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Water Impact Significance

Wastewater is attracting growing attention as a potential source for valuable products. This study describes an integrated system where microalgae is used to treat primary clarified municipal wastewater in different seasons, and the harvested algal biomass is converted to biofuels and fertilizers via hydrothermal processing. Economic analysis of conversion processes is performed and shows promising results of such systems.

Seasonal Treatment and Economic Evaluation of an Algal Wastewater System for Energy and Nutrient Recovery

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Abstract

Algal systems have been proposed for treating wastewater while simultaneously recovering energy and nutrients. In this study, an integrated system with algal treatment of municipal wastewater followed by hydrothermal liquefaction (HTL) conversion and upgrading steps was evaluated. Pilot-scale treatment of primary clarified municipal wastewater effluent was evaluated in different seasons (cold, warm, and a transitional period in between) with different strains of algae selected for each season, and the warm season strain successfully met all local discharge regulations. The collected wastewater algae biomass was subjected to HTL at 300 and 350°C and both energy and nutrient recoveries were much improved at the higher temperature. The transitional batch was found to have the highest biocrude oil yields, and its co-products had the highest nutrient (nitrogen and phosphorus) contents. Economic analysis of conversion processes informed by the observed

HTL product yields was conducted. While this revealed that targeting biofuel products alone was not profitable, adding nutrient co-products (e.g., ammonium sulfate fertilizer), adjusting algae harvesting time, and incorporating component-specific conversion processes could substantially improve system economics. Overall, this study highlights connections between treatment and conversion processes, and demonstrates how these connections can be leveraged for more efficient resource recovery without compromising treatment operations.

1 Introduction

Growing demands for energy, food, and water are placing new stresses on society, and municipal wastewater is attracting increasing interest as a potential resource that can be exploited to help meet these needs.[1–3] However, conventional wastewater treatment plants use energy-intensive treatment strategies (e.g., aeration-based heterotrophic biological treatment) that focus on meeting discharge requirements through simple removal or down-cycling.[4] For example, secondary treatment via activated sludge processes converts nearly 50% of the dissolved organic carbon to CO_2, nitrification-denitrification operations aim to remove nitrogenous constituents as N_2 gas, and chemical precipitation sequester phosphorus as poorly bioavailable solids–all terminal products with limited market value.[4,5]

As an alternative to dissimilative bacterial treatment processes, algae can purify wastewater by metabolically assimilating both organic carbon and nutrients present in wastewater, and the resulting biomass that accumulates can be harvested, concentrated, and processed with biorefinery technologies to generate valuable products, including fuels and fertilizers.[6] Revenues from these product streams can potentially reduce the cost of wastewater treatment or even flip the economic balance. Toward this end, several recent studies have explored the economic feasibility of large-scale biorefineries featuring a central hydrothermal liquefaction (HTL) conversion process

followed by a suite of upgrading steps for algal fuels.[7–9] It is concluded that the cost for algae production accounts for >50% of the overall cost,[7–9] which is expected to be lower for wastewater-algae given the co-location with wastewater treatment plant and the reduction in carbon and nutrient substrates.[10] However, existing studies that investigated the economic feasibility of wastewater-derived algal biofuels predominantly used product yields reported for freshwater-cultivated algae or other biomass.[11–13] As conversion yields and characteristics of the HTL products – which can vary significantly for different algal biomass[14] – have great influence on the overall economic performance of the system,[7–9] and wastewater algae are reported to have different properties from algae cultivated in other media (e.g., freshwater, saltwater).[15,16] This will result in over-estimation of total biofuel yields and more optimistic economic performance. Further, as nutrient co-products (e.g., fertilizers) are needed for algal fuels to be economically competitive,[7,8,17] it is important to examine potential revenues from these co-products, which have been largely overlooked in previous studies. Finally, as wastewater algae produced from different treatment operations (e.g., cold versus warm seasons, different treatment times) can have distinguishable properties and lead to variations in valuable product yields and system economics,[4,18] it is critical to study algae treatment and conversion systems as a whole to understand and leverage the dynamic interactions between treatment and conversion processes for more robust system performance.

In this study, a pilot-scale algal system was experimentally evaluated for treatment of primary municipal wastewater. The treatment experiments were conducted in different seasons (cold, warm, and a transitional period in between) with algal strains adapted for each season. The harvested algal biomass was then subjected to HTL conversion. Experimental yields and characteristics of the HTL products were used to inform model predictions of energy and nutrient

recoveries via aqueous conversion processes that were recently demonstrated with wastewater algal feedstocks.[9] Finally, economic analysis of conversion processes was conducted with sensitivity and uncertainty analyses and evaluated under different scenarios to assess economic feasibility of the system and identify future research priorities.

2 Materials and Methods

2.1 Pilot-scale algal wastewater treatment

Treatment experiments were conducted with primary effluent from the Jacob A. Hands Wastewater Treatment Plant (Las Cruces, NM, USA). Local discharge standards at the treatment facility are 30 mg $O_2 \cdot L^{-1}$, 10 mg $N \cdot L^{-1}$, and 1 mg$\cdot L^{-1}$, for 5-day biochemical oxygen demand (BOD_5), NH_4^+-N, and PO_4^{3-}, respectively. A mixotrophic algal species, *Galdieria sulphuraria,* was selected due to its versatile metabolism that could leverage both CO_2 and organic substrates as the carbon source,[19] thus realizing the removal of organic carbon and nutrients in a single stage.[20] To evaluate treatment efficiency and energy and nutrient recovery potentials of the harvested algae during different seasons, two strains of *G. sulphuraria* – Soos and CCMEE 5587.1 – were selected for cold (mid-December to March) and warm (May to September) seasons, respectively. Soos was originally isolated from a diatomite shield site in the National Nature Reserve Soos, Czech Republic[21] and CCMEE 5587.1 was identified by the Culture Collection of Microorganisms from Extreme Environments at the University of Oregon.[5] Soos was chosen for its ability to maintain comparable properties (e.g., ultrastructure, fatty acid composition, thermostability of enzymes) at much lower temperatures than other *G. sulphuraria* strains with similar substrates;[21] and CCMEE 5587.1 was selected as previous studies had reported promising organic carbon and nutrient removal rates at pilot scale.[5]

94 *G. sulphuraria* was grown in three 700 L parallel pilot-scale photobioreactors in a raceway
95 configuration. The photobioreactors were enclosed in translucent polyethylene growth bags to
96 minimize evaporation and keep culture temperature at much higher levels than ambient air. The
97 photobioreactors were operated with natural photoperiod and light intensity with headspace filled
98 with 2% CO_2-enriched air. Each treatment experiment was initiated with 400 L of wastewater and
99 300 L of cultures (preadapted with wastewater for 5 days prior to experiments). Four batches of *G.*
100 *sulphuraria* biomass were harvested from different treatment experiments and subjected to HTL
101 reactions: one composed of cold strain Soos generated under batch operating conditions (referred
102 to as Cold-B, culture temperature during treatment at 13–37°C, a summary of treatment conditions
103 is provided in Table S1 in the Electronic supplementary information, ESI); one composed of warm
104 strain CCMEE 5587.1 generated under batch operating conditions (referred to as Warm-B, culture
105 temperature during treatment at 31–46°C); one composed of a polyculture generated during the
106 transitional period from CCMEE 5587.1 to Soos under batch operating conditions (referred to as
107 Trans-B, culture temperature during treatment at 10–27°C, made from 1:1 volumetric ratio mixture
108 of Cold-B and Warm-B seed culture of similar biomass densities); and the last sample consisted
109 CCMEE 5587.1 collected during warm season under fed-batch operating conditions (referred to
110 as Warm-FB, culture temperature during treatment at 27–42°C). Under batch operation, all pilot
111 treatment tests were terminated after 10 days; under fed-batch operation, upon meeting all
112 discharge standards, 400 L of the algal-treated wastewater in each reactor was discharged (and
113 biomass solids collected) and the reactors were replenished with fresh primary effluent to start a
114 new treatment cycle. Depending on the influent contaminant levels, each fed-batch cycle took 2–
115 3 days to reach all discharge standards, and the fed-batch test lasted 5 cycles over a period of 20
116 days. BOD_5, NH_4^+-N, and PO_4^{3-} were monitored according to a previous study.[5] BOD_5 was

117 measured in duplicate, and NH_4^+-N and PO_4^{3-} were measured in triplicate. For purposes of
118 experimental expediency, all harvested biomass samples were freeze-dried, ground to powder, and
119 preserved at 4°C for subsequent analyses or use in HTL experiments.

