The individual and synergistic impacts of feedstock characteristics and reaction conditions on the aqueous co-product from hydrothermal liquefaction

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Abstract

We examined the individual and synergistic impacts of reaction conditions and microalgal feedstock characteristics, including previously unreported combinations of variables, on the yield and properties of aqueous co-product (ACP) from hydrothermal liquefaction. Explicitly, we measured the effects of temperature (150 to 350°C), reaction time (1 to 100min), slurry concentration (30 and 120 g L−1), biochemical composition (5.2 to 28.5 wt.% lipid, 14.7 to 50.9 wt.% protein), and species identity (Nannochloropsis, Chlorella, and Spirulina) on ACP characteristics. Measured properties included gravimetric yield, elemental (CHNSOP) recoveries, NH4+-N recovery in the ACP and promote N recovery in the biocrude. High-lipid, 30 g Lrxn−1 slurries reacted at 200°C for 31.6 min are a potential “win-win” set of conditions for both maximizing key ACP-recyclability metrics while limiting N and S recovery in the biocrude to < 5 and < 8%, respectively, and increasing the yield of high-quality biocrude. The results herein further illuminate ways in which reaction conditions and feedstock characteristics for HTL could be manipulated to engineer the ACP for desired nutrient content for direct recycling.

1. Introduction

Hydrothermal liquefaction (HTL) uses hot (150 < T < 400°C), compressed (5 < P < 250 bar) water to process whole algae into an energy-dense biocrude oil, a nutrient-rich aqueous co-product (ACP), a solid by-product, and gases. Using water as the reaction medium, HTL obviates energy otherwise spent on biomass drying. Subcritical, high-temperature water features an increased ion product to facilitate acid- and base-catalyzed reactions and decreased dielectric constant to solvate significantly less polar compounds than possible at ambient conditions [1]. These properties enable water to degrade the lipid, protein, and carbohydrate biomolecules into smaller compounds that further react with water or each other to form the biocrude, aqueous, solid, and gas products [2].

A significant challenge for algal biofuel production at scale is the availability of nutrients required for growth [3]. Therefore, it is imperative that fresh nutrient requirements are minimized to reduce resource constraints. As a result, the ACP is of great importance for overall process sustainability because it contains high concentrations of such nutrients, including N, P, and S [4,5] and their bioavailable forms NH4+-N, PO43−-P, and SO42−-S [6]. These nutrients each enhance the properties of ACP for direct recycling, hereafter referred to as the “recyclability” of the ACP. ACP can be directly recycled both with or without dilution to grow microalgae with positive, synergistic effects on algal growth rates and biomass productivity, most notably with monocultures [7], although also with some monocultures under certain
conditions [8,9]. In addition to high nutrient abundance, there are also organic and inorganic compounds in the ACP that inhibit growth, which can sometimes, but not always, necessitate ACP dilution prior to recycling [7,10,11]. Aqueous organic compounds represent up to 38% of the biomass C [12,13] and possess energy content that could be recovered. Therefore, another route of ACP utilization is through hydrothermal gasification to convert these organics to fuel gases, such as methane, before recycling the N, P, and S for algal growth [14]. An optimal algal biorefining process in terms of maximizing energy return on investment and minimizing environmental impacts may require a combination of direct recycling, which requires relatively trivial energy inputs but retains aqueous C in a recycle loop, and indirect recycling via hydrothermal gasification, which requires significant energy-inputs, but also recovers energy via methane formation from aqueous C.

Aqueous-phase-product yield and recyclability depend on variables such as HTL temperature and time [9,12,13,15–24], concentration [20,24,25], feedstock biochemical composition [17,23,26–30], and microalgal species [17,31]. Previous examinations of the impacts of these variables have various shortcomings, however. Long reactor heat-up times (>3 min) often observed in previous studies [13,15,17,19,20] obscured the coupled effects of temperature and time. At 300°C, ACP formation occurs on the timescale of seconds [21], so it is crucial that heat-up time is minimized to improve kinetic analyses at such high temperatures. Effects of concentration have been examined generally only at one or two reaction conditions [24,25] or a relatively narrow range of several temperatures and times at high reaction severity [20]; without a holistic exploration of concentration effects over a wide range of reaction conditions, any deductions about its impacts are tentative. Biochemical composition has been frequently explored at high reaction severity (temperature and time), but also often for only one or two reaction conditions [26–30]. The impact of this variable on ACP properties has yet to be fully explored at low and mild reaction severities, which are relevant for kinetic analyses.

Prior efforts showed that any differences in HTL product distribution at high reaction severity can be largely explained by proximate biochemical composition (lipids, proteins, carbohydrates) [27,29,32]. Recently, we demonstrated that the identity of the microalgae species can explain variation in biocrude yield and properties beyond that of biochemical composition alone at high reaction severity [33]. We also recently expanded the quantification of this variability induced by species to a much wider range of temperatures, times, and slurry concentrations [34]. Variability in ACP properties between different species after controlling for biochemical composition has received little attention to date [17,31]. In the hydrothermal environment, especially at low-to-moderate reaction severities conducive to hydrolysis, differences in cell morphology (e.g., cell wall strength and surface-area-to-volume ratio) may affect microbial reactivity, independent of proximate biochemical composition. At high reaction severity, proximate biomolecule distribution may also be impervie for correlating product characteristics, and more detailed information about biochemical composition (e.g., DNA, RNA, and unsaponifiable lipid contents) may be important for explaining ACP-property variation.

Although the aforementioned variables are typically only examined individually, they can be coupled during HTL. As an example, different types of biomolecules exhibit different rates of hydrolysis as a function of reaction severity, yielding myriad types of degradation products [2]. Increased slurry concentration can promote interactions between these degradation products through reaction pathways such as the Maillard reaction between reducing sugars and amino acids [11,34,35]. Differences in cell wall strength and possibly cell morphology could further affect reactivity of microalgae during HTL [31], regardless of similarities or differences in proximate biochemical composition. These differences may be compounded by slurry concentration (increased concentration reduces exposure to high-temperature water) and reaction conditions (increased severity increases rate of degradation). We previously examined how these factors individually and synergistically affect the yield and properties of the biocrude, solid, and gas products [34]; however no previous study has probed all of these factors together when examining ACP yield and properties. There could, therefore, be previously unidentified synergistic or antagonistic effects between different feedstock characteristics and reaction conditions. Generally only a few of these variables are considered over relatively narrow ranges, which limits the ability of the field to holistically understand and ultimately model HTL kinetics and may miss sets of HTL parameters that optimize aqueous phase recyclability metrics.

In this study, we examined the individual and synergistic impacts of multiple HTL variables on the distribution of nutrients into the ACP with the objective of engineering improved nutrient recovery. We conducted experiments using fast-heating batch reactors (1-min heat-up) and logarithmically spaced reaction times (10−6, 10−5, 10−4, 10−3, and 10−2 min) over a large range of temperatures (150, 200, 250, 300, and 350°C). Biomass feedstocks included six microalgae with different biomolecule distributions (5.2 to 28.5 wt.% lipid, 14.7 to 50.9 wt.% protein) at two different slurry concentrations (30 and 120 g L−1rxn). Three of these microalgae, including a high-lipid Nanochloropsis, high-lipid Chlorella, and high-protein Spirulina, contain different proximate biochemical compositions to evaluate the impacts of different lipid, protein, and carbohydrate contents at different reaction severities and slurry concentrations. The other three, including a high-protein Nanochloropsis, a high-protein Chlorella, and a mixture of high-protein Spirulina and high-lipid Chlorella, contain a similar proximate biomolecule distribution, enabling quantification of the variability between different species over different reaction conditions and slurry concentrations after controlling for biochemical composition. Likewise, the two variants of Nanochloropsis and Chlorella enable examination of the effects of biochemical composition while controlling for microalgal species. Additionally, differences between the two-species-mixture measured and predicted effects elucidate how the different allotments of biomolecule components in high-protein Spirulina and high-lipid, high-carbohydrate Chlorella react dynamically at different reaction severities and slurry concentrations, compared to how they react alone. The experimental results reported herein enable a greater extent of reaction engineering with respect to key ACP recyclability metrics than previously possible.

