Demonstration and Evaluation of Hybrid Microalgae Aqueous Conversion Systems for Biofuel Production

Yalin Li[†,‡], Shijie Leow[†,‡,§], Tao Dong[‖], Nicholas J. Nagle[‖], Eric P. Knoshaug[‖], Lieve M. L. Laurens[‖], Philip T. Pienkos[‖], Jeremy S. Guest[§], Timothy J. Strathmann[,†,‡,‖]*

[†]Colorado School of Mines, Department of Civil and Environmental Engineering, 1012 14th Street, Golden, Colorado 80401, USA.

[‡]Engineering Research Center for Re-inventing the Nation's Urban Water Infrastructure (ReNUWIt), Colorado School of Mines, 1012 14th Street, Golden, Colorado 80401, USA.

[§]University of Illinois at Urbana-Champaign, Department of Civil and Environmental Engineering, 205 North Mathews Avenue, Urbana, Illinois 61801, USA.

[‖]National Renewable Energy Laboratory, National Bioenergy Center, 15013 Denver West Parkway, Golden, Colorado 80401, USA.

*Corresponding Author: T. Strathmann, E-mail: strthmnn@mines.edu, Phone: +1 (303) 384-2226

Abstract

As an effort to develop affordable and sustainable energy sources, algae-derived biofuels have attracted considerable interest. As use of individual conversion processes targeting a sub-set of biochemical components (e.g., extraction and upgrading of lipids) have been shown to be economically unfeasible, there is a recognized need for integrated conversion systems that can valorize algal feedstocks with varying cell compositions. In this study, two hybrid systems (HBD-

1, HBD-2) are proposed to enable more efficient conversion of all biomass components (lipids, proteins, carbohydrates) by leveraging two complementary systems: direct hydrothermal liquefaction (DHTL) and combined algal processing (CAP). Demonstrative experiments with *Scenedesmus acutus* show a 12.2–34.3% increase in fuel yields relative to individual systems (DHTL, CAP). Subsequent modeling efforts reveal substantial improvements stemming from CAP valorization of carbohydrates and lipids and DHTL valorization of proteins and CAP residuals. The maximum biomass-to-fuel conversion efficiencies for lipids/proteins/carbohydrate cell components are 79%/34%/75% (HBD-2), and techno-economic analysis suggests a 3.2–62.1% reduction in minimum fuel selling prices (MFSPs). The increased fuel yields and reduced MFSPs highlight the flexibility of the hybrid systems for biofuel production, revealing advantages of these systems for broader ranges of feedstocks, including ones not traditionally considered for fuel production.

Keywords

Algal Biofuel, Hydrothermal Liquefaction (HTL), Combined Algal Processing (CAP), Valorization, Techno-economic Analysis (TEA), Minimum Fuel Selling Price (MFSP)

Introduction

With advantages including high productivity, adaptability to various water streams, and potential for being cultivated to a wide range of biochemical compositions,[1–3] microalgae have attracted strong interest as feedstocks for production of biofuels. Continuous governmental support has led to the development of a portfolio of aqueous downstream processes for valorizing algal biomass. These include, but are not limited to, anaerobic digestion (AD, a table of abbreviations was included as Table S1 in the Supporting Information, SI),[4] carbohydrate fermentation,[5,6] lipid

extraction/hydrothermal liquefaction (HTL) followed by associated upgrading processes (e.g., catalytic hydrotreating).[7–10] However, these individual processes were typically targeted at only a sub-set of biochemical components (lipids, proteins, or carbohydrates only) of the feedstock and neglected others, leading to limited fuel yields and the partial wastage of feedstock energy. For example, carbohydrate fermentation targets only carbohydrates by converting hydrolyzed monomeric sugars to ethanol as the fuel product,[11] and lipid extraction followed by upgrading processes only targets the lipid fraction of biomass for the production of hydrocarbon fuels. Admittedly, algal biomass can be engineered (e.g., by gene editing[12]) or cultivated (e.g., by nutrient depletion[1]) to promote accumulation of specific components, but this often comes at the expense of slower growth rates, higher feedstock costs, and increased risk of strain contamination.[13] It follows that opportunities exist to improve fuel productivity by combining component-specific unit processes for more efficient valorization of whole algal biomass and improved economic performance.

To this end, two existing systems – direct HTL and upgrading (DHTL)[14,15] and combined algal processing (CAP)[6,11] – have been designed to take advantage of complementary macromolecule conversion efficiencies to achieve higher fuel yields. While DHTL produces renewable diesel blendstock (RDB) and naphtha (paraffins primarily in the C_6–C_{12} range[11,13]) from direct whole-cell HTL conversion and upgrading, CAP produces ethanol from carbohydrates via fermentation and RDB and naphtha from lipids via extraction and upgrading. However, a recent modeling study by our group[13] evaluated performance of DHTL and CAP for algae cultivated to a wide range of biochemical compositions, concluding that feedstock properties had a major influence over fuel productivity and process economics of both systems.[13] While DHTL was found to be more

65 favorable for biomass with high protein content (typically at the early stage of cultivation), CAP
66 could yield more biofuel at lower cost for mid- and late-harvest biomass that was characterized by
67 higher lipid and carbohydrate contents. On the other hand, during HTL, over 80% of the
68 carbohydrates were converted to gaseous (predominantly CO_2) and solid biochar products with
69 minimal fuel potential,[16,17] while CAP routed the bulk of the protein fraction to AD[5] that yielded
70 no additional liquid fuel (though this did reduce fuel production costs by providing an internal
71 source of combined heat and power and allows for nutrient recycling).[4,13]

72 Thus, major improvements are required for both systems to be competitive for a broader range
73 of algal biomass. As DHTL has high to moderate fuel conversions for lipids and proteins, and CAP
74 is recognized as efficient for both lipid and carbohydrate conversion, it follows that hybrid systems
75 combining the two pathways may more efficiently capture the full energy embedded in algal
76 biomass, further reducing the cost of algal biofuel production. In this study, two hybrid systems
77 (HBD-1 and HBD-2; **Figure 1**) are proposed to leverage the complementary advantages of DHTL
78 and CAP to valorize each set of macromolecules. In HBD-1, most carbohydrates are first converted
79 to ethanol via dilute acid treatment and carbohydrate fermentation, and the resulting low-
80 carbohydrate residuals are processed by DHTL. In HBD-2, the low-carbohydrate residuals after
81 fermentation are subjected to lipid extraction and upgrading before subjecting the protein-rich
82 residual fraction to DHTL. Demonstrative experiments were conducted for all systems (DHTL,
83 CAP, HBD-1, and HBD-2) with one batch of *Scenedesmus acutus* biomass. Experimental results
84 were used to inform techno-economic analysis (TEA) and predict the minimum selling prices of
85 biofuels produced from the competing systems. TEA models for algal biofuel production that
86 incorporated biomass cultivation and downstream processing steps[13] were modified and applied to

extend the TEA for algal biomass of varying biochemical composition. Finally, conclusions from these analyses were synthesized to provide recommendations for algal biofuel production and future research needs.

Experimental Methods and Modeling Approaches

Downstream processes descriptions (Figure 1)

Pathway a. Direct hydrothermal liquefaction (DHTL)[14]

Harvested and dewatered algae (20% algal biomass solids on an ash-free dry weight basis, afdw%) as a pumpable slurry are directly sent to HTL (350°C with 15 min retention time, operating parameters of unit processes were described in detail in Leow et al.[13]), and the resulting biocrude oil (upgradable to liquid fuels), aqueous (rich in organics and nutrients), gaseous (predominantly CO_2[17,18]), and biochar (solid residuals) products are separated. Biocrude oils are further upgraded to RDB and naphtha (403°C with liquid hourly space velocity at 0.5 hr^{-1}, CoMo/alumina as the catalyst). Energy in aqueous products is reclaimed by catalytic hydrothermal gasification (350°C with liquid hourly space velocity at 2 hr^{-1}, 7.8% Ru/C as the catalyst), which converts the aqueous organics into gaseous products rich in CH_4 and H_2.[15,18] All gaseous products (mainly CH_4, H_2, CO_2, and other minor amounts of volatile hydrocarbons) from HTL, catalytic hydrothermal gasification, and upgrading processes are collected and used to generate H_2 by steam reforming through an on-site hydrogen plant. Fuel products from DHTL include RDB and naphtha produced from upgraded biocrude oils.