120 **2.2 Characterization of wastewater algal biomass**

121 Biochemical and elemental compositions of the harvested biomass were analyzed following
122 procedures described previously.[14] Briefly, moisture contents were determined by mass loss at
123 105°C. Ash contents were determined by remaining mass after ignition at 550°C. Elemental
124 carbon, hydrogen, and nitrogen contents were analyzed using an Exeter CE-440 Elemental
125 Analyzer (University of Illinois Microanalysis Laboratory, Urbana, IL). Phosphorus contents were
126 measured by ICP-AES (PerkinElmer 5300DV) following acid digestion according to EPA method
127 3052.[22] Volatile oxygen contents were estimated by difference (100 - C% - H% - N% - P% - Ash%,
128 all on dry weight basis, dw%). Gross lipid contents were estimated by solvent extraction with a
129 2:1 (v/v) chloroform:methanol mixture. Protein contents were calculated from nitrogen contents
130 using a nitrogen-to-protein conversion factor of 6.25;[23] carbohydrate contents were measured by a
131 colorimetric assay using 3-methyl-2-benzothiazolinone hydrazone. All characterizations were
132 conducted in duplicate (moisture and ash contents were measured in triplicate).

133 **2.3 HTL experiments and product analyses**

134 HTL experiments were conducted in tube reactors with dimension of 3" × 1/2" (L × OD, wall
135 thickness was 0.049") and volume of 6.24 mL. Algal slurries of 20 dw% were prepared with
136 freeze-dried algae and deionized (DI) water, and 4.2 g and 3.0 g of slurries were added to reactors
137 for experiments conducted at 300 and 350°C, respectively (to account for water density differences
138 at the two conditions[24]). Reactors were placed into a preheated kiln (Paragon Sentry 2.0) at the

designated temperature for 60 min. Tubes were then placed in room-temperature water to quench further reactions. Detailed protocols used for separation and recovery of HTL products are described elsewhere.[14] Briefly, the reactor was opened to vent gaseous products, and the gas yield was determined by weighing the reactor before and after venting. Contents of the reactor were then poured into a beaker, and the reactor was rinsed sequentially with dichloromethane (DCM) and DI water to recover the biocrude and aqueous residuals, respectively. The resulting mixture was then filtered (0.45 μm PTFE) to separate the solid biochar product, the yield of which was determined by the mass difference of dried filters before and after filtration. Biocrude oil (in DCM) and aqueous products in the filtrate were then separated by a separatory funnel and dried to determine the biocrude and aqueous total dissolved solids (TDS) yields.

Carbon, hydrogen, and nitrogen contents of biocrudes and biochars were determined in the same manner as biomass, and volatile oxygen contents of biocrudes were estimated by difference (100 - C% - H% - N% - Ash%, all on weight basis, wt%). Total organic carbon (TOC) of the aqueous products was analyzed by a Shimadzu TOC-LCSH analyzer, and total nitrogen (TN) by a Shimadzu TNM-L analyzer. NO_2^-, NO_3^-, and PO_4^{3-} contents of aqueous products were analyzed by a Thermo Fisher Scientific Dionex 900 system (NO_2^- and NO_3^- contents were below detection limits for all batches). NH_4^+-N was analyzed using a phenate colorimetric method.[25]

2.4 Energy calculations

Higher heating values (HHVs) of algal biomass samples and the HTL biocrude products were calculated from elemental composition using Dulong's equation.[14] For 1 kg of dry algae, feedstock energy was represented by HHV of the algae; energy recovered in biocrude was calculated as the product of biocrude yield (dw%) and biocrude HHV; energy recovery of biocrude was defined as the percentage of feedstock energy recovered in biocrude (dw%); input heating requirements were

162 defined as the energy required to heat 5 kg of algae slurries (containing 1 kg of dry biomass and 4
163 kg of water) from 25°C to the designated temperatures (300 or 350°C), assuming a heat recovery
164 efficiency of 0.5, and combustion energy efficiency of 0.7.[26] Energy content of the biochar
165 products were not considered due to their low organic contents.

166 **2.5 Economic analysis of conversion processes**

167 Experimental yields and characteristics of HTL products obtained from this study were used
168 as inputs for a techno-economic analysis model[7] developed for production of algal biofuels
169 (energy-related processes in **Figure 1**). Unlike previous model, which assumed a zero net present
170 value to calculate the minimum selling price of biofuels, price of biofuel was set at \$2.79 gal^{-1} as
171 commercial gasoline, which is the average price for gasoline for the past 10 years (2010–2019)
172 reported by U.S. Energy Information Administration (EIA).[27] This adjustment would allow
173 comparison of revenues between biofuels and potential nutrient co-products (revenues from the
174 latter not included in the original model[7], but were calculated separately in this study) under
175 different scenarios. Only conversion of algal biomass was included (i.e., neither costs nor credits
176 associated with the treatment processes were considered) due to lack of well-established large-
177 scale studies on economics of algal wastewater treatment processes. Production of wastewater
178 algae was set to 25.6 U.S. ton per day on dry weight basis (TPD) based on a recent study, which
179 equaled the amount of algae biomass generation expected from a 15-million-gallon-per-day (MGD)
180 wastewater treatment plant.[28] Harvested wastewater algae was sent to a nearby algal conversion
181 plant (i.e., no transportation cost was considered) with a processing rate of 576 TPD.[7] It should be
182 noted that because of the economy of scale, it is unlikely for a conversion plant to be operated at
183 a size that can be supported by a single algal wastewater treatment plant,[7,28] therefore size of the
184 conversion plant was set based on recent studies on algal biofuel production.[7,29] The conversion

185 plant was assumed to use algal biomass feeds from other sources (e.g., algal farms) to compensate
186 the differences, which were assumed to have a biochemical composition of 32.1% lipids, 14.3%
187 proteins, 53.6% carbohydrates (on ash-free dry weight basis, averaged composition of *Chlorella*
188 species modeled in the previous study on algal biofuel).[7] All costs were calculated using
189 composition of the mixed algae stream (weighted average of *Chlorella* and wastewater algae from
190 this study), but revenues were calculated from biofuel yields based on experimental results for
191 wastewater algae reported in this study. In the conversion plant, the algal biomass was first
192 processed via HTL to generate biocrude, aqueous, gaseous, and biochar products. Then the HTL
193 biocrude was further upgraded to liquid hydrocarbon fuels in the gasoline and diesel range via
194 established refinery hydrotreating and hydrocracking operations (**Figure 1**). Residual organic
195 carbon in the aqueous product phase were converted to fuel gas via catalytic hydrothermal
196 gasification (CHG). The generated liquid biofuels were sold for revenues while fuel gases
197 generated in hydrotreating and CHG (rich in H_2 and CH_4) were used internally (e.g., for production
198 of H_2 for hydrotreating).[30,31]

