Bulk Properties of HTL Product Fractions

Elemental composition of the biocrude and aqueous products from HTL of the commercially obtained *Nannochloropsis* (Batch 1) are similar to those reported by Valdez et al.\textsuperscript{53} Individual %C, %H and %O for the biocrude of each batch saw small increases that compensated for the more significant decrease in %N (Table 4). Batches with a smaller fraction of proteins tend to give biocrude yields with lower %N, in agreement with previous reports.\textsuperscript{15,16} The fairly constant CHO content of the biocrude products produced from different batches results in HHVs that vary little, other than a dip in Batch 2. Small changes to the HHVs have typically been observed for different microalgae biomass sources, and appears to be insensitive to either HTL conditions or biomass composition for reasons that remain unclear.\textsuperscript{25,29,62}

3.5 Biocrude Properties

SEC analysis of biocrude molecular weight (MW) distributions show a similar pattern across the seven batches (Figure 4), with one key feature being the centering of peaks around the 200–300 Da region where FAs would fall under (C14:0 – 228 Da, C20:5n3 – 300 Da). In general, the fraction of biocrude shifts towards the 200–300 Da range as biomass lipid content increases, as indicated by the taller peaks. The weight-averaged molecular weight, $M_w$, weighted more heavily towards the larger molecules in the biocrude, is fairly constant across the batches (Table 5), indicating some similarity in the amount of larger oligomers from the partial hydrolysis of proteins and carbohydrates in the seven biocrude products.\textsuperscript{25,63} The number-average molecular weight, $M_n$, weighted more heavily towards smaller molecules, decreases with increasing lipid content of biomass feedstock, which is likely due to the increased presence of FAs with lower MWs. The polydispersity index (PDI), which indicates the spread of MWs
among all the constituents in the biocrude, increases along the batches. Together with the \( M_w \), this again shows the growing discrepancy between higher amounts of low MW FAs against fairly static amounts of other larger MW compounds in the biocrude when lipid content of the feedstock increases.

Biocrude oil boiling point (BP) distribution as analyzed by Sim-Dist is shown in Figure 5. For all batches the largest percentage of biocrude falls in the 300–400°C range, which is typical for HTL biocrudes.\(^{16,25}\) Focus was placed on the 300–400°C fraction given that it showed the largest difference across batches (45.9–76.2% of biocrude). Sim-Dist peaks for reference FAs (C14:0, C16:0, C18:0 and C18:1) matched peaks observed in the biocrude samples (Figure 6A), suggesting that the TAGs in biomass may have hydrolyzed to free fatty acids and partitioned with other biocrude components during HTL. That is, biomass with a higher FAMEs content produces biocrude with larger peak areas at retention times corresponding to the 300–400°C fraction (Figure 6B).\(^{64,65}\) C18:0 (stearic acid) and C18:1 correspond to one peak in the biocrude profile and cannot be identified since monounsaturated FAs could be hydrogenated under subcritical water even without the presence of catalysts.\(^{28}\)
3.6 Figures and Tables

Figure 1. Major cell compositions of harvested batches (%dw). Results are reported as average of duplicate analysis with standard deviations (SDs) shown as error bars.
Figure 2. HTL product distribution of Batch 2, replicate batches, and the average results of the 3 batches indicated as 2(avg). Results reported as average of duplicate HTL reactions and SDs indicated by error bars. Proximate analysis results are shown inset.
Figure 3. HTL product distributions of Batch 1 to 7 in ascending order. Biocrude oil and aqueous phase yields plotted as a function of biomass FAMEs content (%dw) shown inset. Results reported are the average of duplicate HTL conversions. Vertical error bars indicate SD in yield and horizontal error bars indicate SD in FAMEs content.
Figure 4. Molecular weight distribution of biocrude products via SEC analysis. Peaks center at ~250 Da indicated by vertical line.
Figure 5. Sim-Dist boiling point distribution of biocrude oil. Results reported as duplicate analysis and SDs shown as error bars. Inset shows the fraction of biocrude yield with boiling points in the 300–400°C range as a function of the C14–C20 FAMEs in biomass feedstock.
Figure 6. Sim-Dist boiling point distributions. (A) Model compounds comparing to a single biocrude sample (Batch 7). (B) Comparisons of the biocrude samples of Batch 1 to 7.
Table 1. Elemental analysis, HHV, and lipid fractions of the *Nannochloropsis* batches