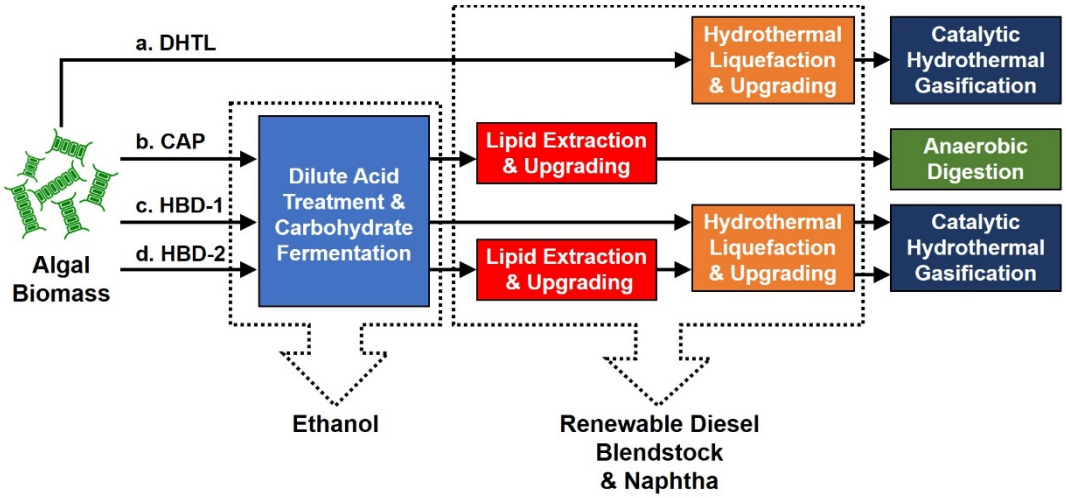


Figure 1. Scheme of four systems compared in this study: (a) direct hydrothermal liquefaction (DHTL), (b) combined algal processing (CAP), and the two hybrid systems HBD-1 and HBD-2 (c)–(d). Fuel products include ethanol, renewable diesel blendstock (RDB) and naphtha (C_6–C_{12} paraffins[11,13]).

Pathway b. Combined algal processing (CAP)[6,11]

Harvested algae are pretreated by H_2SO_4 (2 wt% in demonstrative experiments, 1 wt% in model simulations, 150°C with 5 min retention time) to hydrolyze carbohydrates to monomeric sugars, followed by fermentation (37°C) of the sugars present in the algal hydrolysate to ethanol. The fermented hydrolysate is distilled to recover ethanol and the residual wet biosolids (stillage) are extracted with hexane to collect lipids (5.0 solvent/dry algae weight ratio), which are further processed to RDB and naphtha via degumming by 0.19 wt% phosphoric acid at 110°C, demetallization with 0.1 wt% silica, and bleaching with 0.2 wt% clay. Finally, the remaining biosolids (extracted stillage) are combined and processed by AD (35°C with 25 d retention time) to generate biogas (mostly CH_4 and CO_2). H_2 needed for the upgrading process is purchased from an external source.[11] Fuel products from CAP include ethanol from fermentation, and RDB and naphtha produced from upgrading of the extracted lipids.

Pathway c. Hybrid system-1 (HBD-1)

Harvested algae follow the same hydrolysis and fermentation steps as in CAP, but the stillage following distillation is processed by DHTL (as described in Pathway a) instead of lipid extraction. Fuel products from HBD-1 include ethanol from fermentation, and RDB and naphtha produced from upgraded biocrude oils.

Pathway d. Hybrid system-2 (HBD-2)

Harvested algae are hydrolyzed, fermented, and lipids are extracted and upgraded in the same manner as CAP, but extracted stillage is processed by DHTL (as described in Pathway a) in place of AD. It should be noted that as extracted lipids and HTL biocrude oils are of different nature and no experimental or literature data are available on the co-upgrading of lipids and biocrudes, and therefore they are treated separately in this study. Fuel products from HBD-2 include ethanol from fermentation, as well as RDB and naphtha produced from extracted lipids and HTL-derived biocrude oils.

Feedstock characterization and HTL experiments

To evaluate performance of the proposed hybrid systems, one batch of high-lipid, high-carbohydrate *S. acutus* biomass previously evaluated for CAP[5,6] was first used for experimental demonstration. The systems were later evaluated for algal biomass with varying biochemical compositions using TEA models that incorporated the separate unit processes combined in the hybrid systems. The biomass was cultivated in outdoor flat panel (650 L) photobioreactors in nitrate deplete media. Additional details regarding the biomass can be found elsewhere.[5,6] Raw *S. acutus* biomass and residual biosolids after fermentation (stillage) and fermentation followed by lipid extraction (extracted stillage) generated from the same batch were subjected to HTL. The

146 biomass was characterized for biochemical (extractable lipid, fatty acid, protein, carbohydrate, and
147 ash) and elemental (C, H, and N) compositions following established procedures,[16,17] and
148 analytical details were provided in Section S1 in the SI.

149 HTL experiments of the raw *S. acutus* biomass, stillage, and extracted stillage were conducted
150 following an established protocol.[17] Briefly, the reaction was carried out in stainless steel tube
151 reactors (5.93 mL working volume) with algae slurries (20 wt% solid loading, 4 g slurry was
152 loaded for each run) at 300°C for 30 min. After reaction, four products – including a biocrude oil
153 product, aqueous, gaseous, and biochar products – were generated. Aqueous product was poured
154 out of the reactor and dichloromethane was added into the reactor to recover biocrude oil. Both
155 biocrude (in dichloromethane) and aqueous products were filtered with 0.45 μm syringe filters to
156 separate out the biochar product. Biocrude (dichloromethane removed by evaporation) and biochar
157 products were analyzed for elemental (C, H, and N) composition and aqueous product was
158 analyzed for total organic carbon and total nitrogen contents. Additional details on product
159 recovery and analyses are provided in Section S1 in the SI.

160 **Techno-economic analysis (TEA) models for system evaluation**

161 The four different systems described in **Figure 1** were evaluated using models previously
162 developed for DHTL and CAP.[13] For DHTL and CAP, previous models were directly used without
163 any modifications; for HBD-1, material flows after the fermentation process in CAP were used as
164 inputs for DHTL; for HBD-2, material flows after the lipid extraction and upgrading process were
165 used as inputs for DHTL. For HBD-1 and HBD-2, costs and fuel yields from relevant DHTL and
166 CAP processes were combined for final results. The models considered costs from all aspects
167 (capital, operating, financing, etc.) for a biorefinery facility (including the algal farm and

168 downstream processing) to calculate the minimum selling prices of the produced fuels in order to
169 achieve a net present value of zero. All costs and prices were expressed in 2011 U.S. dollars to be
170 consistent with previous reports and literature that this study was based upon,[5,11,13,14] and can be
171 converted to other years as described in literature.[13] Uncertainty and sensitivity analyses were
172 included in the previous study for the original DHTL and CAP models that this study were based
173 upon.[13] All systems were evaluated for two scenarios: (a) *S. acutus*-demonstrated scenario where
174 experimental measurements of HTL conversion and CAP were used as inputs for fuel yields; and
175 (b) model-predicted scenario where predictive models were used to evaluate feedstocks with
176 varying biochemical compositions (lipids, proteins, carbohydrates). Predictions for fuel yields
177 were made for all possible combinations of lipid, protein, and carbohydrate contents (i.e., 0–100%
178 lipids, proteins, carbohydrates); but predictions for fuel prices were limited to combinations that
179 were practically achievable and feedstock costs of which could be predicted by a cultivation model
180 introduced previously.[13] Additional details on the models can be found in Section S2 in the SI.

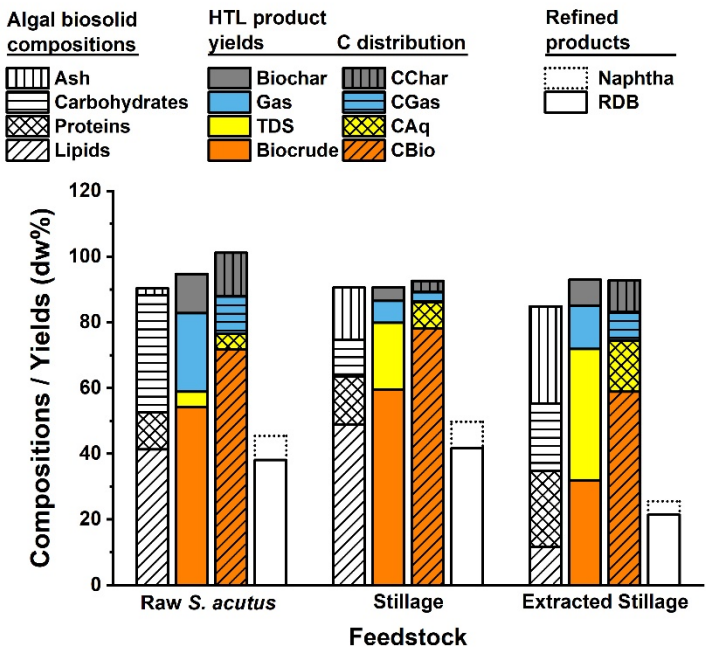
181 **Results and Discussion**

182 **Feedstock characteristics and experimental HTL yields**

183 Characterization of the raw *S. acutus*, stillage, and extracted stillage showed changes of the algal
184 biosolid properties at different stages of CAP, which resulted in changes in product yields and
185 characteristics, leading to changes in C distribution between products and ultimately different
186 refined fuel yields (**Figure 2**, Tables S2 and S3 in the SI). This supported the close relationship
187 between feedstock properties and HTL outcomes as proposed in previous literature.[17] The raw *S.*
188 *acutus* biomass was characterized by high lipid (41.3±0.3 dw%) and carbohydrate (35.6±0.1 dw%)
189 contents with lower protein (11.4±0.1 dw%) and ash (2.0±0.02 dw%) contents. HTL processing

of the biomass resulted in a biocrude yield of 54.3±0.7 dw%, along with 24.0±2.1 dw% of gaseous, 11.9±0.4 dw% of biochar, and only 4.6±0.2 dw% of aqueous total dissolved solid products. These findings showed that this particular batch of algae provided high yield of biocrude, though at the same time nearly 40 dw% of the feedstock mass was diverted to gaseous (predominantly CO_2[17,18]) and biochar products with limited value, indicating a margin for further improvement in conversion efficiency.