199 The total cost included capital, operating, and financial costs. All costs were first calculated
200 for the entire conversion plant at the size of 576 TPD, then prorated to the 25.6 TPD of the assumed
201 wastewater algae inputs. Capital costs included costs of equipment (including installation),
202 warehouse buildings, site development, additional piping, and other indirect costs (e.g., project
203 contingency, working capital). Total capital costs (excluding working capital, which would be paid
204 back at the end of operation) were calculated and annualized to each year as capital depreciation;
205 operating costs included labor, maintenance, insurances and taxes, catalysts, utilities (water,
206 natural gas, electricity); financial costs included income tax (35%) and loan payments based on a
207 40% equity (i.e., 40% of the capital investment was provided by shareholders and 60% from loan),

8% loan interest rate for 10 years, and a 10% internal rate of return (IRR, determines the present value of future cashflows). No cost or credit were considered for the algal biomass, which would be generated from the wastewater treatment process. All prices were converted to 2014 U.S. dollars using GDP chain-type price index reported by U.S. EIA.[27] Detailed breakdown of the costs and calculates are provided in the ESI. To evaluate impacts from key assumptions, sensitivity and uncertain analyses were conducted for biofuel price, plant scale, total capital cost, total operating cost, project equity, and IRR. The range for biofuel price was determined based on the minimum ($2.00 gal^{-1}) and maximum ($3.59 gal^{-1}) gasoline prices reported by U.S. EIA between 2010–2019;[27] project equity was evaluated for a minimum of 0% (all capital investment from loan) and a maximum of 100% (all capital investment from shareholders); IRR was evaluated for a minimum of 0% and a maximum of 20%; plant scale, total capital cost, and total operating cost were evaluated for a minimum of 90% and a maximum of 110% of the baseline values.

Based on a previous study,[9] ammonium in CHG aqueous product could be recovered as ammonium sulfate fertilizer following electrochemical stripping, and phosphorus in HTL biochar product could be extracted and combined with a portion of HTL aqueous product for the production of magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$, MAP or struvite) fertilizer (nutrient-related processes in **Figure 1**). Due to the lack of well-established large-scale studies on electrochemical stripping and struvite production by similar approaches, capital and operating costs for these two processes were not available, but revenues from the nutrient co-products were calculated based on properties of HTL products generated in this study and HTL product upgrading efficiencies reported previously.[9] As biochemical composition of algae changes during the treatment, additional analyses were conducted to understand the implication of different harvesting time. Biochemical composition of algae at their peak storage levels (i.e., highest levels of lipid and

carbohydrate contents) were calculated based on changes of lipid:protein and carbohydrate:protein ratios during their growth to represent the maximum lipid and carbohydrate contents (ash contents on dry weight basis assumed to be the same).[18]

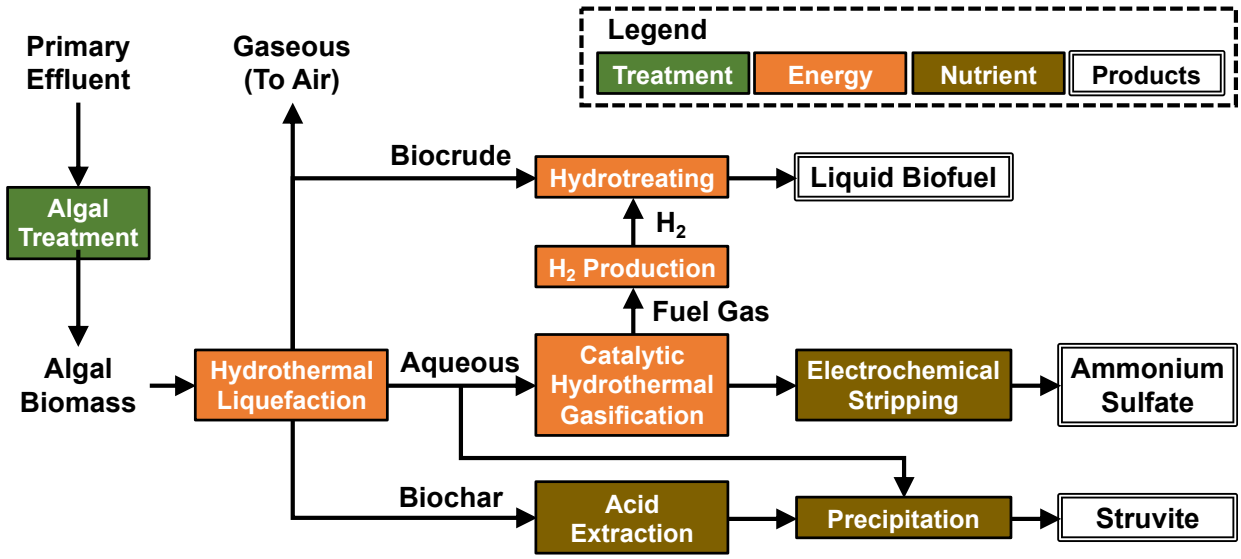

Figure 1. Scheme of processes investigated in this study, energy-related processes were included in the techno-economic analysis model,[7] costs for nutrient-related processes were not included, but revenues from nutrient co-products are discussed in Section 3.5.

3 Results and Discussion

3.1 Characteristics and treatment of wastewater

Characteristics of the primary wastewater effluent used as influent to the photobioreactors were generally consistent throughout the year for NH_4^+-N (22.7–29.2 mg N·L^{-1}) and PO_4^{3-} (2.1–3.9 mg·L^{-1}), but fluctuations were observed in BOD_5, which nearly doubled for the Warm-B test compared to others (111.5 mg O_2·L^{-1} versus 61.5–68.5 mg O_2·L^{-1}, **Figure 2** and Table S1 in the ESI). However, the elevated BOD_5 level didn't compromise the quality of algal-treated effluent, and though the treatment experiment lasted for 10 days, all discharge standards were met within 4 days. This finding corresponded well with past reports showing that the warm season strain of *G. sulphuraria* was capable of reducing all contaminants to their respective discharge levels within

248 3–4 days.[5] In sharp contrast to the Warm-B experiments, the Cold-B and Trans-B tests containing
249 the cold strain Soos were not as effective in NH_4^+-N removal. While both BOD_5 (30 mg $O_2 \cdot L^{-1}$)
250 and PO_4^{3-} (1 mg·L^{-1}) discharge standards were met, NH_4^+-N levels exceeded the discharge limit
251 of 10 mg N·L^{-1} for these two experiments (19.5 and 19.9 mg N·L^{-1} for Cold-B and Trans-B,
252 respectively). Therefore, though all three batch runs resulted in comparable performance for BOD_5
253 and PO_4^{3-}, removal of NH_4^+-N followed the order of Warm-B>> Trans-B > Cold B. As the Trans-B
254 test was seeded with a mixture of warm and cold strains of *G. sulphuraria*, it can be concluded
255 that while both strains are robust in reducing BOD_5 and PO_4^{3-} levels, the warm strain coupled with
256 higher temperature is more efficient in reducing NH_4^+-N contents. Hence, selection of the cold
257 strain and the associated operation conditions need to be further optimized to ensure reliable and
258 efficient year-round algal wastewater treatment. For example, strategies such as increasing initial
259 algae cell concentration, micro-nutrient supplementation, or improved solar heat retention are
260 expected to enhance the treatment performance.[5,32]

261 As for the fed batch experiment (Warm-FB), all three target contaminants were reduced to
262 the discharge standards within 2–3 days during the five repeated treatment cycles over a 20-day
263 period (**Figure 2** and Table S1 in the ESI). This shows further improvement from batch treatment
264 experiments with the same strain (3–4 days based on this study and literature[5]). Therefore, it is
265 expected that the cold and transitional season treatment performance can also be improved by
266 switching to the fed-batch treatment mode. In general, these results indicate that that a single-stage
267 algal wastewater treatment system can be potentially engineered utilizing *G. sulphuraria* to serve
268 as an alternative to the current secondary treatment systems, particularly in hot and sunny regions
269 suitable for the warm strain CCMEE 5587.1.