<table>
<thead>
<tr>
<th>Batch</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>%O²</th>
<th>HHV (MJ/kg)</th>
<th>Neutral (%dw)</th>
<th>Polar (%dw)</th>
<th>NL PL</th>
<th>PL Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.8</td>
<td>5.7</td>
<td>6.9</td>
<td>21.4</td>
<td>18.2</td>
<td>3.7</td>
<td>13.5</td>
<td>0.28</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>54.6</td>
<td>7.9</td>
<td>8.7</td>
<td>23.3</td>
<td>25.7</td>
<td>12.2</td>
<td>18.4</td>
<td>0.66</td>
<td>0.36</td>
</tr>
<tr>
<td>3</td>
<td>59.2</td>
<td>8.8</td>
<td>4.6</td>
<td>24.3</td>
<td>28.3</td>
<td>31.0</td>
<td>15.9</td>
<td>1.95</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>60.1</td>
<td>8.8</td>
<td>5.4</td>
<td>23.4</td>
<td>28.9</td>
<td>29.3</td>
<td>19.6</td>
<td>1.50</td>
<td>0.61</td>
</tr>
<tr>
<td>5</td>
<td>60.3</td>
<td>9.2</td>
<td>4.3</td>
<td>22.8</td>
<td>29.5</td>
<td>36.1</td>
<td>14.9</td>
<td>2.42</td>
<td>0.56</td>
</tr>
<tr>
<td>6</td>
<td>62.9</td>
<td>9.3</td>
<td>3.9</td>
<td>21.8</td>
<td>30.7</td>
<td>43.3</td>
<td>12.8</td>
<td>3.38</td>
<td>0.55</td>
</tr>
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<td>7</td>
<td>62.3</td>
<td>9.3</td>
<td>2.8</td>
<td>23.5</td>
<td>30.3</td>
<td>50.0</td>
<td>8.6</td>
<td>5.81</td>
<td>0.50</td>
</tr>
</tbody>
</table>

1) All values reported as mean of duplicate analysis with SDs <±0.5% or %dw
2) %O = 100% − %C − %H − %N − Ash%
3) Lipid fractionation method recovery was 96.2–99.7% for all batches, and corrected to 100% for each batch

Table 2. FAMEs¹ profile of the *Nannochloropsis* batches

<table>
<thead>
<tr>
<th>Batch</th>
<th>Total FAMES</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C18:1</th>
<th>C20:3n5</th>
<th>C20:3n3</th>
<th>Total Saturated</th>
<th>Total MUFA²</th>
<th>Total PUFA³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.5±0.5</td>
<td>0.24</td>
<td>1.97</td>
<td>1.14</td>
<td>0.27</td>
<td>0.24</td>
<td>2.34</td>
<td>2.32</td>
<td>1.80</td>
<td>5.94</td>
</tr>
<tr>
<td>2</td>
<td>19.8±2.4</td>
<td>1.02</td>
<td>4.73</td>
<td>4.65</td>
<td>1.10</td>
<td>1.09</td>
<td>5.82</td>
<td>6.15</td>
<td>5.92</td>
<td>7.45</td>
</tr>
<tr>
<td>3</td>
<td>38.6±0.2</td>
<td>2.24</td>
<td>9.80</td>
<td>11.51</td>
<td>2.76</td>
<td>2.78</td>
<td>7.66</td>
<td>12.46</td>
<td>14.62</td>
<td>11.30</td>
</tr>
<tr>
<td>4</td>
<td>39.9±0.3</td>
<td>2.37</td>
<td>11.20</td>
<td>9.93</td>
<td>1.67</td>
<td>2.49</td>
<td>9.61</td>
<td>14.17</td>
<td>12.19</td>
<td>13.07</td>
</tr>
<tr>
<td>5</td>
<td>42.4±0.5</td>
<td>2.38</td>
<td>12.94</td>
<td>12.76</td>
<td>2.51</td>
<td>1.84</td>
<td>7.48</td>
<td>15.91</td>
<td>16.14</td>
<td>10.31</td>
</tr>
<tr>
<td>6</td>
<td>50.3±0.0</td>
<td>3.17</td>
<td>16.34</td>
<td>14.73</td>
<td>2.89</td>
<td>2.14</td>
<td>8.38</td>
<td>20.24</td>
<td>18.44</td>
<td>11.50</td>
</tr>
<tr>
<td>7</td>
<td>52.0±0.4</td>
<td>4.03</td>
<td>14.63</td>
<td>16.71</td>
<td>4.82</td>
<td>2.25</td>
<td>6.73</td>
<td>19.45</td>
<td>22.30</td>
<td>10.23</td>
</tr>
</tbody>
</table>