After fermentation, the majority (73.3%) of the carbohydrates in raw *S. acutus* was converted to ethanol, leaving behind stillage biosolids with much lower carbohydrate content (11.3±0.5 dw%) and comparably higher lipid (48.9±1.6 dw%) and protein (14.5±0.1 dw%) contents. Ash content also increased significantly due to the introduction of acids (H_2SO_4) and the subsequent pH neutralizing step (with the addition of NaOH) before fermentation. As oxygen accounted for nearly half of the carbohydrate ($C_6H_{11}O_5$) component, fermentation of the carbohydrates led to a substantial reduction in feedstock volatile oxygen content (from 30.6±0.1 dw% for raw *S. acutus* to 14.9±1.0 dw% for stillage, Table S2 in the SI), but very minor changes in other elements. This led to a positive change in feedstock higher heating value (HHV), increasing from 26.2±0.1 $MJ \cdot kg^{-1}$ for raw *S. acutus* to 29.8±0.5 $MJ \cdot kg^{-1}$ for stillage (all HHVs expressed on a dry weight basis).



206

207 **Figure 2.** Biochemical composition of *S. acutus* biosolids (raw *S. acutus*, stillage, and extracted
208 stillage) generated from different stages of CAP (black and white hatched columns), their
209 experimental HTL product yields (colored columns), C distribution between HTL products
210 (colored and hatched columns), and expected refined fuel products (unfilled columns) based on
211 upgrading yields from previous literature.[13,14] Refined products included renewable diesel
212 blendstock (RDB) and naphtha (C_6–C_{12} paraffins[11,13]) that were derived from HTL biocrude oils.
213 TDS referred to total dissolved solids in the aqueous product of HTL. Both compositions and
214 yields were expressed on a dry weight (dw%) basis, and detailed data with associated uncertainties
215 were provided in Tables S2 and S3 in the SI.

216

217 Notably, this carbohydrate conversion step not only transformed a majority of the carbohydrates
218 into fuel products, but also greatly increased the fraction of fatty acids (more amenable to be
219 upgraded to fuel products[10,11]) in total extractable lipids. The raw *S. acutus* had a fatty acid-to-
220 extractable lipid ratio of 84.4%, and it increased to 96.1% in the carbohydrate-reduced stillage
221 (Table S2 in the SI), likely due to the hydrolysis of some polar fractions (e.g., phosphatidic acid
222 from phospholipids, carbohydrate groups in glycolipids[19]), or a reduction on non-lipid co-
223 extractives. As fatty acids are more stable in a hydrothermal environment[20,21] and result in near-

complete conversion to biocrude oils in HTL,[16] the increased fatty acid-to-extractable lipid ratio, together with other changes in biochemical composition, such as increased protein content in the stillage after removing the carbohydrate fraction, was shown to further improve the HTL performance of stillage. Not only did the biocrude yield increase (from 54.3±0.7 dw% for raw *S. acutus* to 59.6±0.8 dw% for stillage), but the generated biocrude oil was also of higher energy content, as indicated by the increase in HHV from 38.5±0.1 $MJ \cdot kg^{-1}$ to 40.9±0.2 $MJ \cdot kg^{-1}$. Though N content of the stillage biocrude oil (1.9±0.03%) increased from raw *S. acutus*' (1.7±0.03%), the increase was minor and was not expected to greatly affect the upgrading process. Besides biocrude oils, there were evident changes in other product yields. The reduction of carbohydrate content in the stillage also led to considerable reductions in HTL gaseous and biochar product yields; gas yield dropped substantially to 6.6±0.1 dw% and biochar yield dropped to 4.2±0.1 dw% (from 24.0±2.1 dw% and 11.9±0.4 dw% for raw *S. acutus*, respectively). In contrast, a much higher total dissolved solid yield (20.3±2.6 dw% vs. 4.6±0.2 dw% for raw *S. acutus*) was observed, mostly due to the introduction of salts during dilute acid treatment. In all, the added carbohydrate conversion processes generated stillage biosolids of higher and improved lipid content, higher protein content, and lower carbohydrate content, thereby yielding a more desirable HTL product distribution.

Following lipid extraction in CAP, the extracted stillage biosolids were characterized by much higher protein (23.1±0.7 dw%) and ash (29.6±0.2 dw%) contents due to the removal of a large fraction of both carbohydrates (73.3% removed) and lipids (87.1% removed). This process also led to lower feedstock carbon (40.8±0.1 dw% for extracted stillage vs. 57.9±0.9 dw% for stillage) and hydrogen (5.4±0.01 dw% for extracted stillage vs. 9.0±0.2 dw% for stillage) contents,

246 resulting in a substantial reduction in feedstock HHV, dropping to 17.8±0.05 $MJ \cdot kg^{-1}$ from
247 stillage's 29.8±0.5 $MJ \cdot kg^{-1}$. The evident increase in biocrude N content (4.6±0.3%) from raw *S.*
248 *acutus* and stillage's was a result of higher protein content of extracted stillage, and was anticipated
249 to decrease the yield of upgraded fuels. In addition, most fatty acids were removed in the solvent
250 extraction process,[6] and the fatty acid-to-extractable lipid ratio decreased to 69.2%. Consequently,
251 HTL reaction of the extracted stillage yielded 31.9±0.6 dw% biocrude, 40.0±1.3 dw% total
252 dissolved solids (due to the high ash content), 13.2±0.9 dw% gaseous, and 7.9±0.1 dw% biochar
253 yields.

254 As HTL products had different energy values, C distribution between products could be used as
255 a surrogate to evaluate flows of feedstock energy to different products (**Figure 2**). Regardless of
256 the feedstock, majority of the C was transferred to biocrude oil products. This was expected for
257 raw *S. acutus* and stillage, where yields of biocrude oil exceeded 50 dw%, but was particularly
258 notable for extracted stillage. For extracted stillage, yield of biocrude oil was only 31.9 dw%, but
259 it nonetheless contained 58.9% of feedstock carbon. Another noteworthy point was that from raw
260 *S. acutus* to stillage, C distribution to gaseous and biochar products decreased from 11.5% and
261 13.1% to 3.1% and 3.3%, and C distribution to biocrude oil increased from 71.9% to 78.1%,
262 respectively. This again showed the advantage of carbohydrate diversion (to ethanol) prior to HTL
263 – where less energy was "wasted" as undesired products. In all, distribution of C between HTL
264 products confirmed the effectiveness of HTL in transferring majority of feedstock energy in
265 biocrude oil, which can be upgraded into RDB and naphtha using established catalytic
266 hydrotreating processes (**Figure 2**).[14,15] Based on yields of RDB and naphtha from biocrude oils
267 of similar properties,[14,15] stillage was expected to have a biomass-to-RDB and naphtha conversion

efficiency of 41.7 dw% and 8.0 dw%, respectively, resulting in a total HTL conversion efficiency of 49.7 dw%, which was higher than the 45.4 dw% from raw *S. acutus* and the 25.2 dw% from extracted stillage. Collectively, 83.7% of the biocrude oil derived from raw *S. acutus* is expected to be converted into either RDB or naphtha,[14,15] followed by stillage's 83.4% and extracted stillage's 80.0%, reflecting the impacts of varying biocrude N contents on the final fuel yields. As most of the N in biocrude oil is typically removed in the form of NH_3 during upgrading,[14,15] the higher biocrude N contents of stillage and extracted stillage (1.9% and 4.6%, respectively, compared with raw *S. acutus'* 1.7%) resulted in the predicted lower biocrude-to-fuel conversion efficiency. It should be noted that the lower biocrude-derived fuel yield of the extracted stillage was due to diversion of lipids in the preceding extraction step, which could be offset by RDB and naphtha generated from the extracted lipids.

Assuming a starting feed of 1 afdw ton (U.S. ton) of raw *S. acutus*, product yields were tracked along each system (**Figure 3** and Table S4 in the SI), which were then used to estimate yields of refined liquid fuel products. DHTL yielded 0.55 ton of biocrude oils, which could be upgraded to 0.39 ton of RDB and 0.08 ton of naphtha, whereas CAP yielded 0.14 ton of ethanol and 0.37 ton of extracted lipids and could be upgraded to 0.29 ton of RDB and 0.01 ton of naphtha. In comparison, more fuel products could be generated from the hybrid systems. For HBD-1, the diversion of carbohydrates for ethanol production did not obstruct the HTL process. The biocrude yield of 0.51 ton was very close to the 0.55 ton in DHTL, which resulted in similar biocrude-derived fuel (RDB and naphtha) yields (0.43 ton for stillage vs. 0.46 ton for raw *S. acutus*). Additionally, the comparison of CAP and HBD-2 revealed the advantage of applying HTL over processing of the extracted stillage with AD. From the same 0.37 ton of extracted stillage, AD only

generated 0.24 ton of biogas (mainly CH_4 and CO_2) which could not be easily stored or transported and was of low market value. In comparison, applying HTL to the same amount of extracted stillage generated an additional 0.17 ton of biocrude oils, and ultimately 0.13 ton of liquid fuels (0.11 ton of RDB and 0.02 ton of naphtha).