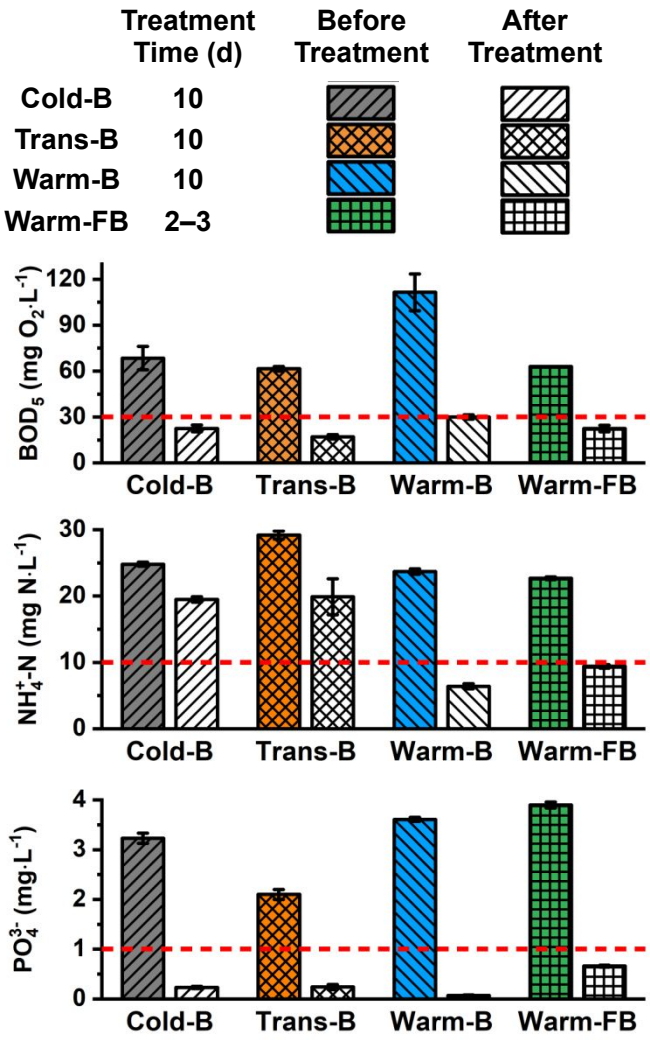

Figure 2. Characteristics of primary clarified municipal wastewater before and after treatment with mixotrophic algal species *G. sulphuraria*. Though the treatment experiment for Warm-B was conducted for 10 days, all discharge standards were met within 4 days; treatment for Warm-FB was conducted for 5 cycles over a 20-day period and each cycle lasted 2–3 days (depending on time needed to reach discharge standards. Levels of BOD_5 (mg $O_2 \cdot L^{-1}$), NH_4^+-N (mg $N \cdot L^{-1}$), and PO_4^{3-} (mg$\cdot L^{-1}$) are shown for batch test at cold (Cold-B), transitional (Trans-B), and warm (Warm-B) seasons, and a fed-batch test at warm season (Warm-FB). Red dashed lines denote discharge standards; error bars show max/min values observed for duplicate BOD_5 tests, and standard deviations for triplicate NH_4^+-N and PO_4^{3-} tests.

3.2 Algal biomass properties and HTL yields

The harvested algal biomass exhibited characteristics typical of wastewater algae with much lower lipid but higher ash contents compared to freshwater/saltwater-cultivated species.[33–36] At the same time, distinctive variations were observed among biomass harvested in different seasons

284 (**Table 1** and Table S2 in the ESI). Specifically, the Cold-B biomass had the lowest lipid (1.3
285 dw%) and protein (30.6 dw%) contents and the highest carbohydrate (27.5 dw%) and ash (29.2
286 dw%) contents, which were expected to result in the lowest biocrude yields but higher co-product
287 yields.[14] The Trans-B biomass, on the other hand, possessed the highest lipid (7.3 dw%) and
288 protein (56.1 dw%) contents and lowest ash (10.3 dw%) content, and therefore was expected to
289 yield the most biocrude and lower quantities of co-products. Biomass from Warm-B and Warm-
290 FB experiments were similar in composition, with lipid, protein, and ash contents between Cold-
291 B and Trans-B, and much lower carbohydrate contents (11.1 and 9.8 dw% for Warm-B and Warm-
292 FB, respectively). The resemblance between Warm-B and Warm-FB batches support the
293 feasibility of operating the algal wastewater treatment systems with partial biomass recirculation
294 and shorter retention time. In addition, as the Trans-B was a polyculture of the cold and warm
295 strains but possessed characteristics that were more favorable for HTL conversion, the current
296 treatment conditions are likely to be suitable for producing high-quality biomass during
297 transitional period only; further optimization will be needed to improve biomass characteristics
298 during warm and cold seasons. As it has been reported that algal biomass properties can be
299 regulated by adjusting operating conditions while maintaining robust nutrient removal,[4] it follows
300 that further optimization of the reactor system conditions can potentially lead to cold and warm
301 season biomass with similar properties as the Trans-B that is favorable for HTL conversion.

302 **Table 1** Algal biomass properties and HTL yields (dw%)[a]

		Cold-B	Trans-B	Warm-B	Warm-FB
Biochemical composition	Lipid	1.3±0.7	7.3±0.3	5.3±0.5	4.8±0.03
	Protein[b]	30.6±0.3	56.1[c]	45.7±0.1	51.6[c]
	Carbohydrate	27.5±1.0	21.4±1.7	11.1±0.5	9.8±1.9
	Ash	29.2±0.3	10.3±0.1	17.3±0.2	17.0±0.1
HTL yields (300°C)	Biocrude	7.6±0.4	27.5±3.2	13.2±1.0	15.5±1.7
	TDS[d]	9.6±0.9	23.2±0.6	25.4±0.2	24.1±2.7
	Gas	23.7±2.5	17.1±1.7	21.5±2.6	16.7±1.8

	Biochar	43.0±4.6	12.0±0.5	23.8±1.5	26.0±1.3
HTL yields (350°C)	Biocrude	13.5±2.0	33.9±0.6	26.6±0.2	21.4±2.5
	TDS[d]	10.8±0.5	13.4±0.2	15.1±0.9	14.8±2.5
	Gas	19.9±0.7	21.1±0.8	29.1±4.3	24.3±4.6
	Biochar	39.0±3.2	10.5±0.5	22.9±1.2	26.9±1.6

[a] Results represent average±max/min of duplicate analysis; errors for volatile oxygen contents and higher heating values (HHVs) calculated by error propagation methods.
[b] Calculated by using 6.25 × feedstock nitrogen contents.[23]
[c] No error value because duplicate analysis yielded the same results.
[d] Aqueous total dissolved solids.