1) All values as %dw. Individual FAME only shown if >1.0% of total FAMEs. SDs of individual FAMEs <±0.1%dw except for Batch 2 (±0.8, 1.5, 0.7, 1.4 %dw for C16:0, C16:1, C18:1, C20:3n5, respectively)
2) Mono-unsaturated Fatty Acids
3) Poly-unsaturated Fatty Acids
Table 3. Distribution of carbon from biomass to HTL products

<table>
<thead>
<tr>
<th>Batch</th>
<th>Biocrude</th>
<th>Aqueous</th>
<th>Gas</th>
<th>Solids</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54.3±0.9</td>
<td>27.2±1.0</td>
<td>4.3±0.6</td>
<td>0.7±0.0</td>
<td>86.6±0.8</td>
</tr>
<tr>
<td>2</td>
<td>70.4±1.9</td>
<td>21.5±0.2</td>
<td>8.2±0.7</td>
<td>0.6±0.0</td>
<td>100.7±1.3</td>
</tr>
<tr>
<td>3</td>
<td>80.2±0.1</td>
<td>11.9±1.6</td>
<td>7.7±0.3</td>
<td>0.3±0.0</td>
<td>100.1±1.4</td>
</tr>
<tr>
<td>4</td>
<td>78.5±0.2</td>
<td>11.7±0.0</td>
<td>7.2±0.1</td>
<td>0.3±0.0</td>
<td>97.8±0.2</td>
</tr>
<tr>
<td>5</td>
<td>75.1±1.4</td>
<td>10.8±1.3</td>
<td>10.0±0.5</td>
<td>0.3±0.0</td>
<td>96.3±3.2</td>
</tr>
<tr>
<td>6</td>
<td>78.3±1.4</td>
<td>10.1±0.1</td>
<td>7.0±0.7</td>
<td>0.5±0.1</td>
<td>95.9±2.0</td>
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<tr>
<td>7</td>
<td>83.0±0.1</td>
<td>9.6±2.0</td>
<td>5.1±0.5</td>
<td>0.8±0.0</td>
<td>98.4±1.4</td>
</tr>
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</table>

1. %C calculated from elemental analysis
2. %C calculated from TOC
3. Assumed 100% CO<sub>2</sub>
4. %C assumed 10.75%

Table 4. Elemental analysis and HHV of biocrude product and ECR and ER% calculations<sup>1</sup>