Overall, **Figure 3** revealed the system-specific product distribution patterns. With the same amount of starting biomass, though CAP generated less RDB and naphtha than DHTL, an additional ethanol product was generated. Therefore, DHTL and CAP were expected to produce similar total fuel (RDB, naphtha, and ethanol) yields on a mass basis, with DHTL slightly higher at 0.46 ton and CAP at 0.43 ton. Both HBD-1 and HBD-2 generated 0.43 ton of RDB and naphtha, close to DHTL's 0.46 ton. However, the slight reduction in RDB and naphtha yields was offset by production of 0.14 ton of ethanol product, resulting in significantly higher total liquid biofuel yield of 0.57 ton for both HBD-1 and HBD-2.

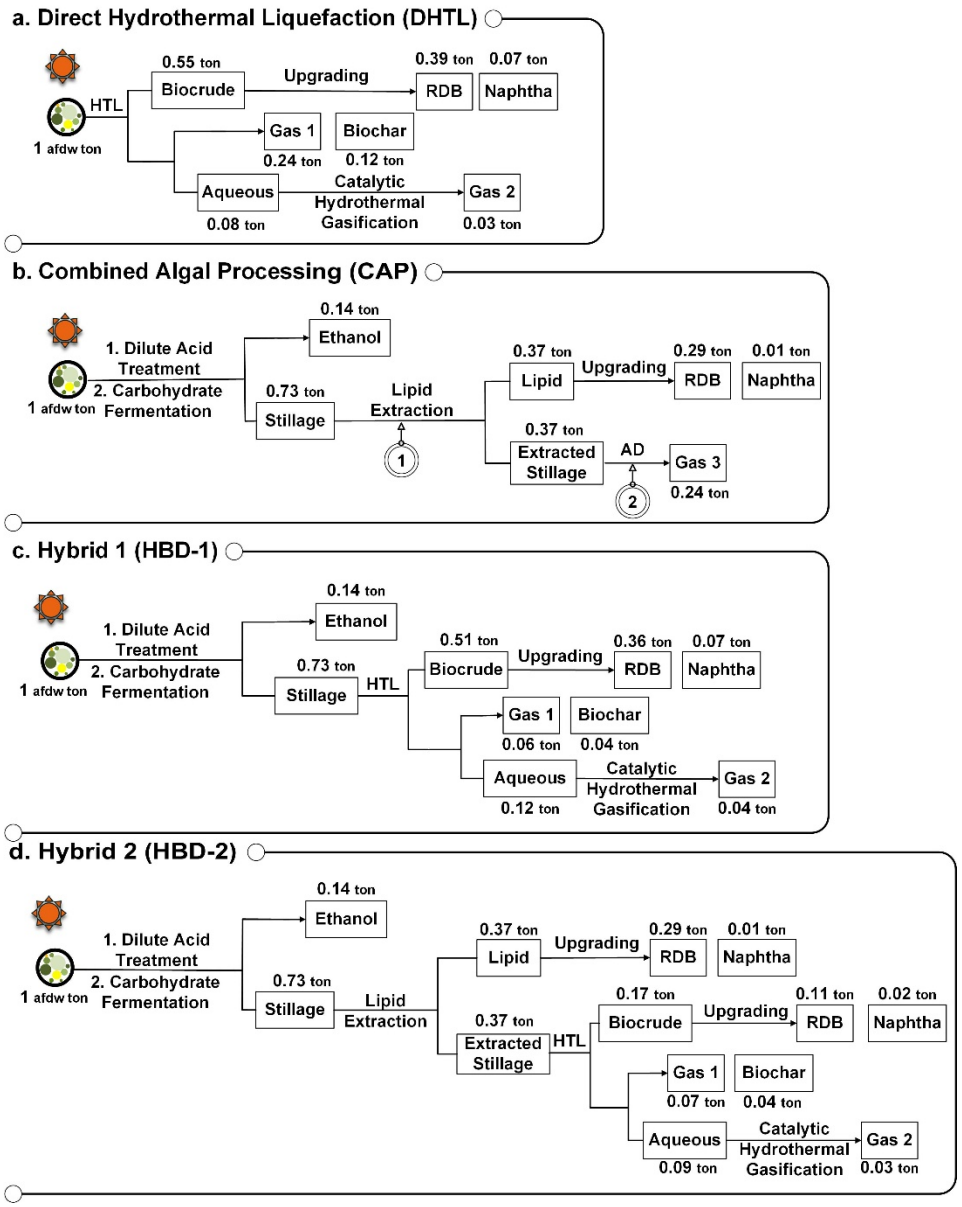


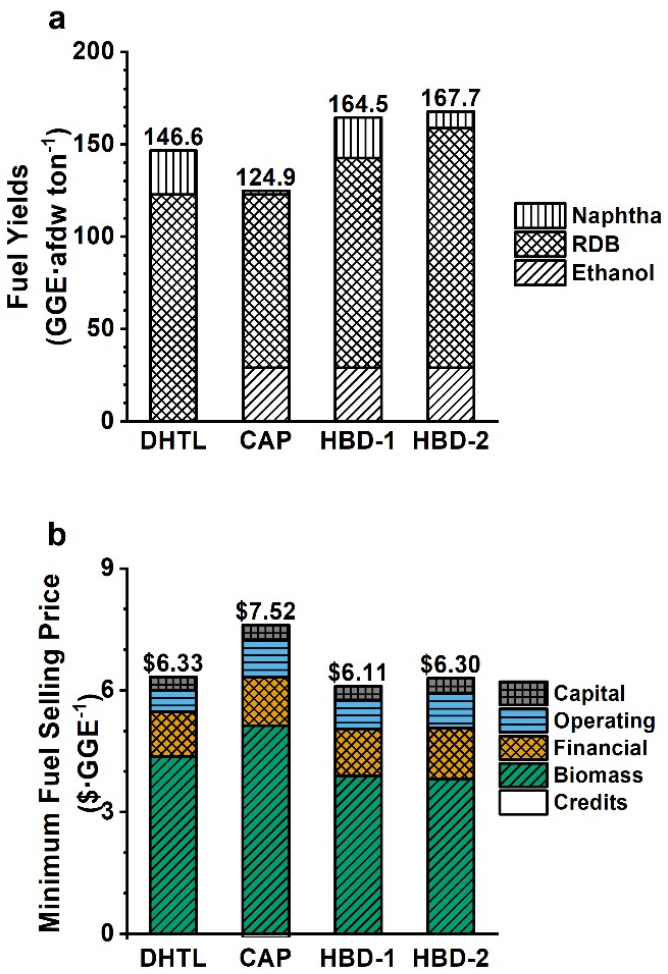
Figure 3. Illustration of system and feedstock flows from 1 afdw ton of harvested *S. acutus* through (a) DHTL, (b) CAP, and two systems that integrate DHTL at different stages of CAP (c)–(d). Quantity of products were tracked and labeled in numbers along the process for the experimentally demonstrated scenario, yields of stillage and extracted stillage were expressed on an afdw basis. Mode 1 (before lipid extraction) and 2 (before AD) in (b) indicated the integration point of DHTL and CAP for the two hybrid systems HBD-1 and HBD-2, respectively. RDB referred to renewable diesel blendstock; Gas 1 was gaseous product from HTL (predominantly CO_2); Gas 2 was gaseous product from catalytic hydrothermal gasification of HTL aqueous product (mainly CH_4 and H_2), Gas 3 was gaseous product from anaerobic digestion (mainly CH_4 and CO_2).[11,14] Detailed product yields with associated uncertainties were provided in Tables S3 and S4 in the SI.

***S. acutus*-demonstrated scenario**

To provide a more systematic and practical comparison, experimental data were used as inputs for TEA to obtain the total fuel yields and calculate the associated minimum selling prices of produced biofuels (**Figure 4** and Table S4 in the SI). Similar to the observations in product yields noted in the previous section, the hybrid systems were expected to produce more fuels from the same *S. acutus* batch than DHTL or CAP alone, with HBD-1 generating 164.5 gasoline gallon equivalent per afdw ton of raw *S. acutus* feed (GGE·ton^{-1}) and HBD-2 generating 167.7 GGE·ton^{-1}, which were 12.2% and 14.4% higher than DHTL's 146.6 GGE·ton^{-1}, respectively (**Figure 4a**). Fuel yields for CAP for this particular *S. acutus* composition were lower with 124.9 GGE·ton^{-1} due to the incomplete hydrolysis of carbohydrates and extraction of lipids (Table S4 in the SI), and the complete diversion of extracted stillage to AD, which accounted for nearly 40% of the feed material but would only generate a CH_4 and CO_2-rich biogas stream instead of liquid fuel products. Notably, HBD-1 and HBD-2's fuel yields were 87.2% and 88.9% of the theoretical 188.6 GGE·ton^{-1} of potential energy in the feed *S. acutus* (estimated by Dulong's equation[17] using elemental composition), which were increased from DHTL's 77.7% and CAP's 66.2%, showing promising improvement for valorization of the *S. acutus* feedstock.