HTL experiments were conducted to provide accurate information on yields and characteristics of the HTL products, which had been identified as critical impactors toward overall economic performance.[8,9,17] Reactions were carried out using 20 dw% algal slurries, which has been reported as a reasonable tradeoff between the capital cost for larger HTL system (due to more dilute biomass), the operating cost for dewatering biomass to a higher solid content, and the heating need.[8] Two previously reported optimum reaction temperatures (300 and 350°C)[37,38] were examined to determine the more suitable one for this application. Distinguishable product patterns were observed as a result of varying biomass properties and reaction temperatures (**Table 1** and Tables S2–S4 in the ESI). As expected from the trends in feedstock lipid and protein contents, processing biomass from Trans-B yielded the most HTL biocrude (27.5 dw% at 300°C and 33.9 dw% at 350°C, respectively), followed by Warm-B and Warm-FB (13.2 and 15.5 dw% at 300°C, 26.6 and 21.4 dw% at 350°C, respectively), and lowest yields for the low-lipid, low-protein Cold-B biomass (7.6 dw% at 300°C and 13.5 dw% at 350°C, respectively). For Trans-B, Warm-B, and Warm-FB, yields of biocrude were higher or comparable to other HTL products, but biochar was the dominant product for Cold-B with yields of 43.0 dw% at 300°C and 39.0 dw% at 350°C due to its high carbohydrate (27.5 dw%) and ash (29.2 dw%) contents. As for the effects of temperature, HTL at 350°C generated more biocrude and gaseous products and less aqueous total dissolved solids (TDS) and biochar products than 300°C, both of which were in agreement with

321 previously reported trends.[37,39] Although higher HTL reaction temperature is demonstrated to
322 promote biocrude formation (critical to process economics[7,8]), greater energy inputs are required
323 to heat the algal slurries to these conditions. Hence, there are tradeoffs associated with processing
324 temperature selection, which are discussed in following sections.

325 **3.3 Energy recovery**

326 Distribution of carbon among HTL products can reflect the allocation of initial energy in the
327 wastewater algae feedstock, and substantial variations were found for different batches and HTL
328 temperatures (**Figure 3a**). In general, 17.0–51.4% of the feedstock carbon was transferred to the
329 desired biocrude products. This wide range results primarily from differences in biocrude yields
330 since there were only small variations in carbon contents of the different biocrudes (70.2–73.5%
331 C). HTL was most efficient in transferring carbon from the Trans-B biomass into biocrude, with
332 41.2 and 51.4% of feedstock's carbon converting into biocrude products at 300 and 350°C,
333 respectively. In contrast, only 17.0% (300°C) and 31.5% (350°C) of the carbon in the Cold-B
334 biomass transferred into the HTL biocrude product; a greater fraction of the carbon in this
335 feedstock transferred to the biochar product (up to 36.5%). 15.0–31.2% of the carbon ended up in
336 aqueous co-products, representing a non-negligible portion of the feedstock energy. Though not
337 directly upgradable to liquid biofuels, aqueous organic compounds can be recovered as
338 energetically valuable fuel gas products (mostly CH_4 and H_2) by CHG, which can be used for
339 onsite co-generation of heat (for reactor heating)/electricity and catalytic upgrading of biocrude to
340 refined fuel products.[8,30,31] Other technologies like anaerobic digestion[40] and microbial electrolysis
341 cells[41] can also be used to recover the energy embedded in aqueous organics as biogas or H_2.
342 Lastly, 10.0–20.6% of the feedstock carbon was converted to gaseous products during the HTL
343 conversion. As the gaseous products are predominantly CO_2[31] and of little energetic value, they

can be recycled to support biomass growth.[8] With regard to reaction temperature, it was found that more carbon from the feedstock could be transferred to biocrudes at 350°C as a result of increased yields and higher biocrude carbon contents observed at this temperature. At the same time, increasing temperature reduced the level of carbon that ended up in the biochar products, a result of both the lower biochar yields and biochar carbon contents. It follows that performing HTL at the higher temperature is advantageous for improving feedstock conversion and energy recovery in the biocrude product.

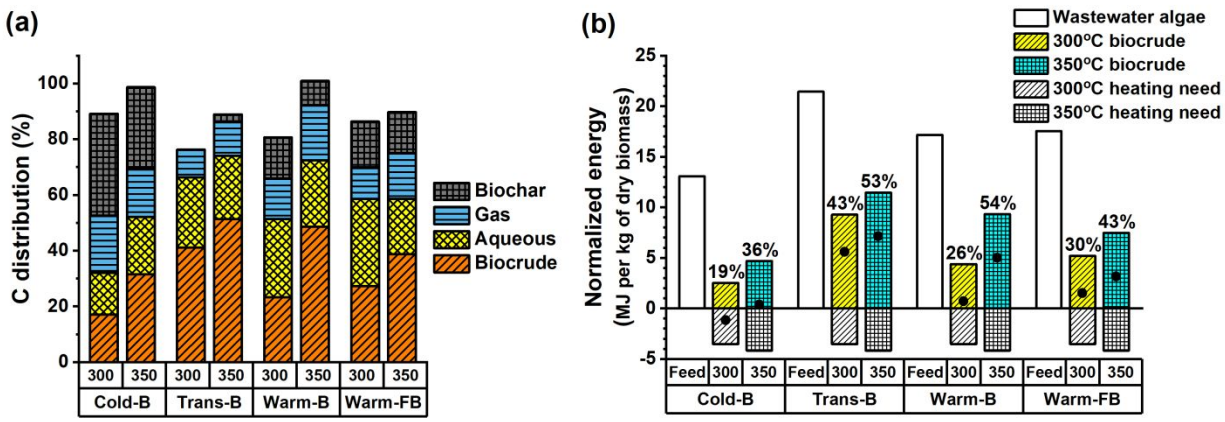

Figure 3. Carbon distribution between HTL products (a) and energy normalized to 1 kg of dry algae (b). Four batches of harvested *G. sulphuraria* (Cold-B, Trans-B, Warm-B, and Warm-FB) and two HTL reaction temperatures (300 and 350°C) were included. In (a), carbon content of biochar for the Trans-B at 300°C could not be determined due to the small amount of sample generated. In (b), open columns show feedstock energy; colored and hatched columns show energy in biocrude generated from 1 kg of dry algae; uncolored hatched columns show heating needs; black dots show the net energy that could be recovered from biocrude after subtraction of heating need; data labeled at the top of columns indicate energy recovery of biocrude (percentage of feedstock energy that can be recovered in biocrude, calculated on dry weight basis). Results are average of duplicate experiments and detailed data with uncertainties can be found in Tables S2–S4 in the ESI.

To give a straightforward illustration of the energy flows during HTL reactions, energy in algal biomass, HTL biocrudes, and the amount required to heat the algal slurries to each reaction temperature were calculated (**Figure 3b**). For 1 kg of dry biomass, the Trans-B biomass had the

366 highest energy content (21.4 MJ·kg^{-1} on dry weight basis), followed by the biomass for Warm-FB
367 (17.5 MJ·kg^{-1}) and Warm-B (17.2 MJ·kg^{-1}), all of which were comparable to previously reported
368 freshwater-cultivated algal biomass.[14,42] The biomass from Cold-B had a lower energy content
369 (13.1 MJ·kg^{-1}) due to its high ash content, which is not uncommon among wastewater algae.[33,35]
370 For the HTL reactions, it was estimated that the heating needs (3.5 and 4.2 MJ per kg of dry algae
371 for 300 and 350°C, respectively) represented 16.5–31.9% of the energy embedded in the starting
372 biomass. The generated biocrudes would contain 2.5–11.5 MJ of energy (colored columns in
373 **Figure 3b**), representing 19.2–54.3% of the energy embedded in the initial algae (data labeled in
374 **Figure 3b**). Subtraction of the heating needs revealed the net amounts of energy that could be
375 recovered in the form of biocrude (black dots and labels in **Figure 3b**), which followed the order
376 of Trans-B > Warm-B and Warm-FB > Cold-B, and 350°C > 300°C. Thus, although HTL
377 conversion at 350°C required more input energy, it would be more than offset by the increased
378 biocrude yield and HHV. Notably, for HTL at 350°C, all four batches of algae generated biocrudes
379 containing more energy than heating needs, supporting the feasibility of HTL-based conversion
380 process.