2. Materials and methods

We describe experimental methods in great detail in our previous work and therefore limit the information presented here to the most important details, many of which are reproduced from before [34].

2.1. Microalgae feedstocks and slurry preparation

Microalgal species for this study include a high-protein Nanochloropsis oculata (Nan-1), high-lipid Nanochloropsis salina (Nan-2), high-protein Spirulina platensis (Spi-1), high-protein Chlorella sorokiniana (Chl-1), and high-lipid Chlorella sorokiniana (Chl-2). We pre-mixed slurries of each biomass type such that their concentrations were either 30 or 120 g L−1rxn at reaction conditions (150 to 350°C) and froze them prior to each reaction (upon which they were thawed at room temperature). See our previous work for the equivalent solids contents and calculation of the concentrations on a g L−1rxn−1 basis [34]. We prepared the two-species mixture slurries (Mix-m) by combining roughly three parts low-protein Chlorella (Chl-2) with seven parts Spirulina (Spi-1) for an average Chl-2 content of 30.1 wt.% We calculated the predicted dependent variables for the two-species mixture (Mix-p) using a weighted average of the dependent variables for Chl-2 and Spi-1 with weights 0.301 and 0.699, respectively.

2.2. Hydrothermal liquefaction

We reacted 30 and 120 g L−1rxn−1 slurries of all biomass types at 200
and 300°C for 3.2 (10^{0.5}) and 31.6 (10^{1.5}) min, amounting to 48 sets of conditions. We also performed some additional reactions using other temperatures (150, 250, and 350°C) and times (10^{0.0}, 10^{1.0}, and 10^{2.0} min) to probe a wider range of reaction severities. In total, we conducted HTL at a total of 91 unique sets of reaction conditions in at least duplicate, and in some cases triplicate or more to generate enough product mass for subsequent analysis. All reactions were conducted in a completely random order.

We conducted HTL in 1.30 mL stainless-steel batch reactors using 1/2-in. o.d., 0.049in. thick Swagelok tubing. We constructed additional 1.16mL proxy reactors fitted with a K-type thermocouple for temperature measurements. Individual reactions proceeded by immersing both a slurry-loaded reactor and proxy reactor into a Techne IFB-51 fluidized sand bath, preheated to the specified temperature. It typically took reactors about 58 s to achieve 98% of the maximum temperature change relative to ambient conditions. At the end of the holding time (10^{0.0}, 10^{1.0}, 10^{2.0}, or 10^{3.0} min), we quenched the reactors in a cold water bath. We define this holding time as the holding time from the moment the reactor starts to heat up to the moment the reactor starts to cool down.

We measured 5.5 mL of dichloromethane (> 99.9% optima grade, Fisher Scientific) and enough deionized water (4.3 to 4.9 mL) such that the total volume of water was approximately 5.5 mL to facilitate product collection via pipette. The type of recovery solvent(s) used to collect the reaction mixture can affect the properties of the products from HTL [36]; however this variable was out of scope for the present study. Following product recovery, we vortexed and centrifuged the product mixture twice to separate out the biocrude (dichloromethane-soluble products), aqueous phase, and solid products into separate glass tubes. We transferred a 500 μL aliquot of aqueous phase into a small plastic vial to be frozen for subsequent ammonium and phosphate analyses. We measured the pH of the aqueous phase using a Fisher Scientific Accumet [36] K-type thermocouple calibrated using pH 4, 7, and 10 buffer solutions. Note that the measured pH of the ACP could differ from that of the uncollected aqueous phase in the reactor due to the addition of deionized water. However, we assume that the overall trends of pH with respect to changes in independent variables are representative of those of the uncollected reaction mixture. We dried the ACP under nitrogen at 70°C for 1 h or until dry. We calculated the ACP mass as the measured dried mass, adjusted to account for the 500 μL aliquot set aside for further characterization.

2.3. Elemental content, ammonium, and phosphate

We previously reported elemental contents (C, H, N, O, S, and P) for the biomass samples [34]. We combined and homogenized replicate dried ACP fractions before Elemental Microanalysis Ltd. measured their C, H, N, and S content. These measurements should be interpreted as the properties of the non-volatile components of the aqueous phase, as some volatile components may be lost from drying. We measured total P in the ACP using persulfate digestion and the ascorbic acid molybdenum method [37,38]. We measured ACP ammonium and phosphate also using this ascorbic acid molybdenum method. To quantify any ammonium that may not have evaporated from the ACP during the drying step, we also rehydrated an aliquot of dried sample with deionized water. However, we assume that the overall trends of pH with respect to changes in independent variables are representative of those of the uncollected reaction mixture. We dried the ACP under nitrogen at 70°C for 1 h or until dry. We calculated the ACP mass as the measured dried mass, adjusted to account for the 500 μL aliquot set aside for further characterization.

2.4. Biomass biochemical content

We approximated biomass lipid content as the total fatty acid content of the biomass averaged over five replicates [34], with the acknowledgement that unsaponifiable lipids and minor components derived from the lipid structures, such as phosphate and glycerol, will be neglected. We estimated biomass protein content by multiplying biomass N content by 4.78 [39]. We measured biomass ash content by combusting the samples at 550°C for 30 h and calculating the percentage of mass retained, minus biomass P content, averaged over five replicates. We calculated biomass carbohydrate content by difference from unity and the sum of lipid, protein, and ash content.

2.5. Statistical analysis

We performed all statistical analyses on subsets of the data using the function LocationTest in Mathematica 11.1. All comparisons between subsets of the data are on an absolute deviation basis, not relative, unless otherwise stated. Calculations of dependent variables for Mix-p are not employed in any statistical analyses, except for those that directly compare those values to the measured dependent variables for Mix-m.

3. Results and discussion

Table 1 summarizes the microalgal species and their biochemical profiles, shorthand identifiers, and symbols used in figures. Ternary diagrams (Fig. 1) show these biochemical profiles (dry, ash-free basis) color-coded with red, green, and blue intensity mapped to lipid, protein, and carbohydrate contents, respectively. Each type of biomass follows this color scheme throughout this article to facilitate evaluation of biochemical composition effects. We report the elemental composition and fatty acid profile for each biomass sample in our previous work [34]. We report data for aqueous-phase elemental recoveries graphically in the following sections and refer the reader to Tables A.3 and A.4 in Appendix A for those data in tabular form. We also report aqueous yields, total ammonia yields and dried-aqueous-phase elemental contents in Tables A.1 and A.2. Biocrude, solid, and gas yields and the overall mass balance appear in the main text and supporting information of our previous work [34].

3.1. Aqueous-phase-product yield

3.1.1. Temperature and time

The yield of non-volatile aqueous-phase products (hereafter referred to as aqueous yield) is shown in Fig. 2 and includes ammonia-free products retained after drying under nitrogen at 70°C. Aqueous yield increased with increasing temperature and time until a maximum was reached at low-to-moderate reaction severity (200°C, 31.6 min to 250°C)

<table>
<thead>
<tr>
<th>ID</th>
<th>Symbol</th>
<th>Genus</th>
<th>Biochemical composition [wt.%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lipid</td>
</tr>
<tr>
<td>Nan-1</td>
<td>•</td>
<td>Nannochloropsis</td>
<td>11.6 ± 0.1</td>
</tr>
<tr>
<td>Nan-2</td>
<td>•</td>
<td>Chlorella</td>
<td>28.5 ± 0.5</td>
</tr>
<tr>
<td>Chl-1</td>
<td>•</td>
<td>Chlorella</td>
<td>9.4 ± 0.3</td>
</tr>
<tr>
<td>Chl-2</td>
<td>•</td>
<td>Chlorella</td>
<td>19.9 ± 0.1</td>
</tr>
<tr>
<td>Spi-1</td>
<td>•</td>
<td>Spirulina</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Mix-m</td>
<td>△</td>
<td>Mixture</td>
<td>9.6 ± 0.1</td>
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<tr>
<td>Mix-p</td>
<td>▼</td>
<td>Mixture</td>
<td>9.6 ± 0.1</td>
</tr>
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Other studies have shown a maximum to occur at similar conditions [15,21,23]. This maximum appears to be a function of biochemical composition, with the high-protein Nan-1 reaching a maximum later than the low-protein Chl-2 at 250 °C. Beyond this point, aqueous yield decreased monotonically with increasing reaction severity, a trend consistent with numerous previous studies [12,13,15,17,21,23]. The magnitudes of aqueous yields in the present study were also generally consistent with these studies form the literature, with some variability due to differences in drying conditions for the aqueous phase (e.g., temperature).