Minimum fuel selling prices (MFSPs) for each system were also calculated (**Figure 4b**) based on feedstock cost (specific to each biochemical composition) from a previous study.[13] HBD-1 had the lowest fuel cost of \$6.11 GGE^{-1}, followed by HBD-2's \$6.30 GGE^{-1}. While DHTL close at \$6.33 GGE^{-1}, CAP's MFSP was comparably higher at \$7.52 GGE^{-1}. Breakdown of the MFSPs showed that for all systems the cost of feedstock biomass remained the largest contribution (>60% of total cost), consistent with previous studies.[11,13,14] As the total feedstock costs per year were the

same for all systems (i.e., the same amount of algal biomass was purchased at the same price for all systems), the differences in $\$\cdot GGE^{-1}$ values reflected the differences in total fuel yields. Hence for CAP with lowest fuel yields, \$5.13 GGE^{-1} out of the total \$7.52 GGE^{-1} was for feedstock acquisition, while only \$3.82 GGE^{-1} out of the total \$6.30 GGE^{-1} was required for HBD-2 with the highest fuel yields. When considering the process-specific costs of operating and capital, the relatively small contribution (<20% of overall MFSPs for all systems) suggested that while hybrid systems included more unit processes that would have associated costs, these costs could be more than offset by the higher total fuel yields obtained by these added processes. Therefore, opportunities existed for the costlier (with regard to total investment and operating expenses) hybrid systems to be more competitive than DHTL or CAP. In fact, the lowest MFSP of HBD-1 showed that though it raised the capital and operating costs by appending more processes, the costs could actually be outweighed by the profits from the added fuel products. Comparing HBD-1 to HBD-2, however, showed that the minor increase in fuel yields from the former (3.2 $GGE\cdot ton^{-1}$) did not offset the additional process costs of this integration strategy. These analyses illustrated the tradeoffs between improved fuel yields and the associated higher capital and operating costs, with the optimal solutions likely dependent on feedstock composition.



352

353 **Figure 4.** Modeled results for *S. acutus*-demonstrated scenario: (a) fuel product yields from 1 afdw
354 ton of raw *S. acutus*, the total yields were labeled at the top of each column; (b) minimum fuel
355 selling prices (MFSPs) breakdown for different systems, the overall MFSPs (in 2011 U.S. dollars,
356 credits included) were labeled at the top of each column; financial costs included taxes and loan
357 payments; excess electricity generated in CAP was sold to the grid and was counted as a credit
358 (i.e., negative in value); biomass costs were separated from other operating costs as they were
359 larger than any other costs. Note that the amount of naphtha produced in CAP in (a) and electricity
360 credit of CAP in (b) were small and thus appeared to be obscure. Details on cost calculation can
361 be found elsewhere.[13]

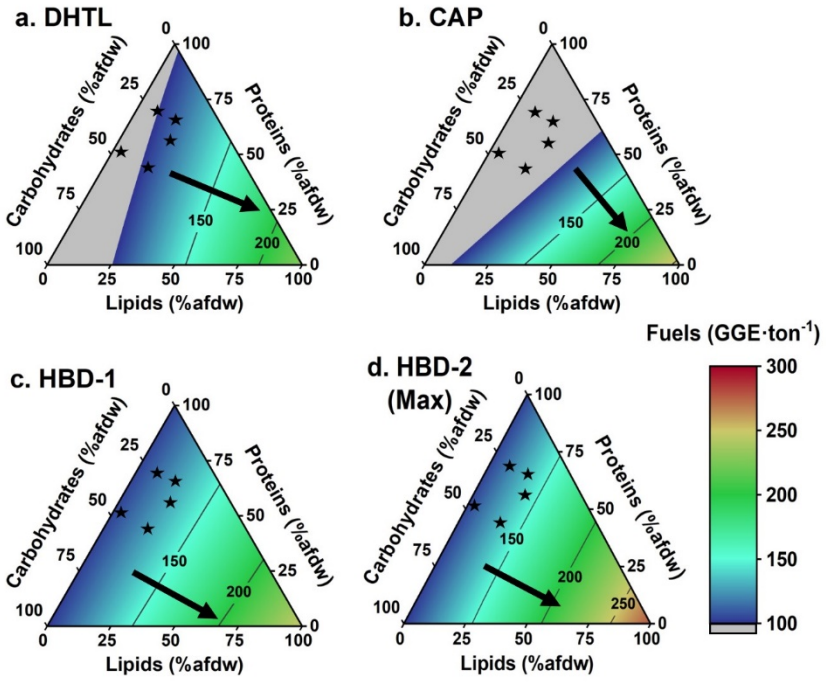
Model-predicted fuel yields

Using TEA models that comprises both cultivation and conversion processes,[13] yields of the fuel products from algae of varying composition were calculated (**Figure 5**). General trends for fuel yields agreed with expectations where the total yields increased with feedstock lipid contents for all systems, and the fuel productivity generally followed the order of HBD-2 > HBD-1 > DHTL and CAP, except for nearly 100%-lipid feedstocks, where CAP generated slightly more fuels than HBD-1. Both DHTL and CAP had gray areas where total fuel yields were considerably compromised due to the inefficient coupling of feedstock and processes: DHTL was unfavorable in high-carbohydrate regions (bottom left corner of the ternary plot) due to the substantial amount energy diverted to gaseous and biochar products (DHTL only yielded 55.6 GGE·ton^{-1} for 100%-carbohydrate feedstock); and high-protein species (top corner of the ternary plot) were greatly penalized in CAP due to the complete diversion of protein to AD (no liquid fuels would be generated for 100%-protein feedstock). Comparing fuel yields of HBD-1 and HBD-2, it was shown that fuel yields of the two systems were very close for low-lipid compositions; but the difference increased when shifting to high-lipid compositions (right side of the ternary plot). While HBD-2 was predicted to yield a maximum of 279.2 GGE·ton^{-1} for 100%-lipid feedstocks, HBD-1 only yields 248.8 GGE·ton^{-1}. Though such an extreme feedstock was out of the practical range of cultivation, this nevertheless suggested that HBD-2, which took a more granular fractionation process into account, was more efficient in fuel production for high-lipid feedstocks. Predictions in **Figure 5** also showed that the proposed hybrid systems could be effective strategies for converting algal biomass from non-traditional sources like wastewater treatment facilities. Based on biochemical compositions reported in literature,[18,22–25] the typical high-protein, high-

carbohydrate, but low-lipid properties of wastewater algae resulted in low fuel yields for both DHTL and CAP, where some or all of the predicted fuel yields were <100 GGE·ton^{-1} (star symbols in **Figure 5**). In comparison, for the hybrid systems, all wastewater algae were predicted to yield >100 GGE·ton^{-1} and even close to 150 GGE·ton^{-1} for HBD-2. This finding supported the development of hybrid systems to process a broader range of algal feedstocks.

When analyzing these systems at the scale of individual unit operations, all above observations could be explained by component-to-fuel conversions of individual processes. Based on the models, HTL-upgrading could convert roughly 65%, 34%, and 16% of the feedstock lipids, proteins, and carbohydrates, respectively, into fuel products, whereas lipids and carbohydrates could be more efficiently converted via lipid extraction-upgrading and fermentation with conversions of 74% and 72%, respectively. Hence, fuel yields for CAP (containing fermentation and lipid extraction-upgrading) were less than DHTL (containing HTL-upgrading) for low-lipid, high-protein feedstocks (left and top corners of the ternary plot), but higher than DHTL for feedstocks with higher lipid contents (right corner of the ternary plot). The hybrid system HBD-1 took advantage of the fermentation process, thus marking a significant improvement in carbohydrate conversion from HTL-upgrading's 16% to 72%; and HBD-2 took a step further by adding the lipid extraction-upgrading step prior to HTL-upgrading to make better use of the lipids. Moreover, as unextracted lipids could still be converted to fuels through HTL-upgrading, the lipid-to-fuel conversion could be further improved from 74% (in lipid extraction-upgrading alone) to 79%. Therefore, the hybrid systems possessed much greater flexibility in terms of valorizing feedstocks of widely varying composition due to the substantial improvement in component-to-fuel conversions.

406 It should be noted that more work is needed to improve the TEA models for more robust
407 prediction of conversion process yields based on properties of the algal feedstock, especially for
408 CAP where fuel yield was over-predicted fuel yields by 16.5%. This deviation stemmed from the
409 lower-than-expected fermentable carbohydrate hydrolysis ratio (73.3% experimental vs. 90%
410 modeled) and fatty acid-to-extractable lipid ratio (84.4% experimental vs. 95% modeled). If
411 experimental values (i.e., 73.3% and 84.4% instead of 90% and 95%, respectively) for these two
412 parameters were used for process modeling, the difference between modeled and experimental
413 values for total fuel yield could be narrowed to 5.4%. Therefore, the models should be calibrated
414 and validated with more experimental results to better guide the development of these aqueous
415 conversion systems. Nonetheless, the current models provided decent predictions for the other
416 three systems (8.4%, 1.2%, and 5.7% deviation for DHTL, HBD-1, and HBD-2, respectively) and
417 critical insights for future optimization.