381 **3.4 Nutrient recycling**

382 Recovery of nutrients (most importantly nitrogen and phosphorus[43–45]) in conversion process
383 is another important aspect for economics and sustainability of the overall system,[46,47] which can
384 vary greatly over different algal biomass[14] but not yet well studied for large-scale systems.[8] **Figure**
385 **4** shows the distribution of nitrogen and phosphorus within the HTL products observed in this
386 study. Unlike the distribution of carbon, which varied widely between different biomass feedstocks
387 (e.g., Cold-B versus Trans-B) and products (e.g., biocrudes versus biochars), much less variation
388 was observed for the distribution of nitrogen (**Figure 4a**). The largest fraction of feedstock

389 nitrogen ended up in the aqueous co-products, close to or exceeding 50% for most reactions. Even
390 aqueous products of the Cold-B, where yields were less than 30% of biochar products', contained
391 most of the feedstock nitrogen among all products. This finding was consistent with earlier reports
392 on HTL of algal biomass[14,48] and revealed a strong tendency of nitrogen-containing compounds to
393 partition into the aqueous phase. Aside from aqueous products, a substantial fraction of the
394 feedstock nitrogen also transferred into the biocrude and biochar products (10.7–24.6% for
395 biocrudes and 3.3–25.2% for biochars). Similar to the case of carbon, these observed variations
396 resulted mostly from the distinctive product yields rather than nitrogen contents of the different
397 products, which were found to be narrowly constrained (6.1–7.0% for biocrudes and 2.2–3.3% for
398 biochars). On the impacts of reaction temperature, generally more nitrogen was directed to
399 biocrude and aqueous products and less to biochar products at 350°C. It should be noted that the
400 increase in nitrogen distribution to biocrudes resulted from the increase in biocrude yields rather
401 than higher biocrude nitrogen contents. In fact, nitrogen contents of the biocrudes generated at
402 350°C (6.1–6.5%) were found to be slightly lower than biocrudes generated at 300°C (6.4–7.0%),
403 which is preferred, as elevated nitrogen content is detrimental to biocrude quality, negatively
404 influencing refined fuel yields from hydrotreating processes.[8] Aqueous nitrogenous constituents
405 mainly existed in the form of NH_4^+ and organonitrogen compounds,[49] and substantial increases in
406 aqueous NH_4^+-N contents were observed with increasing HTL temperature (3350–8450 mg $N \cdot L^{-1}$
407 at 350°C versus 1980–4870 mg $N \cdot L^{-1}$ at 300°C). This can be attributed to the further degradation
408 of organonitrogen compounds (both in the biocrude and aqueous products) at more severe reaction
409 conditions, which is preferred because NH_4^+ can be more readily recovered for production of
410 commercial fertilizers (e.g., ammonium sulfate) or recycled for algae cultivation.[46,50,51]

411 Phosphorus contents of the biochar products could only be directly determined for half of the
412 experiments (because of the small amounts of biochar generated during some reactions), but
413 analyses of the aqueous products (all batches) and available biochar products revealed a clear
414 tendency for phosphorus species to be incorporated into the solid phase products (**Figure 4b**),
415 likely in the form of polyvalent phosphate salts (e.g., calcium phosphate).[49] For all experiments,
416 around or less than 5% of the feedstock phosphorus transferred into the aqueous phase after HTL
417 reaction, compared to 93.1–104.6% in the biochar products (phosphorus contents of biocrude and
418 gaseous products were assumed to be negligible[49]). The strong tendency for phosphorus
419 distribution to the biochar was even more evident for reactions conducted at 350°C, where aqueous
420 phosphorus species only accounted for 1.3–3.1% of the phosphorus originally present in the
421 feedstock. The diversion of almost all phosphorus to biochar is advantageous for subsequent
422 recovery of energy from the HTL aqueous products through catalytic processes (e.g., CHG) where
423 phosphorus can poison and deactivate catalysts.[52,53] Additionally, the concentration of phosphorus
424 in biochar is beneficial for the direct use of biochar as a soil amendment and fertilizer,[54,55] or as a
425 source material for production of other phosphorus-containing fertilizers.[43,46]

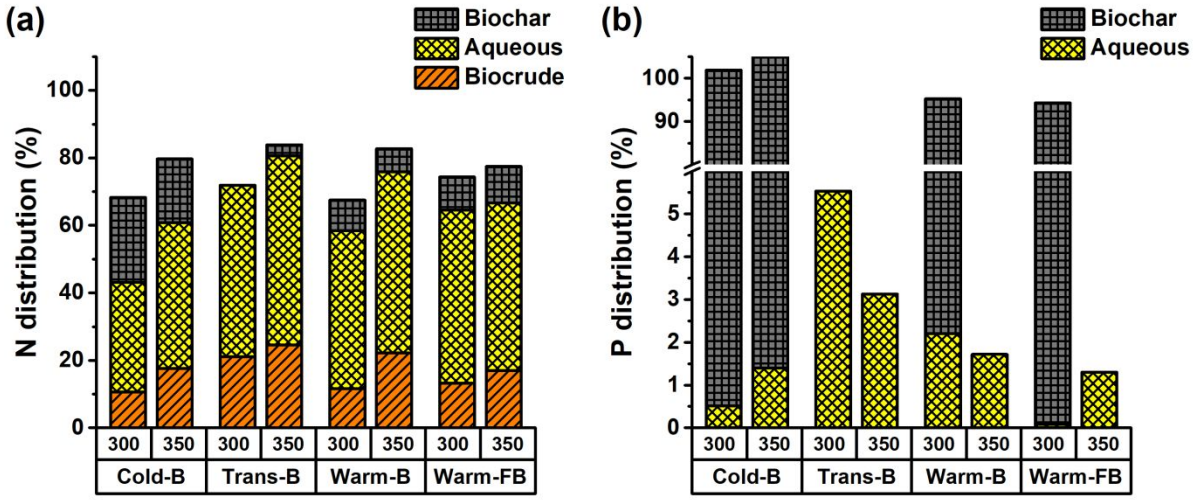

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Figure 4. Nitrogen (a) and phosphorus (b) distributions between HTL products. Four batches of harvested *G. sulphuraria* (Cold-B, Trans-B, Warm-B, and Warm-FB) and two HTL reaction temperatures (300 and 350°C) were included. Biochar nitrogen content of the Trans-B at 300°C, and phosphorus contents of Trans-B, Warm-B, and Warm-FB batches at 350°C could not be measured due to small quantities of biochar generated. Results are average of duplicate experiments and detailed data with uncertainties can be found in Tables S2–S4 in the ESI.