### 3.1.2. Slurry concentration

Across all temperatures and times, decreasing slurry concentration led to an average increase in aqueous yield of 3.9 wt.% ($p < 10^{-8}$). This effect is largest between 200 and 300 °C (4.9 wt.%, $p < 10^{-5}$), and is lower but still statistically significant at 350 °C, 10 min and higher (1.4 wt.%, $p < 0.02$). One explanation for this effect is that the more dilute slurry facilitates hydrolysis of proteins and carbohydrates into smaller peptides/amino acids and saccharides, respectively, which as a general rule become increasingly soluble in water with decreasing size. We posit that this hydrolysis facilitation could occur due to shifts in the relative proportions of conjugate acids and bases of compounds in solution owed to the four-fold difference in slurry concentration that in turn affect the relative amounts of $H^+$ and $OH^-$ ions that catalyze hydrolysis. This explanation is supported by the variability in aqueous pH as a function of slurry concentration discussed later in Section 3.6, which shows that at room temperature, the ACP is significantly more basic for the 30 g L$^{-1}$ slurries than the 120 g L$^{-1}$ slurries. This may suggest that the perceived increase in hydrolysis rate is due to base-catalysis, however without knowing the pH of the mixture at reaction and drying conditions.

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**Fig. 1.** Biochemical profile ternary diagrams (dry, ash-free basis) of six different microalgal feedstocks presented in Table 1, reproduced from [34]. L (red), P (green), and C (blue) denote color-shaded profile of lipids, proteins, and carbohydrates, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 2.** Non-volatile aqueous-phase-product yield versus reaction time grouped by temperature and initial concentration. See Table 1 for microalgae types. The bottom row depicts the first row (30 g L$^{-1}$) minus the second row (120 g L$^{-1}$). Data shown as mean ± standard error, n ≥ 2, including data where the error bars are smaller than the plot markers.
temperature this categorization is tentative. Another possibility is that the more concentrated slurry significantly increases the rates of reaction between biomolecule-degradation products at the expense of hydrolysis with the bulk high-temperature water.

It is also possible that the 120 g L\textsuperscript{-1} slurries produced ACP that was saturated with certain compounds that possess limited solubility in water. All product mixtures were dissolved in approximately the same volume of water (including fresh DI-water diluent to facilitate with product recovery); therefore, a hypothetical solubility limitation for the more concentrated slurries would result in relatively more compounds precipitating out of the ACP than observed for the less concentrated slurries, on a per-unit-biomass basis. However, we would expect to see this concentration effect predominantly at the points of maximum aqueous yield, where the amount of dissolved material is the greatest, but instead this trend exists for a range of aqueous yields. This suggests that solubility limitations are unlikely to be systematically affecting this trend.

3.1.3. Biochemical composition

Aqueous yield generally increased with increasing protein content (color-coded in Fig. 2; see Fig. A.1 in Appendix A for explicit yield vs. protein plots); however, there were several exceptions at temperatures of 250°C and lower. This trend was more consistent at 300°C and higher, a finding that has been seen previously [23,26,29] and would be expected due to the high aqueous solubility of protein-degradation products, including most peptides and amino acids. Controlling for species identity (i.e., comparing effects of biochemical composition between *Nannochloropsis* and *Chlorella*, respectively) at 300°C, higher biomass-protein content led to an average aqueous yield increase of 9.9 wt.% ($p < 0.0004$).
3.1.4. Species identity

Despite comparable biochemical profiles, the aqueous yields of Nan-1 (■), Chl-1 (▲), and Mix-m (▼) varied considerably at 200°C. However, complete separation of the solid and aqueous phase for samples produced from Spi-1 (the primary constituent of the two-species mixture Mix-m) was difficult at 200°C and lower, so aqueous yields for these samples (which were generally higher than other biomass types at those temperatures) may contain solids. Excluding Mix-m, the average difference between Nan-1 and Chl-1 was only significant at 3.2 min (9.2 wt.%, p < 0.01) rather than at 31.6 min (1.2 wt.%, p < 0.78). At 300°C, variability due to species identity between Nan-1, Chl-1, and Mix-m was on average only ± 1.0 wt.% for the 30 g \( L_{rxn}^{-1} \) slurries, whereas it was ± 3.3 wt.% for the 120 g \( L_{rxn}^{-1} \) slurries. [31] reported aqueous yield variabilities of ± 6.2 and ± 2.4 wt.% for 50 g \( L_{rxn}^{-1} \) slurries at 250°C, 9 min and for 30 g \( L_{rxn}^{-1} \) slurries at 375°C, 11 min, respectively, for five different species with similar biochemical composition (52 ± 2 wt.% protein content, dry ash-free basis). These variabilities are comparable in magnitude and trend with increasing temperature as those of the present study. Both the data in the present study and that of [31] demonstrate that biochemical composition is a reasonable predictor of aqueous yield at reaction severities of 300°C and higher, especially for dilute slurries.

3.1.5. Two-species mixture interactions

The measured aqueous yield for the two-species mixture (Mix-m, ▼) only deviated from predicted (Mix-p, ◆) at 200°C, 3.2 min (− 6.2 wt.%, p < 0.14), although not with statistical significance. There was no difference at all other reaction conditions (0.3 wt.%, p < 0.52), suggesting that there are no significant interactions between the protein-degradation products of Spi-1 (■) and carbohydrate- and lipid-degradation products of Chl-2 (▲) affecting aqueous yield.

3.2. Nitrogen recovery

3.2.1. Temperature and time

\( \text{NH}_4^+ \) -N recovery (Fig. 3a), or the percentage of biomass N recovered as aqueous \( \text{NH}_4^+ \), monotonically increased with increasing reaction severity for all biomass types and slurry concentrations, reaching a maximum of 81.2% (30 g \( L_{rxn}^{-1} \) extrmL \( L_{rxn}^{-1} \) slurry of Spi-1 (■) at 350°C, 100 min). In contrast, organic N recovery (Fig. 3b) followed a similar trend as that of aqueous yield, increasing to a maximum at moderate severity (200°C, 31.6 min to 250°C, 10 min) before decreasing thereafter. Total N recovery (Fig. 3c) shows that at 300°C and above, any decrease in organic N recovery with increasing severity is matched with an approximately equivalent increase in \( \text{NH}_4^+ \) -N recovery. Magnitudes and trends with respect to reaction severity for total N recoveries are comparable to other reports [9,12,15-18,22], with some variation due to differences in measurement technique (namely dry oxidation versus digestion, the latter of which can underestimate organic N content). The percentage of ACP N as \( \text{NH}_4^+ \) (Fig. 3d) followed the same trend as \( \text{NH}_4^+ \) -N recovery, reaching a maximum of 97.0%. At high reaction severity, these percentages were somewhat higher than some other studies [12,23,26] but equivalent to others [17,22], although less severe conditions were more comparable. At high reaction severity, other sources of volatile N, such as methylamine and ethylamine, have been reported as degradation products of amino acids [40]; however, most of these compounds are likely lost during the drying step and would be unaccounted for in the aqueous total N, which may explain the higher percentages of aqueous N as ammonium. Note that, with the exception of Chl-2 (▲), N mass closure (from all products) was 80% or higher at 300°C and higher (Fig. 8, discussed in Section 3.7).