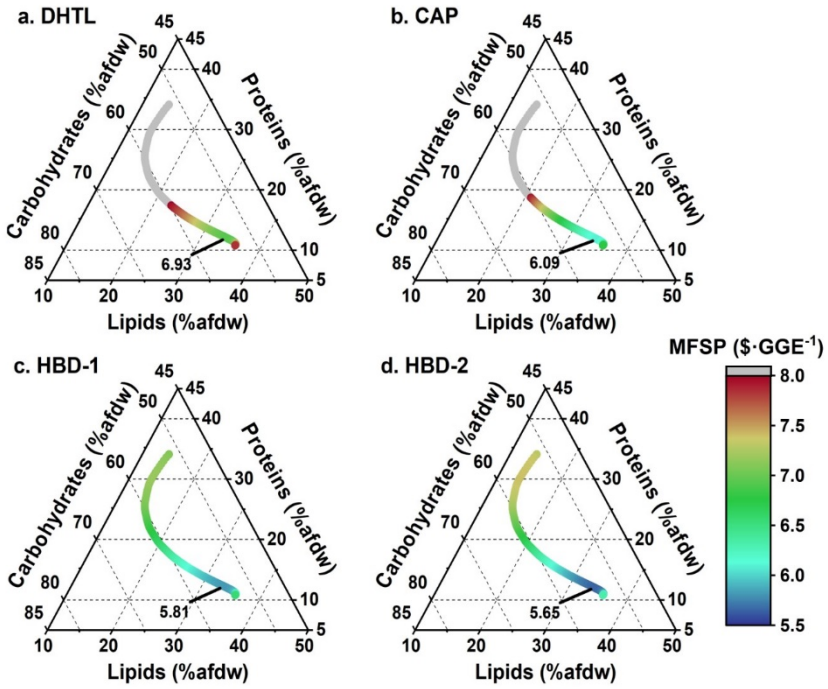
418

Figure 5. Model-predicted fuel yields for 1 afdw ton of algal biomass for (a) DHTL, (b) CAP, (c) HBD-1, and (d) HBD-2; gray areas indicated fuel yields <100 GGE·ton^{-1}. Predictions were not species-specific and included all biochemical compositions. HBD-2 was projected to produce the most fuel products for all compositions. Contour lines for 150, 200, and 250 GGE·ton^{-1} were labeled for easy interpretation; black arrows perpendicular to contour lines indicated directions of increasing fuel yields; black stars indicated biochemical compositions of wastewater algae reported in literature.[18,22–25]

Model-predicted MFSPs

When comparing the MFSPs, cultivation models need to be incorporated to account for changes in costs associated with producing algae of varying compositions. As reported in a previous study,[13] compositions of the *S. acutus* species through the course of cultivation involved steadily decreasing protein content due to nutrient depletion. At the same time, the lipid content kept increasing, while the carbohydrate content initially increased to around 60 afdw% before decreasing. Based on predictions from **Figure 5**, these changes in biochemical compositions led to increases in fuel yields, which could generally be translated to lower MFSPs (**Figure 6**). With costs of algal feedstocks calculated by a previously developed cultivation model,[13] MFSPs were predicted along the growth trajectory of *S. acutus* species, which started from the high-protein composition (top corner) to the high-lipid composition (bottom right corner). Remarkably, MFSPs for both HBD-1 and HBD-2 were lower than those for DHTL and CAP throughout the entire trajectory. This was more evident for feedstocks with lower lipid contents, where DHTL and CAP were predicted to have MFSPs >\$8.0 GGE^{-1}, but MFSPs for both two hybrid systems were similar at \$7.0–7.5 GGE^{-1}. This suggested that for hybrid systems, benefits from the increased fuel yields outweighed the higher capital and operating costs associated with the more complicated systems. For all systems, the lowest MFSP occurred for feedstocks with compositions of ~35 afdw% lipids, ~12 afdw% proteins, and ~53 afdw% carbohydrates. HBD-2 was predicted to be the most

444 economically competitive one with a minimum MFSP of \$5.65 GGE^{-1}, which was followed by
445 HBD-1 with a similar \$5.81 GGE^{-1}, CAP at \$6.09 GGE^{-1} and DHTL at a much higher \$6.93 GGE^{-1}. For feedstocks with >35 afdw% lipids, further increases in lipid contents led to higher MFSPs,
447 which was a result of the increased feedstock costs (due to the longer cultivation time and lower
448 biomass productivity) not compensated by the limited increases in fuel yields (due to the minimal
449 changes in biochemical compositions). When lipid contents of the feedstocks reached the
450 maximum toward the lower right end of the growth trajectory, drastic increases in MFSPs were
451 observed for all systems, as further increase in cultivation time only contributed to higher feedstock
452 costs but not fuel yields. Finally, it should be noted that the differences between maximum and
453 minimum MFSPs for the modeled feedstocks were only \$1.77 GGE^{-1} for HBD-1 and \$1.74 GGE^{-1} for HBD-2, much lower than the variabilities experienced with DHTL (\$2.23 GGE^{-1}) and CAP
455 (\$5.60 GGE^{-1}) alone. The less variable MFSPs would allow more flexible selection of feedstocks
456 and reduce risks related to unexpected changes in supplies of specific feedstocks, further
457 supporting the hybrid systems to be promising strategies for production of algal biofuels.



458

459 **Figure 6.** Model-predicted MFSPs for feedstocks of varying biochemical compositions for (a)
460 DHTL, (b) CAP, (c) HBD-1, and (d) HBD-2. Predictions were made for the *S. acutus* species based
461 on feedstock costs predicted by the cultivation model in a previous study.[13] The drastic increase in
462 MFSPs toward the lower right end for feedstocks with the highest lipid contents was due to
463 substantial increases in feedstock costs with minimal changes in feedstock biochemical
464 compositions and fuel yields. The lowest MFSP for each system was labeled in the respective plot;
465 gray areas indicated MFSP > \$8.0 GGE^{-1}.

466

467 **Implications and future research needs**

468 Microalgae have been identified as a promising feedstock for renewable liquid fuels, and various
469 processes have been promoted for the conversion of algal biomass into biofuels via an aqueous
470 route. However, individual processes are often limited to certain biochemical components of the
471 feedstock while neglecting others, leading to poor valorization of the whole algal biomass.[13] To
472 further increase fuel production potential of algae and provide flexibility for various feedstock

compositions, innovations and optimizations in aqueous conversion system design are needed to improve fuel conversion efficiencies for all feedstock lipid, protein, and carbohydrate components.

In this study, two hybrid systems were proposed to leverage advantages from existing systems and mitigate impacts from their individual weaknesses, guaranteeing moderate- to high-efficiency conversion of feedstocks across the entire biochemical composition regime. This is especially beneficial for early-harvesting species with high protein contents and low lipid and carbohydrate contents, whose biofuels are currently penalized by high MFSPs due to the lower fuel yields. A combination of the fractionation strategy in CAP and whole-cell conversion strategy in DHTL in hybrid systems leads to high conversion of carbohydrates and lipids, while the residual biomass which is inconvertible in CAP can be more efficiently valorized through DHTL. This advantage is particularly pertinent to algal wastewater treatment processes, where the primary goal is to remove aqueous nutrients rather than cultivate the algal biomass to high-lipid content via nutrient depletion. Therefore, the harvested wastewater algae often have high protein but low lipid content due to the lack of a carbon accumulation stage.[26] In some cases, changes in the treatment operations may be required in different seasons to meet regulatory discharge standards, leading to variations in feedstock compositions and a necessity for flexible downstream systems. The adaptability of hybrid systems to feedstocks of varying composition while managing to achieve low MFSPs for optimized feedstocks enables the facilities to acquire biomass from different sources, which can reduce feedstock costs (largest contributor to MFSPs). For example, costs for wastewater algae are expected to be much lower than algae from cultivation farms due to the co-location with wastewater treatment plants, the use of organic carbon and nutrients derived from wastewater purification, and any credits from wastewater treatment.[18,27–29] Additional work is needed to

495 determine costs of algal treatment of wastewater at various scales, and incorporate these costs into
496 the TEA models to evaluate the economic feasibility of such integrated systems. This broader
497 range of feedstocks, along strategies like storing biomass from higher productivity seasons for use
498 in lower productivity seasons can reduce the variability in feedstock flow and increase the size of
499 the facility to take advantage of economies of scale.[11,14]

500 Further, MFSPs of the hybrid systems can be further reduced by streamlining the facilities and
501 processes. For example, for HBD-1, the hydrolysate after ethanol distillation is at an elevated
502 temperature, which can decrease the energy demand for reaching HTL process conditions; for
503 HBD-2, upgrading of the extracted lipids and generated biocrude oils can be combined to save
504 capital investment and labor expenses. Advancement in technologies can also increase fuel yields
505 thus contribute to lower MFSPs (e.g., by reducing evaporation of volatile fractions of biocrude
506 oils[30] and improving lipid extraction efficiency). Admittedly, more research is needed to further
507 improve the protein-to-fuel conversion, which is only 34% for both hybrid systems and much less
508 than that of lipids and carbohydrates (65–79%). This may be achieved by feedstock pretreatment
509 (e.g., sequential HTL[31]) to pre-extract proteins, which can be biologically converted to biofuels at
510 higher conversions.[32] Moreover, higher valuable products (e.g., succinic acid from fermentation
511 of hydrolyzed carbohydrates[33]) and non-fuel co-products (e.g., protein feed[34]) can also be included
512 to boost system economics.