3.5 System evaluation and path forward

Following HTL experiments, economic analysis was conducted to compare the costs and revenues of the conversion processes (**Figure 5** and Tables S5 and S6 in the ESI). For all batches, a similar cost of 2.0–2.1 million dollars per year (MM $\$\cdot yr^{-1}$) was calculated (annualized results assuming year-round operation with only the respective strain, adjusted to 25.6 TPD). This was expected as properties of algae had minor effects on size of the major equipment (therefore total capital cost).[56] However, much larger variations were observed when comparing revenues derived from conversion of these wastewater algae. While less than 0.8 MM $\$\cdot yr^{-1}$ was calculated for Cold-B due to the low biofuel yields, 1.6 MM $\$\cdot yr^{-1}$ was expected for Warm-B and 2.0 MM $\$\cdot yr^{-1}$ for Trans-B. Assuming a year-round operation with a third of Cold-B, Trans-B, and Warm-B each, an average revenue (Average-B) of 1.4 MM $\$\cdot yr^{-1}$ was predicted. Overall, annual loss of 0.1–1.3 MM$\$\cdot yr^{-1}$ was predicted and was consistent with existing literature. Previous research concluded that in order for the plant to break even, the minimum selling price of HTL-derived biofuel needs to be \$4.49–32.60 gal^{-1} with an average of \$10.23 gal^{-1}.[7,28,57–59] Assuming 70% of the costs were attributed to feedstock,[29] the conversion process accounted for \$1.35–9.78 gal^{-1} with an average of \$3.07 gal^{-1}, higher than the value of \$2.79 gal^{-1} used in this study, thus leading to negative net revenues. For wastewater algae examined in this study, biofuel should be priced at \$3.10–6.47 gal^{-1} for the plant to break even, which is in the range reported in literature.

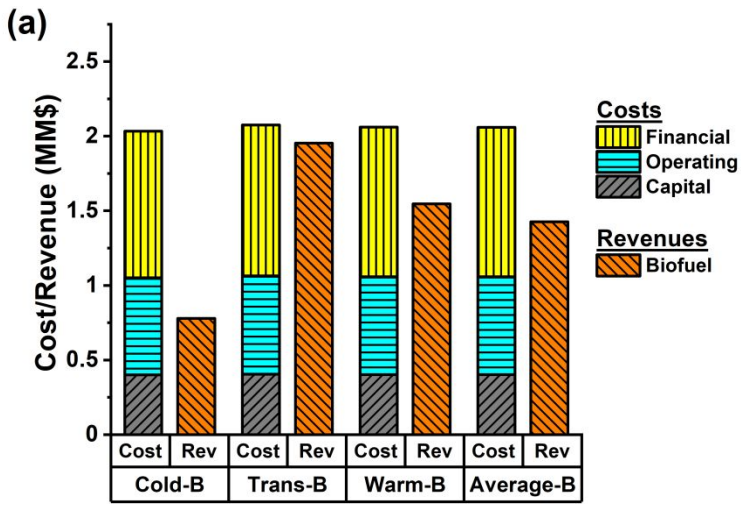

Figure 5. Process economic analysis for harvested *G. sulphuraria*. Results of Cold-B, Trans-B, and Warm-B were calculated using the respective experiment data (350°C HTL conversion) as inputs of the model described in a previous study;[7] results for batch Average-B were calculated using the average HTL results of Cold-B, Trans-B, and Warm-B as model inputs. Warm-FB gave similar results as Warm-B thus not presented. Costs/credits from algal treatment of wastewater were not included due to lack of large-scale studies on such processes. Detailed cost and revenue breakdown can be found in Tables S5 and S6 in the ESI.

To characterize the impact from key factors on the overall system, sensitivity and uncertainty analyses were conducted for the baseline scenario of Average-B (**Figure 6a**), which better represented the composition of wastewater algae that could be harvested from year-round operation. Financial cost coming from loan payments was found to be the largest contributor (0.7 MM $\$\cdot yr^{-1}$), thus changes in project equity would affect the overall cost in the most significant way. As biofuel was the only product in the baseline scenario, the system was sensitive to the selling price of biofuel, and a ~±0.2 MM $\$\cdot yr^{-1}$ (~±15% of total revenue) was estimated based on the changes of commercial gasoline price over the past 10 years. ±10% of the baseline plant scale would shift the overall economics in a similar way as biofuel price, but if algae from the wastewater treatment plant was the only feedstock source (i.e., plant scale decreased to 25.6 TPD, or only 4.4% of the baseline value), then the total cost would be more than doubled with no changes in revenues. This economy of scale reveals the necessity to have central conversion plants for large

quantities of feedstocks, and additional feedstock sources should be considered. In fact, a broad spectrum of organic materials (e.g., food wastes,[60] manure,[15] waste plastics[61]) can be included as HTL is widely applicable. Moreover, as yields and characteristics of HTL products are determined by feedstock biochemical compositions (lipid, protein, carbohydrate, and ash contents) rather than feedstock type (e.g., algae versus manure), results from this study can be extended to other waste biomass with similar properties (e.g., sludge and manure which have low lipid but high ash contents).[14] IRR evaluates the attractiveness of the project and 10% has been recommended for renewable energy-related projects.[62] An IRR of 0% could increase the revenue by 0.16 MM $\cdot$yr^{-1}, but investors would not be interested in this project considering inflation and project risk. Total capital and total operating costs were expected to have smaller effects (<0.1 MM $\cdot$yr^{-1}) and the effects were symmetric for minimum and maximum boundaries. Overall, as the baseline scenario of Average-B had a negative revenue of -0.63 MM $\cdot$yr^{-1}, which was larger than the scales of changes that could be brought by these factors other than 100% equity, the project was not likely to be profitable under the current design.

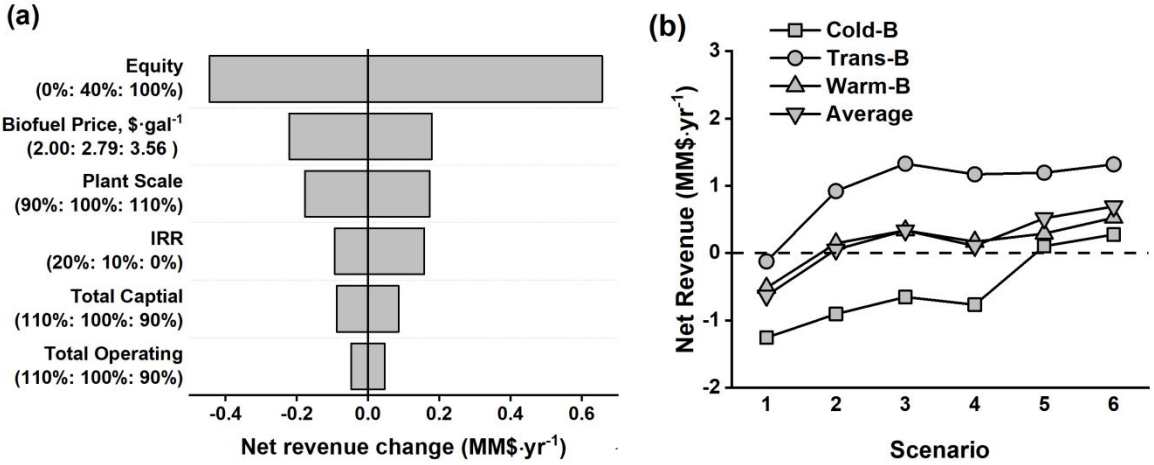

Figure 6. (a) Sensitivity and uncertainty analyses for Average-B in **Figure 5**; examined factors included percentage of project equity, selling price of biofuel, scale of the biorefinery plant, internal rate of return (IRR), total capital investment, and total operating cost. (b) Changes in net

revenues under different scenarios. Scenario 1 was the baseline scenario in **Figure 5**; Scenario 2 included revenue from ammonium sulfate; Scenario 3 included revenues from both ammonium sulfate and struvite; Scenario 4 included revenues from both ammonium sulfate and struvite, and assumed wastewater algae were harvested at their peak storage level (maximum lipid and carbohydrate contents); Scenario 5 and 6 included revenues from both ammonium sulfate and struvite, and assumed carbohydrates in algae were hydrolyzed and fermented to ethanol, lipids in algae were extracted and hydrotreated to green diesel prior to HTL conversion based on model in[29], Scenario 5 was calculated for the baseline composition and Scenario 6 was calculated for the peak-storage composition.[29] Detailed cost breakdown can be found in Tables S5–S7 in the ESI.