Notably, the ACP total N recovery produced by Chl-2 (▲) reaches a maximum at 200°C and 31.6 min before decreasing at higher reaction severity, while all other biomass types continue to increase or plateau in N recovery through 300°C and 31.6 min. We previously showed that, for Chl-2, this N is recovered in both the biocrude and solid phases to a greater extent than the other biomass types [34]; however, the N recovery in the solid fraction monotonically decreased with reaction severity (albeit slowly), so the decrease in ACP total N recovery corresponds directly with an increase in biocrude N recovery. This suggests that nitrogen-containing compounds in the ACP are reacting to form biocrude, perhaps via a Maillard reaction pathway. See Section 3.2.3 for further explanation.

At 350°C for Nan-1 (■), aqueous total N recovery increased by 9.2% (p < 0.03) from 10 to 100 min despite having decreased from 1 to 10 min by 6.3% (p < 0.18). Fig. 3a and b shows that this increase is entirely due to increasing \( \text{NH}_4^+ \) -N recovery. Moreover, we previously found that biocrude N recovery for Nan-1 decreases in this range as well [34]. Therefore we conclude that at these reaction conditions (350°C, 10 to 100 min), N-containing biocrude-soluble compounds are losing N to the ACP in the form of ammonium, possibly through deamination reactions. This result suggests that for higher-protein biomass, extended reaction times at 350°C could both improve ACP recyclability and reduce biocrude heteroatom content, a trend demonstrated previously [12]. Future work with additional high-protein biomass types and longer reaction times could further corroborate this observation.

3.2.2. Slurry concentration

At 200°C and higher, regardless of biomass type, the 30 g \( L_{rxn}^{-1} \) slurries led to on average 3.3% higher (p < 0.0001) \( \text{NH}_4^+ \) -N recovery, 3.9% higher (p < 10⁻⁶) organic N recovery, and as a result 7.3% higher (p < 10⁻⁵) total N recovery. This trend coincides with a previously reported 4.6% decrease in biocrude N recovery at 250°C and higher for the 30 g \( L_{rxn}^{-1} \) slurries relative to that of the 120 g \( L_{rxn}^{-1} \) slurries [34]. The data in Fig. 3 now reveal that this decrease in biocrude N recovery directly benefits both \( \text{NH}_4^+ \) N and organic N recovery in the ACP.

3.2.3. Biochemical composition

\( \text{NH}_4^+ \) N recovery (Fig. 3a) is a strong function of biochemical composition at 250°C and higher; for example, it increases by an average 13.0% (p < 0.001) with increasing protein content at 300°C (see Fig. A.3 in Appendix A for explicit N recovery vs. protein plots). These data support our earlier assertion that carbohydrates are the limiting reactant in the Maillard reaction [34,35]. A greater proportion of proteins to carbohydrates would enable more of the protein fraction to degrade autogenously into products such as ammonium, as is observed here with the more protein-rich biomass samples. In contrast, for the biomass samples with higher proportions of carbohydrates to proteins, such as Chl-2 and Nan-2, relatively more protein-degradation products, such as amino acids, would participate in Maillard reactions rather than undergo deamination.

Organic N recovery (Fig. 3b) generally increased with increasing protein content at all reaction conditions but not universally and to a lesser extent than that of \( \text{NH}_4^+ \) N. This effect was low and statistically insignificant (+ 1.8%, p < 0.3) at 200°C, but greater and statistically significant (+ 5.3%, p < 0.01) at 300°C. As a result, total N recovery generally increased with increasing biomass protein content at 200°C and lower. This trend was more significant at 250°C and higher, a result corroborated by previous work [26-28,30]. Interestingly, the proportion of total N owed to ammonium (Fig. 3d) was on average 4.3% lower (p < 0.01) with increasing protein content at 200°C; this trend occurred largely due to the increased organic N recovery observed for the high-protein biomass types, which lowered the proportion of aqueous N as ammonium. This proportion was on average 4.6% higher (p < 0.07) with increasing protein content at 300°C, suggesting that the extra organic N recovered in the ACP at low severity leads to greater deamination at high severity and thus increased ammonium recovery. [30] showed similar trends at high reaction severity, albeit with even larger increases than observed in the present study; however in contrast, [26] found no clear trend in the percentage of total N as ammonium with increasing protein content at 300°C.
3.2.4. Species identity

Between Nan-1 (a), Chl-1 (g), and Mix-m (v), NH$_4$$^+$-N recovery varied by $\pm$ 1.0% and $\pm$ 4.3% at 200 and 300°C, respectively. Previous work has demonstrated similar NH$_4$$^+$-N recovery variability due to species identity [12,15], indicating that biochemical composition is a reasonable predictor of NH$_4$$^+$-N within those uncertainties. Variability in organic ($\pm$ 2.1%) and total N ($\pm$ 4.8%) were similarly low at 300°C for these three biomass types with similar biochemical composition.

3.2.5. Two-species mixture interactions

At 300°C, the measured (Mix-m, v) NH$_4$$^+$-N recovery in the 120 g L$_{{\text{rxn}}}^{-1}$ slurries was 7.4% ($p < 0.06$) lower than predicted (Mix-p, a). This trend is partially explained by our previous work, which showed that biocure N recovery at these conditions was on average 5.0% ($p < 0.21$) higher than predicted [34]. We proposed that Maillard reactions between the degradation products from the carbohydrate-rich Chl-2 (a) and protein-rich Spi-1 (g) in the two-species mixture were the most likely explanation for the increased N recovery. The data presented here suggest that this increased N recovery in the biocure is directly related to reduced NH$_4$$^+$-N recovery in the aqueous phase. Note that, although the increase in biocure N recovery was not statistically significant, the associated decrease in NH$_4$$^+$-N recovery was statistically significant. This effect was absent or significantly reduced at 200°C and/or for the 30 g L$_{{\text{rxn}}}^{-1}$ slurries. These data demonstrate that the Maillard reactions are favored by concentration increases and likely proceed by an overall reaction order greater than one, given that the recoveries are normalized with respect to biomass N.

3.3. Phosphorus recovery

3.3.1. Temperature and time

Increasing temperature and time increased PO$_4$$^{3-}$-P recovery (Fig. 4a) until a maximum was reached at moderate reaction severity (around 250°C, 10 min). Notably the reaction conditions that maximized PO$_4$$^{3-}$-P recovery were essentially the same for all biomass types, similar to NH$_4$$^+$-N recovery. Total P recovery (Fig. 4c) at this maximum is significantly higher in the present work than one previous study [22]. Further increases in temperature and time led to decreased PO$_4$$^{3-}$-P recovery, in some cases down to single-digit recoveries by 350°C, 100 min. This reduction in P recovery has been documented before [9,12,13,15,22,24], and is likely due to the formation of highly insoluble phosphate precipitates in the solid phase [13], including calcium phosphates like hydroxyapatite [41]. Our previous work presented an increase in P recovery in the solid phase at these conditions [34]. These precipitates likely form as a function of pH, which itself is a function of reaction conditions and feedstock characteristics (discussed later in Section 3.6); pH and PO$_4$$^{3-}$-P recovery were strongly and significantly correlated ($p = -0.47$, $p < 10^{-16}$).