513 The hybrid systems also have the potential to further reduce the environmental impacts of algal
514 biofuels. For example, by enabling the use of wastewater algae as a potential feedstock source,
515 algae biomass can be cultivated with less nutrient inputs, which has been shown to be a main
516 contributor to several life cycle environmental impact categories (e.g., climate change, non-

renewable resources).[35,36] However, it should be noted that though substitution of AD with HTL is found to improve fuel productivity, recycling of nutrients (e.g., N and P) from HTL products may involve more steps[18,37] than required from AD aqueous product, and more work remained in this area to study whether the hybrid systems is able to meet the sustainability targets for algal biofuels. Particularly, life cycle assessment (LCA) of these systems should be conducted to assess the environmental impacts of the proposed hybrid systems. Calculation of key indicators like energy return on investment will also be valuable contribution to assess energy efficiency of the hybrid systems and compare them to traditional fossil fuels and/or other renewable fuels. Further, as LCA may favor different systems than TEA (e.g., one system may have larger environmental impact but lower MFSP), results from both LCA and TEA should be considered for more comprehensive conclusions.

Overall, as demonstrated by experiments and evaluated by TEA models, the proposed hybrid systems HBD-1 and HBD-2 can produce more algal biofuels at lower cost than individual systems DHTL and CAP. These results shed light upon industrially viable and economically feasible solutions for biofuels comparable with traditional energy sources, therefore reducing the reliability on fossil fuels and alleviating associated environmental impacts.

Associated Content

Supporting Information.

The following files are available free of charge: Experimental and modeling method details and supplementary data on algae properties and process yields (PDF).

Author Information

Corresponding Author

Timothy J. Strathmann, E-mail: strthmnn@mines.edu, Phone: +1 (303) 384-2226

ORCID

Yalin Li: 0000-0002-8863-4758

Shijie Leow: 0000-0002-9276-0618

Tao Dong: 0000-0002-4961-7576

Nicholas J. Nagle: 0000-0002-7667-8622

Eric P. Knoshaug: 0000-0002-5709-914X

Lieve M. L. Laurens: 0000-0003-4930-3267

Philip T. Pienkos: 0000-0002-6284-7392

Jeremy S. Guest: 0000-0003-2489-2579

Timothy J. Strathmann: 0000-0002-7299-3115

Notes

The authors declare no competing financial interest.

Acknowledgements

Financial support for work carried out at CSM and UIUC was provided by National Science Foundation (NSF) through the NSF Engineering Research Center for Reinventing the Nation's Urban Water Infrastructure (ReNUWIt; EEC-1028968) and NSF awards CBET-1555549 and CBET-1438667. Work at NREL was supported by the U.S. Department of Energy under Contract No. DE-AC36-08-GO28308 with the National Renewable Energy Laboratory as part of the BioEnergy Technology Office (BETO) task # 1.3.4.201. S. Leow is supported by the National Research Foundation (NRF) Singapore under its NRF Environmental and Water Technologies (EWT) PhD Scholarship Programme and administered by the Environment and Water Industry Programme Office (EWI). Dr. J. McGowen at the Arizona State University is acknowledged for providing algal biomass samples; Anna Fedders (UIUC, CEE), John Scott and Susan Barta (UIUC, ISTC) are acknowledged for providing analytical support.

References

(1) Williams, P. J. le B.; Laurens, L. M. L. Microalgae as Biodiesel & Biomass Feedstocks: Review & Analysis of the Biochemistry, Energetics & Economics. *Energy Environ. Sci.* **2010**, *3* (5), 554–590. https://doi.org/10.1039/B924978H.

(2) Mata, T. M.; Martins, A. A.; Caetano, N. S. Microalgae for Biodiesel Production and Other Applications: A Review. *Renew. Sustain. Energy Rev.* **2010**, *14* (1), 217–232. https://doi.org/10.1016/j.rser.2009.07.020.

(3) Moody, J. W.; McGinty, C. M.; Quinn, J. C. Global Evaluation of Biofuel Potential from Microalgae. *Proc. Natl. Acad. Sci.* **2014**, *111* (23), 8691–8696. https://doi.org/10.1073/pnas.1321652111.

(4) Zhao, B.; Ma, J.; Zhao, Q.; Laurens, L.; Jarvis, E.; Chen, S.; Frear, C. Efficient Anaerobic Digestion of Whole Microalgae and Lipid-Extracted Microalgae Residues for Methane Energy Production. *Bioresour. Technol.* **2014**, *161*, 423–430. https://doi.org/10.1016/j.biortech.2014.03.079.

(5) Laurens, L. M. L.; Nagle, N.; Davis, R.; Sweeney, N.; Wychen, S. V.; Lowell, A.; Pienkos, P. T. Acid-Catalyzed Algal Biomass Pretreatment for Integrated Lipid and Carbohydrate-Based Biofuels Production. *Green Chem.* **2015**, *17* (2), 1145–1158. https://doi.org/10.1039/C4GC01612B.

(6) Dong, T.; Knoshaug, E. P.; Davis, R.; Laurens, L. M. L.; Van Wychen, S.; Pienkos, P. T.; Nagle, N. Combined Algal Processing: A Novel Integrated Biorefinery Process to Produce Algal Biofuels and Bioproducts. *Algal Res.* **2016**, *19*, 316–323. https://doi.org/10.1016/j.algal.2015.12.021.

(7) Davis, R.; Fishman, D.; Frank, E. D.; Wigmosta, M. S.; Aden, A.; Coleman, A. M.; Pienkos, P. T.; Skaggs, R. J.; Venteris, E. R.; Wang, M. Q. *Renewable Diesel from Algal Lipids: An Integrated Baseline for Cost, Emissions, and Resource Potential from a Harmonized Model*; 2012.

(8) Peterson, A. A.; Vogel, F.; Lachance, R. P.; Fröling, M.; Antal Jr., M. J.; Tester, J. W. Thermochemical Biofuel Production in Hydrothermal Media: A Review of Sub- and Supercritical Water Technologies. *Energy Environ. Sci.* **2008**, *1* (1), 32–65. https://doi.org/10.1039/b810100k.

(9) Kumar, G.; Shobana, S.; Chen, W.-H.; Bach, Q.-V.; Kim, S.-H.; Atabani, A. E.; Chang, J.-S. A Review of Thermochemical Conversion of Microalgal Biomass for Biofuels: Chemistry and Processes. *Green Chem.* **2017**, *19* (1), 44–67. https://doi.org/10.1039/C6GC01937D.

(10) Kruger, J. S.; Christensen, E. D.; Dong, T.; Van Wychen, S.; Fioroni, G. M.; Pienkos, P. T.; McCormick, R. L. Bleaching and Hydroprocessing of Algal Biomass-Derived Lipids to Produce Renewable Diesel Fuel. *Energy Fuels* **2017**, *31* (10), 10946–10953. https://doi.org/10.1021/acs.energyfuels.7b01867.

(11) Davis, R.; Kinchin, C.; Markham, J.; Tan, E.; Laurens, L. M. L.; Sexton, D.; Knorr, D.; Schoen, P.; Lukas, J. *Process Design and Economics for the Conversion of Algal Biomass to Biofuels: Algal Biomass Fractionation to Lipid- and Carbohydrate-Derived Fuel Products*; NREL/TP-5100-62368; National Renewable Energy Laboratory (NREL), Golden, CO., 2014.

(12) Posewitz, M. C. Algal Oil Productivity Gets a Fat Bonus. *Nat. Biotechnol.* **2017**, *35* (7), 636–638. https://doi.org/10.1038/nbt.3920.

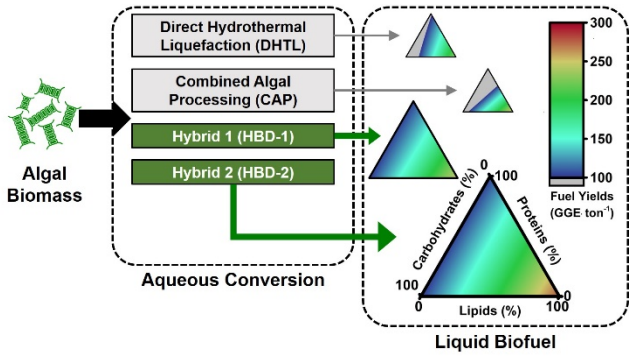
(13) Leow, S.; Shoener, B. D.; Li, Y.; DeBellis, J. L.; Markham, J.; Davis, R.; Laurens, L. M. L.; Pienkos, P. T.; Cook, S. M.; Strathmann, T. J.; Guest, J. S. A Unified Modeling Framework to Advance Biofuel Production from Microalgae. *Environ. Sci. Technol.* **2018**, *52* (22), 13591–13599. https://doi.org/10.1021/acs.est.8b03663.