<table>
<thead>
<tr>
<th>Batch</th>
<th>Yield</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
<th>% O</th>
<th>% N&lt;sub&gt;bm&lt;/sub&gt;</th>
<th>HHV</th>
<th>ECR</th>
<th>ER%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>74.6</td>
<td>10.2</td>
<td>5.0</td>
<td>10.2</td>
<td>6.9</td>
<td>38.1</td>
<td>0.312</td>
<td>62.2</td>
</tr>
<tr>
<td>2</td>
<td>53.6</td>
<td>71.8</td>
<td>10.2</td>
<td>5.5</td>
<td>12.5</td>
<td>8.7</td>
<td>36.7</td>
<td>0.179</td>
<td>76.7</td>
</tr>
<tr>
<td>3</td>
<td>63.9</td>
<td>74.3</td>
<td>11.1</td>
<td>3.4</td>
<td>11.2</td>
<td>4.6</td>
<td>39.1</td>
<td>0.142</td>
<td>87.5</td>
</tr>
<tr>
<td>4</td>
<td>63.5</td>
<td>74.2</td>
<td>11.1</td>
<td>3.7</td>
<td>11.0</td>
<td>5.4</td>
<td>39.1</td>
<td>0.144</td>
<td>85.0</td>
</tr>
<tr>
<td>5</td>
<td>61.1</td>
<td>74.0</td>
<td>11.1</td>
<td>3.4</td>
<td>11.4</td>
<td>4.3</td>
<td>39.0</td>
<td>0.148</td>
<td>80.8</td>
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<tr>
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<td>75.7</td>
<td>11.5</td>
<td>2.7</td>
<td>10.1</td>
<td>3.9</td>
<td>40.4</td>
<td>0.132</td>
<td>86.8</td>
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<tr>
<td>7</td>
<td>68.3</td>
<td>75.6</td>
<td>11.8</td>
<td>2.0</td>
<td>10.5</td>
<td>2.8</td>
<td>40.8</td>
<td>0.127</td>
<td>91.6</td>
</tr>
</tbody>
</table>

1. Results are the average of duplicate analysis. SDs for elemental analysis ≤±0.7% except Batch 5 %C±2.52%. Biocrude yield (%dw) and %N of biomass (%N<sub>bm</sub>) included for comparison
2. %O = 100% − %C − %H − %N
3. Originally shown in Table 1
Table 5. $M_n$, $M_w$ and PDI values from SEC analysis of biocrude product

<table>
<thead>
<tr>
<th>Batch</th>
<th>$M_n$</th>
<th>$M_w$</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1410±20</td>
<td>4250±90</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>1250±60</td>
<td>3910±200</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>1220±40</td>
<td>4340±140</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>1190±20</td>
<td>4010±120</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>1150±70</td>
<td>4210±170</td>
<td>3.7</td>
</tr>
<tr>
<td>6</td>
<td>1040±1</td>
<td>3720±100</td>
<td>3.6</td>
</tr>
<tr>
<td>7</td>
<td>990±2</td>
<td>3810±4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

1. Number Average Molecular Weight
2. Weight Average Molecular Weight
3. Poly-Dispersity Index. SDs (<±0.09) not shown
4.1 Discussion on Results

Based on the collective results of compositional analysis, it appears that for a single species cultivated for lipid accumulation, each harvested batch contains a baseline composition of structural compounds in the biomass, and the major difference among batches at different growth stages is the relative accumulation of NLs in the form of TAGs. Previous studies of the fate of TAGs under subcritical HTL conditions show that these compounds are rapidly hydrolyzed to liberate TAG-bound FAs as their respective free fatty acid form. Nearly quantitative recovery (>97%) of FAs from their corresponding TAGs have been reported for various vegetable oils, while Biller et al. have shown through GC-MS analysis the presence of FAs in the HTL biocrude product of Nannochloropsis. In the context of HTL conversion, the accumulation of lipids in Nannochloropsis biomass and perhaps for any other microalgae could technically be simplified into an increase in FA content on top of a baseline structural composition.

This simplification of changes in biomass composition suggests that biocrude yield for biomass of varying lipid accumulations will be strongly dictated by the FAMEs content, which was tested by plotting both biocrude and aqueous product yields as a function of FAMEs content (Figure 3, inset). Both displayed a fairly good fit to a linear trend ($R^2 = 0.838$). While the sample size might be considered insufficient for rigorous statistical analysis, reasonable conclusions can be made that the biocrude yield is indeed correlated with the FAMEs content of the feedstock, while aqueous phase yields exhibit an inverse trend to balance the increase in biocrude. This
presents a new paradigm in the HTL of varying biomass compositions, where accumulated fatty acids proportionately increases biocrude yield from a baseline value due to structural components, and aqueous phase yield decreases accordingly assuming insignificant changes to the solid and gas phases. HTL of microalgae species that have variable compositions, such as *Nannochloropsis*, can thus be predicted with reasonable accuracy knowing the fatty acid content and non-lipid accumulated (baseline) biomass composition, as long as HTL conditions are maintained.