Non-phosphate P recovery (Fig. 4b) was significant at low reaction severities, similar to that of [22], but generally decreased with increasing reaction severity. At 250°C, 10 min and above, the vast majority of P was present as phosphate (Fig. 4d), consistent with some prior findings [12,15] although others showed 15 to 35% non-phosphate P recovery. Given that hydrolysis is the mechanism by which PO$_4$$^{3-}$ is liberated from the phospholipids, DNA, and RNA (the three main phosphate-containing compounds in microalgae [43]), it is also possible that the 30 g L$_{{\text{rxn}}}^{-1}$ slurry may produce ACP that quickly saturates with PO$_4$$^{3-}$, which would allow a greater proportion of the PO$_4$$^{3-}$ from the 30 g L$_{{\text{rxn}}}^{-1}$ slurry to dissolve; thus increasing its recovery. If solubility is the limiting factor, pH would be the dominant factor controlling PO$_4$$^{3-}$-P recovery. Given the data presented herein support this notion, especially at 300°C. Between the two types of Chlorella, the higher-lipid sample (Chl-2, g) demonstrated 21.9% higher PO$_4$$^{3-}$-P recovery ($p < 0.001$) at 300°C, 3.2 min and milder severities compared to Chl-1 (a). Non-phosphate recovery showed the opposite effect (Fig. 4b), decreasing with increasing lipid content by on average 20.7% ($p < 0.0001$) at 200°C and lower severities, for both types of Nannochloropsis and Chlorella.

3.3.2. Slurry concentration

At moderate reaction severity (200 to 300°C), increased slurry concentration (30 g L$_{{\text{rxn}}}^{-1}$) led to on average 10.2% higher ($p < 10^{-7}$) PO$_4$$^{3-}$-P recovery and 10.3% higher ($p < 10^{-6}$) total P recovery, but no difference in non-phosphate P recovery. This effect was significant enough to increase PO$_4$$^{3-}$-P recovery to > 95% for Chl-2 (a) and to separate instances (Fig. 4a). This trend was previously shown to hold for even higher ranges of slurry concentrations (126 to 422 g L$_{{\text{rxn}}}^{-1}$) [20]. One explanation is that algal slurries in the range of 30 to 120 g L$_{{\text{rxn}}}^{-1}$ may produce ACP that quickly saturates with PO$_4$$^{3-}$, which would allow a greater proportion of the PO$_4$$^{3-}$ from the 30 g L$_{{\text{rxn}}}^{-1}$ slurry to dissolve; thus increasing its recovery. If solubility is the limiting factor, pH would be the dominant factor controlling PO$_4$$^{3-}$-P recovery. Given that hydrolysis is the mechanism by which PO$_4$$^{3-}$ is liberated from the phospholipids, DNA, and RNA (the three main phosphate-containing compounds in microalgae [43]), it is also possible that the 30 g L$_{{\text{rxn}}}^{-1}$ slurry may produce ACP that quickly saturates with PO$_4$$^{3-}$, which would allow a greater proportion of the PO$_4$$^{3-}$ from the 30 g L$_{{\text{rxn}}}^{-1}$ slurry to dissolve; thus increasing its recovery. If solubility is the limiting factor, pH would be the dominant factor controlling PO$_4$$^{3-}$-P recovery. Given the data presented herein support this notion, especially at 300°C. Between the two types of Chlorella, the higher-lipid sample (Chl-2, g) demonstrated 21.9% higher PO$_4$$^{3-}$-P recovery ($p < 0.001$) at 300°C, 3.2 min and milder severities compared to Chl-1 (a). Non-phosphate recovery showed the opposite effect (Fig. 4b), decreasing with increasing lipid content by on average 20.7% ($p < 0.0001$) at 200°C and lower severities, for both types of Nannochloropsis and Chlorella.

3.3.3. Biochemical composition

PO$_4$$^{3-}$ and total P recoveries generally decreased with increasing ash content (Fig. A.4b and d in Appendix A) and generally increased with increasing lipid content (color-coded in Fig. 4a and c; explicitly in Fig. A.4a and c). The concentration of metal cations in solution (originating from the ash fraction), is known to affect phosphate recovery due to precipitation into the solid phase [44]. The data reported herein support this notion, especially at 300°C. Between the two types of Chlorella, the higher-lipid sample (Chl-2, g) demonstrated 21.9% higher PO$_4$$^{3-}$-P recovery ($p < 0.001$) at 300°C, 3.2 min and milder severities compared to Chl-1 (a). Non-phosphate recovery showed the opposite effect (Fig. 4b), decreasing with increasing lipid content by on average 20.7% ($p < 0.0001$) at 200°C and lower severities, for both types of Nannochloropsis and Chlorella.

3.3.4. Species identity and two-species mixture interactions

Variability in PO$_4$$^{3-}$-P recovery due to species identity was on average $\pm$ 6.4% and $\pm$ 15.3% at 200 and 300°C, respectively. Although the proximate biochemical compositions of Nan-1 (e), Chl-1 (m), and Mix-m (w) were similar, their P contents were relatively different (0.94, 1.03, and 1.21 wt.%, respectively) [34]. Additional information, such as phospholipid and/or DNA content, may be necessary to correlate PO$_4$$^{3-}$-P recovery with greater precision. There was no significant difference between the measured (Mix-m, v) and predicted (Mix-p, a) P recoveries for the two-species mixture. This suggests that there are likely no significant interactions between degradation products of the composite Chl-2 (a) and Spi-1 (g) affecting P recovery in the aqueous phase.

3.4. Sulfur recovery

3.4.1. Temperature and time

S recovery in the aqueous phase ranged from 7.7 to 75.5%, shown in Fig. 5. It generally increased with increasing reaction severity to a maximum between 200°C, 31.6 min and 250°C, 10 min. At 250°C, this maximum occurred at 1 min for Chl-2 (m), but at 10 min for Nan-1 (e), the same conditions maximizing aqueous yield and organic N recovery.
for those respective biomass types. Beyond this maximum, S recovery generally decreased with increasing reaction severity, as has been reported previously [13]. In contrast, [24] did not observe a monotonic decrease in aqueous S recovery at 350 °C with increasing time for a 101 g L⁻¹ slurry of Nannochloropsis; instead it decreased from an initial maximum and varied by only ± 3% from 2 to 60 min reaction time. Crucially, they did not analyze the elemental content of the dried aqueous phase, but rather of the undried aqueous phase via ICP-OES. This suggests that volatile forms of S, for example hydrogen sulfide or methane thiol, could be formed at these conditions and would be lost during the drying of the aqueous phase at 70°C.

3.4.2. Slurry concentration

At reaction conditions preceding the maximum aqueous S recovery, decreasing slurry concentration led to an average of 5.4% higher S recovery (p < 10⁻⁵). Beyond that point, there was no universal trend with respect to changing concentration, however certain biomass types showed large, but statistically insignificant differences with decreasing slurry concentration, such as Chl-1 (+10.5%, p < 0.09) and Chl-2 (-9.0%, p < 0.26) at 300°C. At 350°C, 30 min for a strain of Nannochloropsis, [24] observed a decrease in aqueous S recovery of 8% from 30 to 64 g L⁻¹, but no change from 64 to 101 g L⁻¹, which is comparable in magnitude to the differences reported herein at similar reaction severities.

3.4.3. Biochemical composition and species identity

There were no clear trends in aqueous S recovery with respect to changing biochemical composition. Additionally, the three biomass types with comparable biochemical composition demonstrated high variability in aqueous S recovery at both 200°C (± 10.7%) and 300°C (± 5.7%) [17] presented similar variability (± 6.9%) from 300 to 350°C. Moreover, there were no clear trends with respect to changing
biomass S content (Fig. A.5). The reason for the variability could therefore lie with sulfates precipitating in the solids phase as a function of aqueous pH and cation availability. Additionally, further study of the behavior of S-containing compounds in microalgae, such as the amino acids methionine and cysteine [45], as well as some lipids [43] could be useful for understanding S partitioning.

3.4.4. Two-species mixture interactions

Similar to aqueous P recoveries, the measured and predicted two-species mixture aqueous S recoveries were in agreement at both 200 and 300°C. These data provide no evidence of any reactions between the protein-degradation products (some of which contain S) of Spi-1 (①) and the carbohydrate-degradation products of Chl-2 (②).