(14) Jones, S. B.; Zhu, Y.; Anderson, D. B.; Hallen, R. T.; Elliott, D. C.; Schmidt, A. J.; Albrecht, K. O.; Hart, T. R.; Butcher, M. G.; Drennan, C.; Snowden-Swan L.; Davis R.; Kinchin C. *Process Design and Economics for the Conversion of Algal Biomass to Hydrocarbons: Whole Algae Hydrothermal Liquefaction and Upgrading*; PNNL-23227; Pacific Northwest National Laboratory (PNNL), Richland, WA (US), 2014.

(15) Elliott, D. C.; Hart, T. R.; Schmidt, A. J.; Neuenschwander, G. G.; Rotness, L. J.; Olarte, M. V.; Zacher, A. H.; Albrecht, K. O.; Hallen, R. T.; Holladay, J. E. Process Development for Hydrothermal Liquefaction of Algae Feedstocks in a Continuous-Flow Reactor. *Algal Res.* **2013**, *2* (4), 445–454. https://doi.org/10.1016/j.algal.2013.08.005.
(16) Leow, S.; Witter, J. R.; Vardon, D. R.; Sharma, B. K.; Guest, J. S.; Strathmann, T. J. Prediction of Microalgae Hydrothermal Liquefaction Products from Feedstock Biochemical Composition. *Green Chem* **2015**, *17* (6), 3584–3599. https://doi.org/10.1039/C5GC00574D.
(17) Li, Y.; Leow, S.; Fedders, A. C.; Sharma, B. K.; Guest, J. S.; Strathmann, T. J. Quantitative Multiphase Model for Hydrothermal Liquefaction of Algal Biomass. *Green Chem.* **2017**, *19* (4), 1163–1174. https://doi.org/10.1039/C6GC03294J.
(18) Li, Y.; Tarpeh, W. A.; Nelson, K. L.; Strathmann, T. J. Quantitative Evaluation of an Integrated System for Valorization of Wastewater Algae as Bio-Oil, Fuel Gas, and Fertilizer Products. *Environ. Sci. Technol.* **2018**, *52* (21), 12717–12727. https://doi.org/10.1021/acs.est.8b04035.
(19) Laurens, L. M. L.; Van Wychen, S.; McAllister, J. P.; Arrowsmith, S.; Dempster, T. A.; McGowen, J.; Pienkos, P. T. Strain, Biochemistry, and Cultivation-Dependent Measurement Variability of Algal Biomass Composition. *Anal. Biochem.* **2014**, *452*, 86–95. https://doi.org/10.1016/j.ab.2014.02.009.
(20) Kocsisová, T.; Juhasz, J.; Cvengroš, J. Hydrolysis of Fatty Acid Esters in Subcritical Water. *Eur. J. Lipid Sci. Technol.* **2006**, *108* (8), 652–658. https://doi.org/10.1002/ejlt.200600061.
(21) Shin, H.-Y.; Ryu, J.-H.; Park, S.-Y.; Bae, S.-Y. Thermal Stability of Fatty Acids in Subcritical Water. *J. Anal. Appl. Pyrolysis* **2012**, *98*, 250–253. https://doi.org/10.1016/j.jaap.2012.08.003.
(22) Zhou, Y.; Schideman, L.; Yu, G.; Zhang, Y. A Synergistic Combination of Algal Wastewater Treatment and Hydrothermal Biofuel Production Maximized by Nutrient and Carbon Recycling. *Energy Environ. Sci.* **2013**, *6* (12), 3765–3779. https://doi.org/10.1039/C3EE24241B.
(23) Chen, W.-T.; Zhang, Y.; Zhang, J.; Yu, G.; Schideman, L. C.; Zhang, P.; Minarick, M. Hydrothermal Liquefaction of Mixed-Culture Algal Biomass from Wastewater Treatment System into Bio-Crude Oil. *Bioresour. Technol.* **2014**, *152*, 130–139. https://doi.org/10.1016/j.biortech.2013.10.111.
(24) Mehrabadi, A.; Craggs, R.; Farid, M. M. Wastewater Treatment High Rate Algal Pond Biomass for Bio-Crude Oil Production. *Bioresour. Technol.* **2017**, *224*, 255–264. https://doi.org/10.1016/j.biortech.2016.10.082.
(25) Cheng, F.; Cui, Z.; Mallick, K.; Nirmalakhandan, N.; Brewer, C. E. Hydrothermal Liquefaction of High- and Low-Lipid Algae: Mass and Energy Balances. *Bioresour. Technol.* **2018**, *258*, 158–167. https://doi.org/10.1016/j.biortech.2018.02.100.
(26) Pittman, J. K.; Dean, A. P.; Osundeko, O. The Potential of Sustainable Algal Biofuel Production Using Wastewater Resources. *Bioresour. Technol.* **2011**, *102* (1), 17–25. https://doi.org/10.1016/j.biortech.2010.06.035.
(27) Cabanelas, I. T. D.; Ruiz, J.; Arbib, Z.; Chinalia, F. A.; Garrido-Pérez, C.; Rogalla, F.; Nascimento, I. A.; Perales, J. A. Comparing the Use of Different Domestic Wastewaters for Coupling Microalgal Production and Nutrient Removal. *Bioresour. Technol.* **2013**, *131* (Supplement C), 429–436. https://doi.org/10.1016/j.biortech.2012.12.152.
(28) Chiu, S.-Y.; Kao, C.-Y.; Chen, T.-Y.; Chang, Y.-B.; Kuo, C.-M.; Lin, C.-S. Cultivation of Microalgal Chlorella for Biomass and Lipid Production Using Wastewater as Nutrient Resource. *Bioresour. Technol.* **2015**, *184* (Supplement C), 179–189. https://doi.org/10.1016/j.biortech.2014.11.080.
(29) Xin, C.; Addy, M. M.; Zhao, J.; Cheng, Y.; Cheng, S.; Mu, D.; Liu, Y.; Ding, R.; Chen, P.; Ruan, R. Comprehensive Techno-Economic Analysis of Wastewater-Based Algal Biofuel Production: A Case Study. *Bioresour. Technol.* **2016**, *211*, 584–593. https://doi.org/10.1016/j.biortech.2016.03.102.
(30) Dong, T.; Xiong, W.; Yu, J.; Pienkos, P. T. Co-Production of Fully Renewable Medium Chain α-Olefins and Bio-Oil via Hydrothermal Liquefaction of Biomass Containing Polyhydroxyalkanoic Acid. *RSC Adv.* **2018**, *8* (60), 34380–34387. https://doi.org/10.1039/C8RA07359G.

(31) Miao, C.; Chakraborty, M.; Dong, T.; Yu, X.; Chi, Z.; Chen, S. Sequential Hydrothermal Fractionation of Yeast Cryptococcus Curvatus Biomass. *Bioresour. Technol.* **2014**, *164*, 106–112. https://doi.org/10.1016/j.biortech.2014.04.059.
(32) Huo, Y.-X.; Cho, K. M.; Rivera, J. G. L.; Monte, E.; Shen, C. R.; Yan, Y.; Liao, J. C. Conversion of Proteins into Biofuels by Engineering Nitrogen Flux. *Nat. Biotechnol.* **2011**, *29* (4), 346–351. https://doi.org/10.1038/nbt.1789.
(33) Knoshaug, E. P.; Mohagheghi, A.; Nagle, N. J.; Stickel, J. J.; Dong, T.; Karp, E. M.; Kruger, J. S.; Brandner, D. G.; Manker, L. P.; Rorrer, N. A.; Hyman, D. A.; Christensen, E. D.; Pienkos, P. T. Demonstration of Parallel Algal Processing: Production of Renewable Diesel Blendstock and a High-Value Chemical Intermediate. *Green Chem.* **2018**, *20* (2), 457–468. https://doi.org/10.1039/C7GC02295F.
(34) Cruce, J. R.; Quinn, J. C. Economic Viability of Multiple Algal Biorefining Pathways and the Impact of Public Policies. *Appl. Energy* **2019**, *233–234*, 735–746. https://doi.org/10.1016/j.apenergy.2018.10.046.
(35) Beal, C. M.; Gerber, L. N.; Sills, D. L.; Huntley, M. E.; Machesky, S. C.; Walsh, M. J.; Tester, J. W.; Archibald, I.; Granados, J.; Greene, C. H. Algal Biofuel Production for Fuels and Feed in a 100-Ha Facility: A Comprehensive Techno-Economic Analysis and Life Cycle Assessment. *Algal Res.* **2015**, *10*, 266–279. https://doi.org/10.1016/j.algal.2015.04.017.
(36) Tu, Q.; Eckelman, M.; Zimmerman, J. B. Harmonized Algal Biofuel Life Cycle Assessment Studies Enable Direct Process Train Comparison. *Appl. Energy* **2018**, *224*, 494–509. https://doi.org/10.1016/j.apenergy.2018.04.066.
(37) Edmundson, S.; Huesemann, M.; Kruk, R.; Lemmon, T.; Billing, J.; Schmidt, A.; Anderson, D. Phosphorus and Nitrogen Recycle Following Algal Bio-Crude Production via Continuous Hydrothermal Liquefaction. *Algal Res.* **2017**, *26*, 415–421. https://doi.org/10.1016/j.algal.2017.07.016.

683 **For Table of Contents Use Only**



684
685 **Synopsis**

686 Experiments and model predictions show increased algal biofuel yields at lower prices via
687 proposed hybrid conversion systems.