Sim-Dist and SEC results further suggest that the difference in C14–C20 fatty acids explains the main difference in the 300–400°C range of biocrude oil (Figures 4, 5 and 6). In order to make comparisons based on the %dw of biomass, the biocrude yield was corrected by its corresponding percentage in the 300–400°C fraction, and plotted against the sum of C14–C20 FAMEs (Figure 4, inset). The high $R^2$ value (0.976) indicates a strong linear correlation between the specific FAMEs content and the biocrude content in that BP range, which will also corroborate with earlier discussion that the FAMEs are the dominant determinants to biocrude yield and quality.

Influence of varying biomass compositions in HTL extends beyond yield alone with significant effects in product quality (Table 4). Previous microalgae HTL studies have shown that nitrogen-rich biomass would result in the greater partitioning of nitrogenous compounds into the biocrude fraction, leading to a higher %N in the biocrude. If not subjected to hydrodenitrogenation or other upgrading techniques to remove nitrogen, the %N is considered undesirable given the potential for higher NOx emissions during combustion. Other than energy and yield, lower %N in the biocrude could be a significant advantage that lipid-accumulated biomass offers over protein-rich feedstock.
The increasing ER% (62.2–91.6%, Table 4) again alludes to the fact that as biomass FAs partition to the biocrude product, recovery of these high energy compounds leads to a higher ER%. Perhaps through a similar mechanism, ECR decreases from 0.312 to 0.127. A lower ECR implies greater returns in terms of recoverable combustion energy. ECR calculation is highly dependent on moisture content and increases significantly (less favorable) as moisture content increases. Low ECR values in the higher lipid batches (Table 4) suggests that favorable ECR values (<1.0) could still be obtained even with higher moisture biomass. This significantly lowers the energy demand in dewatering steps during the harvesting of microalgae biomass, which has been identified as a major hurdle to the successful implementation of microalgal biofuels. It must be noted that both the ER% and ECR do not account for energy required for cultivation, and so it still remains to be seen if high lipid batches would actually provide net benefits in an overall system.

4.2 Broader Implications and Future Work

Even with the information established on both cultivation and HTL separately, literature that considers the overall process from biomass to biofuels is scarce, and current challenges towards the fundamental understanding of microalgal-derived biofuels cannot be sufficiently tackled if the limited scope of present studies is not addressed. While the exact demands of cultivating varying compositions of Nannochloropsis extend beyond the scope of this study, maintaining a microalgae culture continuously for longer periods (e.g., 14 days for Batch 7) to obtain high lipid contents might entail greater energetic costs as compared to rapidly growing cultures with limited organic carbon storage (e.g., 3 days for Batch 2). In relation to HTL biocrude yield and quality, it is not known if less biomass with higher lipids is actually beneficial
over more biomass with low lipid.\textsuperscript{5,69} Life cycle analyses (LCAs) and design studies on the overall process have up to now only drawn their boundary around a single process, often using overly general estimations as a link to the other and diminishing the potential impact of such studies.\textsuperscript{70} These important questions underscore the current lack of research on the interconnections between upstream cultivation and downstream conversion that we attempted to bridge for the first time.

The results of this study collectively show that cell composition can indeed be utilized as the unifying factor in the scheme of microalgae HTL. One only needs to establish the baseline HTL product distribution for a certain reaction condition and subsequent variations can be predicted if the biomass composition is known. In the same way, cultivation conditions can be controlled to obtain compositions optimized for a certain target product yield or quality. Microalgae lipid accumulation studies could apply the suggested relationship between FA accumulation and biocrude yield to estimate energy productivity. After additional resources for cultivation are accounted for, high lipid biomass might not always be entirely favorable over one with lesser lipids, providing new insights into the seemingly entrenched mentality that having a higher amount of lipids in biomass would always entail greater returns.\textsuperscript{5,17,60} LCAs can examine the overall process better without making assumptions that do not have experimental basis, providing key sustainability and cost insights into the algae biofuel process.