3.5. Carbon recovery

3.5.1. Temperature and time

Aqueous C recovery ranged from 1.3 to 60.8% (Fig. 6a). Trends with changing reaction severity were similar to those of S recovery. C recovery increased with increasing temperature and time, reaching a maximum at moderate reaction severity (200°C, 31.6 min to 250°C, 10 min) before decreasing with further increases in severity. Both the initial increases [18] and subsequent decreases [12,13,17,20,22] in aqueous C recovery are consistent with prior studies. Note that [16] demonstrated that, with just 9 s of residence time, aqueous total organic carbon generally increases with increasing temperature from 205 to 325°C, so it is likely that at temperatures above 250°C, the maximum is achieved on the order of seconds rather than minutes.

3.5.2. Slurry concentration

The ACP of the 30 g L<sub>rxn</sub>⁻¹ slurries recovered on average 2.9% more C (p < 10⁻⁶) than the 120 g L<sub>rxn</sub>⁻¹ slurries at HTL temperatures of 200°C and higher. This effect was more pronounced at lower temperature (e.g., 200°C, +4.5%, p < 0.001) than at higher temperature (e.g., 300°C, +2.3%, p < 0.01). Other studies have shown this trend over both relatively dilute (7 to 70 g L<sub>rxn</sub>⁻¹) [25] and concentrated ranges (126 to 422 g L<sub>rxn</sub>⁻¹) [20], respectively. [25] posed that equilibrium limitations, particularly carbon solubility in the aqueous phase, could be a factor. Given that organic N recovery (Fig. 3b) and S recovery (Fig. 5) are also higher for the 30 g L<sub>rxn</sub>⁻¹ slurries, we suspect that this increased C recovery is due to higher concentrations of peptides and amino acids liberated from the protein fraction via hydrolysis.

3.5.3. Biochemical composition

At 250°C and lower, higher protein content resulted in generally higher aqueous C recovery, however with a significant amount of variability (Fig. 6b). However, at 300°C, there was a strong, positive correlation between C recovery and biomass protein content. For example, the correlation coefficient, ρ, was 0.98 for the 30 g L<sub>rxn</sub>⁻¹ slurries at 3.2 min (p < 10⁻⁶), similar to the data for 120 g L<sub>rxn</sub>⁻¹ slurries and at 31.6 min. This correlation provides strong evidence that the

Fig. 5. Aqueous-phase-product sulfur recovery versus reaction time grouped by temperature and initial concentration. See Table 1 for microalgae types. The bottom row depicts the first row (30 g L<sub>rxn</sub>⁻¹) minus the second row (120 g L<sub>rxn</sub>⁻¹). Data shown as mean ± standard error, n ≥ 2, including data where the error bars are smaller than the plot markers.
protein fraction of the biomass is the primary source of C in the aqueous phase at temperatures of 300°C and higher, corroborating similar results from previous studies at these conditions [26–30].

3.5.4. Species identity
Variability in aqueous C recovery between Nan-1 (●) and Chl-1 (●) was ± 3.0% at 200°C. At 300°C, variability between Nan-1, Chl-1, and Mix-m (✓) was slightly lower at ± 1.9% (we excluded Mix-m at 200°C for the reasons discussed in Section 3.1.4). [17] similarly found that C recovery variability due to species identity was ± 2.4% from 300 to 350°C. Collectively, these data demonstrate that biochemical composition predicts aqueous C recovery with reasonable precision.

3.5.5. Two-species mixture interactions
The two-species mixture measured (✓) and predicted (●) aqueous C recoveries were generally in agreement. One exception was on average 6.2% lower C recovery (p < 0.10) at 200°C, 3.2 min. Given that total N recovery measured and predicted values were equivalent here, this suggests that the carbohydrate fraction may be the source of this discrepancy.

3.6. pH

Aqueous-phase pH (Fig. 7) varied dramatically depending on biomass type and reaction conditions, ranging from 3.90 to 8.24. Note that this pH measurement occurred after the addition of deionized water during product recovery, which represents a dilution factor of 4.7 to 10, depending on reaction temperature and concentration. Tables A.1 and A.2 in Appendix A list the exact dilution factors used for each set of reaction conditions and feedstocks.

3.6.1. Temperature and time
Aqueous pH decreased (became more acidic) with increased reaction severity to a minimum in the range of 200°C, 31.6 min to 250°C, 10 min (Fig. 7a). [16] similarly observed this minimum to be at 240°C, 9 s over a temperature range of 205 to 325°C. Beyond this maximum acidity, the aqueous phase becomes monotonically more basic with increasing reaction severity, a trend demonstrated previously [16,19,22,46].

3.6.2. Slurry concentration
At reaction severities of 250°C, 1 min and lower, the ACP was more basic for the 30 g L⁻¹ slurries than the 120 g L⁻¹ slurries by an average of 0.22 (p < 10⁻⁶). This could be explained by the increased NH₄⁺-N recovery (Section 3.2) at those conditions. At 300°C and higher, however, the trend reversed with the less concentrated slurries producing more acidic aqueous phase by an average of 0.52 (p < 0.01). At these conditions, NH₄⁺-N is generally still favored with decreasing slurry concentration, and would push the pH in the opposite direction of this trend (toward more basic). This suggests that compounds other than the ammonium seem to be responsible for this pH trend. Previous studies have shown these increases in basicity with increasing slurry concentration at high reaction severity [20,46].

3.6.3. Biochemical composition
In general, pH significantly increased (became more basic) with increasing biomass protein content (Fig. 7b), a trend also observed previously [27,28,30,47]. Between the two types of both Nannochloris and Chlorella, higher protein content led to an average increase of 2.91 (p < 0.01) across all reaction conditions. The increased ammonia (Kn = 1.8 × 10⁻⁵) generated from deamination of amino acids liberated from protein likely explains this sharp increase in pH.

3.6.4. Species identity
Nan-1 (●) and Mix-m (✓) produced aqueous phases with nearly identical pH values regardless of concentration, temperature, or time, an average difference of just 0.03 (p < 0.47). However, the pH for Chl-1 (●) was on average 0.64 lower (p < 0.03) than that of Nan-1 and Mix-m at 200°C. It is not immediately clear why the Chl-1 aqueous phase was more acidic, given that ammonium and phosphate recoveries were similar to at least one of the other two biomass types. However, at 300°C, variability between the three species was just ± 0.09, indicating that at high reaction severity, biochemical composition is a strong predictor of aqueous-phase pH.

3.6.5. Two-species mixture interactions
There was a small but significant decrease in pH (−0.16, p < 0.01) for Mix-m (✓) compared to Mix-p (●) across all reaction conditions. This difference was largest (−0.33, p < 0.07) at 200°C, 31.6 min. We
demonstrated earlier (Section 3.2) that at 300°C, there was less ammonium recovery than expected in the aqueous phase from Mix-m, which could explain why its pH is lower (more acidic) than expected. At 200°C however, there was no such difference in ammonium recovery and there are no other trends concurrent with the increase in pH there.

3.7. Engineering nutrient recovery and recycling

In this section, we describe particular sets of reaction conditions and feedstock characteristics that tend to maximize aqueous phase recyclability, while also considering other factors such as the minimization of heteroatoms in the biocrude (as discussed previously [34]). We remind the reader that, in general, the most optimal aqueous phase is one that maximizes recovery of key elements required for microalgal growth, such as N, P, and S [4,5], while minimizing recovery of C. In addition, bioavailable forms of these elements, such as NH$_4^+$ and PO$_4^{3-}$, are preferred to other forms, as they can be directly used during growth [6,7]. Fig. 8 demonstrates how these key elements partition into the different product fractions with respect to changing reaction conditions for the 120 g L$_{\text{LHS}}^{-1}$ slurries of Nan-1 (a) and Chi-2 (b). We chose these two biomass types because we analyzed them over the most temperatures and times and the 120 g L$_{\text{LHS}}^{-1}$ slurries provided sufficient mass for characterizing most product fractions. Note that not only are N, P, and S beneficial to the ACP for recycling, they are also undesirable in the biocrude, as they must be removed through catalytic upgrading prior to traditional refining processes [48].