While revenues from biofuels alone could not cover the anticipated costs of these conversion processes, inclusion of nutrient co-products (ammonium sulfate and struvite) could potentially flip the balance sheet. Specifically, 0.4–1.0 MM $\$\cdot yr^{-1}$ in additional revenue was predicted for ammonium sulfate, which was enough to bring net revenues for Trans-B, Warm-B, and Average-B above 0 (Scenario 2 in **Figure 6b** and Tables S5 and S6 in the ESI**)**, and inclusion of struvite would add another 0.2–0.4 MM $\$\cdot yr^{-1}$ in revenue (Scenario 3 in **Figure 6b** and Tables S5 and S6 in the ESI). Moreover, current ammonium sulfate yields were based on NH_4^+-N contents of HTL aqueous products. Since the subsequent CHG step can convert almost all other non-NH_4^+ N-containing species in HTL aqueous products (e.g., organic nitrogen species) to NH_4^+-N,[8,9,31] revenues from ammonium sulfate could be >50% higher than current estimates. Though costs of generating these nutrient co-products could not be calculated at this stage, these processes were conducted at much milder conditions than the hydrothermal processes, so costs are expected to be relatively small compared to those producing fuels. Overall, revenues from these nutrient co-products were 55–78% of the biofuel sales and demonstrated that nutrient recovery was not only a necessity for environmental sustainability of algal bioproducts,[43–45] but also important for economic considerations. Since algae samples used in this study were harvested after ≥10 days when part of the storage compounds (lipids and carbohydrates) had been consumed, additional evaluations were conducted for algae at their peak storage levels (day 4 of growth based on[18],

516 sufficient for Warm-B and Warm-FB to meet discharge standards[20]). Composition of algae were
517 simulated based on previously reported growth profile[18] and were characterized by higher lipid
518 and carbohydrate contents but lower protein contents (3.1–13.7% lipids, 40.3–62.5% proteins, and
519 23.8–56.6% carbohydrates for peak-storage scenario versus 2.1–8.6% lipids, 51.5–71.5% proteins,
520 and 17.9–46.3% carbohydrates for baseline scenario, all corrected to 100% ash-free dry weight
521 basis, ash contents were assumed to be the same). However, these changes in composition led to
522 0.12–0.23 MM $\$\cdot yr^{-1}$ decrease in revenues (Scenario 4 in **Figure 6b** and Table S7 in the ESI),
523 which was due to the tradeoffs between different products. As HTL is not efficient in converting
524 carbohydrates to fuels (16% carbohydrate-to-fuel efficiency versus 34% for proteins and 65% for
525 lipids),[29] the major increase in carbohydrate contents and decrease in protein contents outweighed
526 benefits from the minor increase in lipid contents, leading to reduction in fuel yields. Moreover,
527 lower protein contents also led to less ammonium in HTL aqueous product that could be recovered
528 as ammonium sulfate. Alternatively, component-specific conversion process other than HTL could
529 be incorporated to improve biomass-to-fuel efficiency and therefore overall revenues. For
530 example, hybrid system featuring fermentation of carbohydrates to ethanol and
531 extraction/hydrotreating of lipids to green diesel prior to HTL conversion has been proposed, and
532 it has been demonstrated with freshwater algae to increase carbohydrate-to-fuel efficiency from
533 16% to 75% and lipid-to-fuel efficiency from 65% to 79% (compared to HTL alone).[29] Assuming
534 the same conversion efficiency, substantial increases in net revenues compared to HTL alone could
535 be achieved for Cold-B (0.8 MM $\$\cdot yr^{-1}$ for the baseline scenario and 1.0 MM $\$\cdot yr^{-1}$ for the peak-
536 storage scenario, respectively) with similar net revenues for Trans-B and Warm-B (Scenarios 5
537 and 6 in **Figure 6b** and Table S7 in the ESI, changes in costs included).[29] On average, these
538 changes led to increase of 0.2 MM $\$\cdot yr^{-1}$ for the baseline scenario and 0.6 MM $\$\cdot yr^{-1}$ for the peak-

storage scenario, demonstrating the benefit of more efficient conversion of individual components, especially for carbohydrates. This application of hybrid system was particularly relevant for Cold-B, whose low lipid and high carbohydrate contents led to negative net revenues even when nutrient co-products were considered, and the much smaller differences in net revenues between different batches would allow steadier year-round operation. However, more research should be conducted in the future to obtain the experimental biomass-to-fuel efficiency for wastewater algae.

When viewed from a systems level, this study revealed the underlying connections between treatment and conversion processes and present the significant effects of the algal strains and operating modes on treated water quality, properties of the harvested algae, and energy and nutrient recoveries thereof. While the warm strain was found to be most effective in wastewater treatment, biomass recovered from the polyculture transitional batch generated the most valuable products and was expected to have the highest profit. This highlights the tradeoffs that can exist between treatment efficacy and downstream energy recovery and suggest a need to customize operating strategies to each season. For example, higher initial algae concentration, micro-nutrient supplementation, fed-batch operating mode, etc. can be considered to improve the removal of NH_4^+-N in cold and transitional seasons, and treatment time can be shortened for the warm season due to the higher contaminant removal rate. In addition to treatment operating decisions, energy and nutrient recoveries can be greatly improved by optimizing conversion conditions and system configuration. Negative impacts resulting from the elevated ash contents algal biomass (e.g., reduced biocrude yields and higher biochar yields) can be alleviated, to a degree, by increasing the HTL reaction severity, where higher biocrude yields and nutrient recovery in desired products were observed. More importantly, as wastewater algae are characterized by high carbohydrate contents that cannot be efficiently converted by HTL, application of hybrid systems with

component-specific conversion techniques can lead to substantial improvement in economics performance. Therefore, treatment experiments with varying solid residence time should be conducted to establish dynamic contaminant removal and algae composition profile, therefore recognizing the optimal time for meeting discharge standards while maintaining high levels of storage compounds in algal biomass for greater biofuel yields. Overall, this work shows the potential of algal wastewater treatment systems for energy and nutrient recovery, which are promising in turning wastewater treatment plants into profitable water resource recovery facilities.

Conflicts of interest

There are no conflicts of interest to declare.

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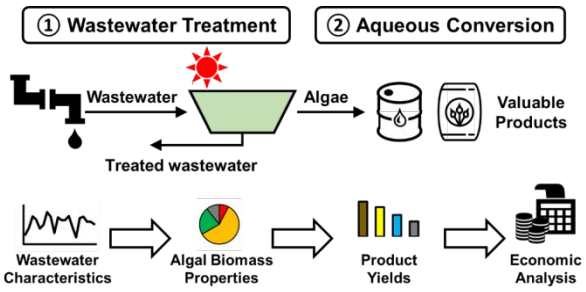

Treatment efficacy and economic performance of an algal wastewater treatment and valorization system were evaluated at different seasons.