Possible future work can quantify the exact resource demands for the cultivation of varying biomass compositions, and determine the value of higher biocrude yields from higher lipid biomass against that of low lipid batches. Further downstream work in terms of HTL products can explore the exact amount of fatty acids in the biocrude by performing FAMEs analysis to fortify conclusions made in this study. Obtaining a biomass that contains mostly
proteins and carbohydrates by defatting will also provide useful data into the baseline product distributions and quality for further comparison and understanding into model as proposed here. Similar studies along the vein of cultivating varying biomass compositions for HTL conversion can explore different species to determine if FAMEs still remains the key determinant, especially for mixed culture scenarios where fatty acid distribution can vary significantly depending on dominant species. These studies will all link up with the work presented here towards cell composition as the central factor in the biomass to biofuels model.
CHAPTER 5
SUMMARY AND CONCLUSIONS

This study examined the influence of varying microalgae biomass compositions on Hydrothermal Liquefaction (HTL) biocrude yield and quality. A single species, *Nannochloropsis oculata*, was cultivated under a series of increasingly nitrogen-depleted conditions to obtain biomass with different amounts of lipids, proteins, and carbohydrates. The batches were subjected to HTL at a single condition (80 wt% moisture, 300°C, 30 min) and product yield and characteristics were analyzed. The objective was to determine any significant relationships that can contribute towards a deeper understanding of the connections between upstream cultivation and downstream conversion, leading to improvements in the overall microalgae biofuels process.

Detailed analysis of the varying biomass compositions revealed that structural components remained relatively constant (carbohydrates 12–22 %dw, polar lipid/protein ratio 0.3–0.61), indicating that differences between batches were largely attributed to the accumulation in fatty acid (FA) content (10.5–52.0 %dw). HTL product yield analysis showed a fairly linear relationship between biocrude oil yield and FAs in the biomass ($R^2 = 0.838$). Together, these results suggest that the accumulated FAs proportionately increases biocrude yield above a baseline contribution from structural components, while the aqueous phase mainly decreases to compensate, given that solid and gas yields are either insignificant or vary just slightly.

Differences in biocrude oil properties were observed in terms of decreasing %N with decreasing protein content, and increasing ER% and ECR values with increasing lipid content. A strong linear relationship ($R^2 = 0.976$) between the FA content and the fraction of biocrude in the
300–400°C boiling point range, together with molecular weight distributions showing increasing amounts of FAs, further corroborate the above findings that FAs are a key determinant to biocrude yield and properties in the HTL of varying biomass compositions, and not as much crude lipids as previously thought. Further studies both in the upstream cultivation and downstream conversion fields can continue to enhance these findings, and can be linked to the results from this study to establish a complete, well-defined microalgal biofuels model.
REFERENCES


## APPENDIX A

**SUPPLEMENTARY INFORMATION**

Table 6. Proximate analysis of harvested batches (%wt)

<table>
<thead>
<tr>
<th>Batch</th>
<th>Moisture</th>
<th>Ash</th>
<th>Crude Proteins</th>
<th>Crude Lipids</th>
<th>Crude Carbohydrates</th>
<th>Summation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.6 ± 0.2</td>
<td>25.7 ± 0.2</td>
<td>43.2 ± 0.5</td>
<td>16.5 ± 0.0</td>
<td>10.3 ± 0.4</td>
<td>102.3 ± 1.5</td>
</tr>
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<td>54.5 ± 0.6</td>
<td>32.3 ± 0.5</td>
<td>13.0 ± 0.3</td>
<td>107.0 ± 0.9</td>
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<td>2(b)</td>
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<td>6.1 ± 0.2</td>
<td>52.3 ± 0.0</td>
<td>33.5 ± 1.2</td>
<td>11.8 ± 0.3</td>
<td>104.2 ± 1.3</td>
</tr>
<tr>
<td>2(c)</td>
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<td>32.0 ± 0.1</td>
<td>12.8 ± 0.3</td>
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<td>50.9 ± 1.5</td>
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</tr>
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<td>7</td>
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<td>60.3 ± 1.1</td>
<td>22.8 ± 0.8</td>
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