Fig. 8a and b show that there is a trade-off between maximizing NH$_4^+$-N recovery (at 350°C, 100 min), and limiting biocruide C recovery, for example to < 5% (around 200°C, 31.6 min or 250°C, 1 min). PO$_4^{3-}$-P recovery (Fig. 8c and d) tends to be maximized at moderate reaction severity (e.g., 200°C, 31.6 min or 300°C, 3.2 min), although total P recovery in the ACP decreases with increasing reaction severity thereafter; at such high severities, P is either precipitated as a solid or incorporated into the biocrude phase. Aqueous S recovery (Fig. 8e and f) is maximized at several moderately severe conditions; however, at 200°C, 31.6 min, biocruide S recovery is additionally limited to < 8% and further increases to reaction severity dramatically increase S partitioning to the biocrude. Aqueous C recovery (Fig. 8g and h) is maximized at moderate reaction severity and minimized at the highest reaction severity examined (350°C, 100 min), whereas biocruide C recovery is maximized at 300°C, and changes very little with increasing reaction severity.

In terms of maximizing aqueous phase recyclability while minimizing heteroatom incorporation into the biocrude, a reaction condition such as 200°C and 31.6 min appears to achieve both. This condition generally maximizes aqueous PO$_4^{3-}$-P, total P, and S recoveries while limiting biocruide N and S recoveries to < 5 and < 8%, respectively. This condition does, however, only recover 50 to 60% of the N in the aqueous phase, with just 20% of that in the bioavailable form of NH$_4^+$. In practice, if the aqueous phase at this reaction condition were recycled continuously, a steady-state amount of organic N in the aqueous phase would be achieved [49]. This steady-state amount will also depend on the extent that algae or heterotrophic bacteria can directly consume the organic C or use extracellular enzymes to liberate the amine groups; such a phenomenon occurs frequently in aquatic environments [50]. Further research and process modeling would be necessary to determine the approximate amount of organic N in this theoretical recycle loop [51]. It is possible that at steady-state, enough ammonium would be liberated to fully replenish the spent media for each cycle. The solids remaining at this condition could also be recycled or reacted at higher severity conditions; this latter scenario has been referred to as two-step HTL, and is a subject of ongoing research [18,52,53].

Another possibility is to operate HTL at very high reaction severity, such as 350°C, 100 min or higher. This condition maximized ammonium recovery, and higher reaction severities approaching hydrothermal gasification are likely to liberate N from the biocrude, as we demonstrated previously [34]. P at these conditions has been demonstrated to form solid precipitates [13,41], and could be a side product used as fertilizer for other applications. It is not immediately clear where the rest of the P partitions, however there are a few possibilities. The most plausible one is that the remaining P exists as a solid precipitate lost during the product recovery step. The majority of solids were likely recovered at these conditions, but because P content in the solid phase is high at high reaction severity [34], small losses in solids result in relatively larger losses in P. Another possibility is that the
method employed for total P measurement in the aqueous phase is not sensitive to certain forms of P. A final possibility is that some of the P is present in more volatile forms, such as phosphine, which could be lost in the gas phase. High-temperature water at those conditions can serve as a hydrogen source [1], for example for reduction of phosphate to phosphine. S recovery at high severity is similarly low, and it is possible that hydrogen sulfide or similarly volatile compounds are similarly unaccounted for there, as suggested earlier in Section 3.4. Further research aimed at fully recovering and characterizing the more volatile components produced during HTL would improve comparisons of aqueous phase recyclability, particularly at higher reaction severities.
Table 2
Summary of differences in aqueous-phase properties as a result of changing six different independent variables from two different reference points. Sensitivity Scale qualitatively denotes the sensitivity of aqueous phase properties to each independent variable at a given reference point. ON, TN, OP, and TP represent organic nitrogen, total nitrogen, other phosphorus, and total phosphorus recoveries, respectively. Yellow and blue intensity denote increases and decreases in the associated property, respectively. Changes in temperature (n = 24), time (n = 24), and concentration (n = 46) are the average of the differences in the six different types of biomass (see Table 1). Changes in biochemical composition (n = 20) show the average of differences in the two different types of Nannochloropsis and Chlorella, respectively. Changes in species (n = 8) denote the standard deviation of values from Nan-1, Chl-1, and Mix-m. *Denotes statistically significant difference at the 0.05 level.

<table>
<thead>
<tr>
<th>Ref. Point</th>
<th>Independent Variable</th>
<th>Change</th>
<th>Sensitivity Scale</th>
<th>Yield [%]</th>
<th>Recovery [%]</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NH₄⁺⁻N</td>
<td>ON</td>
</tr>
<tr>
<td>200 °C</td>
<td>Temperature: 200 → 300 °C</td>
<td>−10</td>
<td>23*[�]</td>
<td>−7</td>
<td>16*[�]</td>
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<tr>
<td>3.2 min</td>
<td>Time: 3.2 → 31.6 min</td>
<td>9*[�]</td>
<td>10*[�]</td>
<td>8</td>
<td>18*[�]</td>
</tr>
<tr>
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<td>Lipid Content: 10.5 → 24.2 wt%</td>
<td>1</td>
<td>3*[�]</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>30 g L⁻¹rxn</td>
<td>Protein Content: 17.4 → 42.8 wt%</td>
<td>−1</td>
<td>−3*[�]</td>
<td>−3</td>
<td>−6</td>
</tr>
<tr>
<td>300 °C</td>
<td>Concentration: 30 → 120 g L⁻¹rxn</td>
<td>−4</td>
<td>0</td>
<td>−6</td>
<td>−7</td>
</tr>
<tr>
<td>3.2 min</td>
<td>Species: Nan-1, Chl-1, Mix-m</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>30 g L⁻¹rxn</td>
<td>Lipid Content: 10.5 → 24.2 wt%</td>
<td>−14</td>
<td>−7</td>
<td>−7</td>
<td>−10</td>
</tr>
<tr>
<td>3.2 min</td>
<td>Protein Content: 17.4 → 42.8 wt%</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>30 g L⁻¹rxn</td>
<td>Time: 3.2 → 31.6 min</td>
<td>−13*[�]</td>
<td>18*[�]</td>
<td>−20*[�]</td>
<td>−2</td>
</tr>
<tr>
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<td>Concentration: 30 → 120 g L⁻¹rxn</td>
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<td>−4</td>
<td>−9*[�]</td>
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<tr>
<td>Species: Nan-1, Chl-1, Mix-m</td>
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<td>3</td>
<td>1</td>
<td>2</td>
<td>13</td>
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4. Further discussion and conclusion

Table 2 summarizes how the yield, elemental recoveries, and pH of the ACP are affected by manipulating reaction time, slurry concentration, lipid content, protein content, microalgal species, and reaction temperature. These changes each occur with all other factors fixed from two reference points at 200 and 300°C, respectively, for 30 g L⁻¹rxn slurries and 3.2 min reaction time.

4.1. Mild-reaction-severity reference point

At the 200°C, 3.2 min, and 30 g L⁻¹rxn reference point, temperature is the most influential input variable affecting ACP properties. An increase of 100°C led to significantly higher NH₄⁺⁻N (+23%) and total N (+16%) recoveries in addition to 7% higher PO₄³⁻⁻P recovery and 7% lower C recovery, all of which are improvements in recyclability. However, at the same time, total P and S recoveries decreased by 10 and 11%, respectively. Reaction time was nearly as broadly significant as temperature at this mild reference point, although crucially, increased time led to nearly universal improvements in aqueous phase recyclability. The recoveries of NH₄⁺⁻N (+10%), organic N (+8%), PO₄³⁻⁻P (+13%), and S (+12%) all increased with increasing time, albeit with a 9% increase in C recovery. Increased biomass lipid content only affected PO₄³⁻⁻P recovery (+21%), S recovery (+11%), and pH (−1.2) to a significant extent at this reference point, although it did also provide a small (+3%) but statistically significant boost to NH₄⁺⁻N recovery. Notably, higher lipid content improved ACP recyclability unilaterally, albeit to a slightly lesser extent than did reaction time, but also without increasing C recovery. We previously showed that at this reference point, increased lipid content also increases C and H recovery to the biocrude by 21% [34]. HTL of high-lipid microalgae at this reference point is a “win-win,” improving nutrient partitioning to the aqueous phase while also increasing the yield of high-quality biocrude. Slurry concentration was less influential than the aforementioned independent variables at this reference point; however, increased concentration led to universal decreases in nutrient recovery to the aqueous phase, likely through promotion of Maillard reactions between protein- and carbohydrate-degradation products discussed in Section 3.2. Variability due to species identity was generally the least influential variable, although aqueous yields and organic N recoveries varied by ±7 wt.% and ±6%, respectively. We assumed that all biomass N resided in the protein fraction, although it is possible that these differences in aqueous yield and organic N recovery could be explained by the true distribution of N between biomass protein and DNA in each of the microalgae. The incorporation of N on a molecular level is generally different in protein and DNA, for example within an amino-acid-linking peptide bond for the former and within a cyclic or polycyclic nitrogenous base structure for the latter. Therefore the total rate of liberation of the N into the aqueous phase could be expected to be different based on the relative abundances of those two biochemical classes. A more granular accounting of biochemical composition may be required to reduce this variability between microalgae that are expected to behave similarly during HTL on these grounds.

4.2. High-reaction-severity reference point

At the 300°C, 3.2 min, and 30 g L⁻¹rxn reference point, biochemical composition is the most influential variable, with approximately as much influence as that of temperature from 200 to 300°C. Increased protein content improved some key recyclability metrics, such as NH₄⁺⁻N (+7%), total N (+14%), and S (+14%) recoveries; however, it also decreased PO₄³⁻⁻P (−10%) and total P (−8%) recoveries and increased C recovery (+14%). Increasing lipid content (necessarily) demonstrated the opposite effect. Aqueous phase characteristics and elemental recoveries were nearly as sensitive to increasing reaction time as biochemical composition. Increasing time at this reference point had mixed effects on aqueous phase recyclability, with decreases to PO₄³⁻⁻P (−12%), total P (−11%), and S (−11%) recoveries, but increases to NH₄⁺⁻N recovery (18%) and decreases to C recovery (−12%). Notably, reaction time, lipid content, and protein content all demonstrated different combinations of effects on N, P and S recoveries. These different combinations could enable optimization of specific nutrient recycling metrics at the expense of others. For example, if N recovery were prioritized, followed by P and S, then high lipid content and longer reaction times could be used together to improve NH₄⁺⁻N recovery (net +11%) and maintain PO₄³⁻⁻P recovery (net −2%) at the expense of S recovery (net −25%).
This section describes some approaches discussed earlier [34] for optimizing biofuel production from a hypothetical biorefinery. We describe those approaches again in brief and contextualize them in terms of the effects of aqueous-phase recyclability. We argued previously that optimizing sustainable fuel production from microalgal HTL necessitates that energy and material inputs and costs are minimized for four major process units, including (A) algal cultivation and (B) dewatering, (C) HTL, and (D) catalytic upgrading. Here we also consider a fifth processing unit, (E) nutrient recycling. We proposed hypothetical, but practical, strategies for operating these process units under a low- or high-input strategy, which we reproduce in Table 3. These “inputs” refer to the aggregate of energy, materials, and costs. The strategies attempt to either minimize local process unit inputs or expend relatively higher local inputs to reduce global inputs.

The first approach we outlined is to (A) maximize biomass productivity while minimizing inputs (which produces high-protein biomass [54]) and (B) concentrate to high levels (e.g., 16 wt.%) to reduce energy expended to heat water for (C) HTL at high reaction severity, which maximizes biocrude yield. Due to the high microalgal-protein content, biocrude N content at these severe reaction conditions is > 5 wt.% and must be (D) reduced to lower levels (< 0.5 wt.%) via catalytic upgrading before conventional refining [55], thereby (E) liberating nitrogen for nutrient recycling which can be supplemented with fresh media to compensate for losses during upgrading. The crux of this collective approach is the minimization of the inputs for Step A, on a per-unit-biomass basis, while choosing parameters for Steps B and C that maximize biocrude yield, assuming that inputs required in Step D to remove the resulting high-heteroatom content are relatively small, and assuming that most of the nitrogen recovered in the upgrading effluent can be recycled and any deficit met by added fresh media in Step E.

Optimizing approaches for an algal biorefinery (i.e., maximizing productivity vs. lipid content) is rooted in assumptions about algal growth (Step A), catalytic upgrading (Step D), and nutrient recycling (Step E). The additional inputs needed to grow high-lipid biomass to enable more direct ACP recycling and simultaneously reduce catalytic upgrading inputs must be rigorously evaluated alongside the fewer inputs needed to cultivate high-protein biomass with enhanced productivity but with significantly more inputs required for nutrient recycling and catalytic upgrading. This study characterizes how decisions for upstream process units (i.e., algal cultivation and dewatering) can affect downstream process units (i.e., nutrient recycling and catalytic upgrading). The results herein bolster those reported previously [34] by suggesting that the benefits of higher biomass-lipid content could more than compensate for reductions in biomass productivity by offering increased aqueous-phase total and bioavailable nutrient recovery and, as a result, decreased biocrude heteroatom content and increased energy density. Detailed life cycle assessments and techno-economic assessments are needed to fully characterize and contextualize the effects of employing the different strategies among the processing steps. In particular, future work is needed to quantify the effect of steady-state nutrient recycling and the inputs needed to remove heteroatoms from the biocrude via catalytic upgrading in bioavailable forms for recycling.

### 4.4. Additional conclusions

In addition to the aforementioned effects, there were several other key takeaways from this article. The tendency for higher protein-to-carbohydrate biomass to produce ACP with higher NH_4^+-N recovery corroborated our earlier claim that carbohydrates are the limiting reactant for Maillard reactions [34,35]. More dilute slurries also tended to

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Table 3

<table>
<thead>
<tr>
<th>Step</th>
<th>Process unit</th>
<th>Low-input strategy</th>
<th>High-input strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Algal growth</td>
<td>Maximize biomass (high-protein content)</td>
<td>Maximize lipid content (less biomass)</td>
</tr>
<tr>
<td>B</td>
<td>Dewatering</td>
<td>Concentrate by factor of 200 to 800 to 4 wt.%</td>
<td>Concentrate by factor of 800 to 3200 to 16 wt.%</td>
</tr>
<tr>
<td>C</td>
<td>HTL</td>
<td>Mild reaction severity (e.g., 200°C, 31.6 min)</td>
<td>High reaction severity (e.g., 350°C, 100 min)</td>
</tr>
<tr>
<td>D</td>
<td>Catalytic upgrading</td>
<td>Upgrade from &lt; 3 wt.% N content</td>
<td>Upgrade from &gt; 5 wt.% N content</td>
</tr>
<tr>
<td>E</td>
<td>Nutrient recycling</td>
<td>ACP with less fresh media</td>
<td>Upgrading effluent with more fresh media</td>
</tr>
</tbody>
</table>
recover more N as NH₄⁺ (plausibly due to less Maillard reaction products formed), suggesting that the overall reaction order for the Maillard reaction is greater than one. This work illuminated additional benefits of employing dilute slurries of high-lipid biomass for HTL, including enhanced ACP recyclability. These results demonstrate that high-lipid and low-carbohydrate biomass could be ideal for maximizing ACP recyclability in addition to maximizing biocrude yield and quality [34].

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Declaration of Competing Interest

No conflicts, informed consent, human or animal rights applicable.

Appendix A. Supplementary data

Appendix A presents tabular data for aqueous yield, elemental composition and recoveries, and pH. Appendix A also contains supplemental figures for these aqueous properties with respect to time or biochemical content. Supplementary data associated with this article can be found in the online version. Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2019.101568.

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[15] A.A. Peterson, R.P. Lachance, J.W. Tester, Kinetic evidence of the Maillard reaction of aqueous properties with respect to time or biochemical content. Supplementary data associated with this article can be found in the online version. Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2019.101568.


