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A Unified Modeling Framework to Advance Biofuel Production from Microalgae

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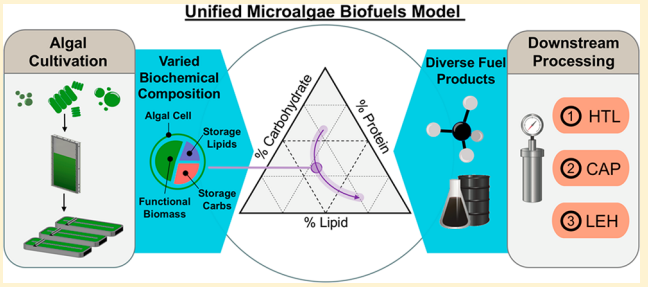
Supporting Information

ABSTRACT: Modeling efforts to understand the financial implications of microalgal biofuels often assume a static basis for microalgae biomass composition and cost, which has constrained cultivation and downstream conversion process design and limited in-depth understanding of their interdependencies. For this work, a dynamic biological cultivation model was integrated with thermo-chemical/biological unit process models for downstream biorefineries to increase modeling fidelity, to provide mechanistic links among unit operations, and to quantify minimum product selling prices of biofuels via techno-economic analysis. Variability in design, cultivation, and conversion parameters were characterized through Monte Carlo simulation, and sensitivity analyses were conducted to identify key cost and fuel yield drivers. Cultivating biomass to achieve the minimum biomass selling price or to achieve maximum lipid content were shown to lead to suboptimal fuel production costs. Depending on biomass composition, both hydrothermal liquefaction and a biochemical fractionation process (combined algal processing) were shown to have advantageous minimum product selling prices, which supports continued investment in multiple conversion pathways. Ultimately, this work demonstrates a clear need to leverage integrated modeling platforms to advance microalgae biofuel systems as a whole, and specific recommendations are made for the prioritization of research and development pathways to achieve economical biofuel production from microalgae.



1. INTRODUCTION

In the pursuit of renewable feedstocks for transportation fuels,[1−3] microalgae possess distinct advantages including the potential for high areal productivities (surpassing those of terrestrial feedstocks),[4] a wide range of microalgae strain choices with tunable biomass compositions through accumulation of lipids or carbohydrates,[5,6] and options for various downstream aqueous conversion processes to produce renewable diesel blendstocks (RDB; a complete list of abbreviations is available in the Supporting Information (SI)) as a supplement to fuel production.[7,8] These processes include hydrothermal liquefaction (HTL), which converts whole wet microalgal biomass thermochemically under high temperature and pressure to HTL biocrude oil, which can then be further upgraded to RDB.[6,7] Conversely, biochemical fractionation methods such as combined algal processing (CAP) enable isolation and conversion of individual biomass constituents to fuels or products through separate processing steps (e.g., fermentation of sugars after acid hydrolysis of the whole biomass, extraction and upgrading of lipids, and anaerobic digestion (AD) of protein).[8−10] Despite the potential and collective research progress in both microalgal cultivation and conversion, a critical barrier to the advancement of microalgal-based biofuels is a lack of understanding of how cultivation approaches, design and operational decisions, and uncertainties in the performance of individual unit processes propagate throughout the integrated feedstock to biofuel system and

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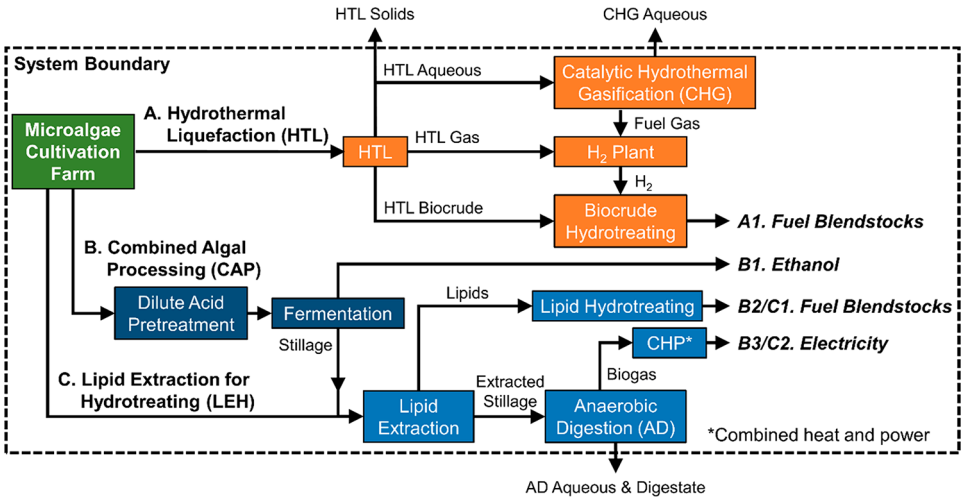

Figure 1. Overview of the processes modeled and their product and coproduct streams. Models of microalgal cultivation (green box), HTL (Process A, orange boxes), CAP (Process B, dark and light blue boxes), and LEH (Process C, light blue boxes) were integrated with biochemical composition (functional biomass and carbon storage products) as a unifying framework.

hence influence process economic viability. As the United States and international entities intensify the pursuit of market-ready renewable transportation fuels at the industrial scale,[3,11] there is a critical need for integrated modeling frameworks which link reactor-scale cultivation models with plant-scale downstream conversion simulations to elucidate the factors governing system-scale optimization and prioritize research and development goals for microalgae biofuels.[12−14]

The current approach to predictions of large-scale microalgal feedstock production relies on assumptions of areal productivity (in terms of $g{\cdot}m^{-2}{\cdot}d^{-1}$), which are applied to both functional biomass (defined as high protein biomass, excluding storage lipids and storage carbohydrates) and storage polymers (i.e., triacylglycerides (TAGs) and starch).[15] To date, most productivity estimates have relied on linear scaling of bench-scale growth and lipid accumulation data, with recent improvements stemming from more robust geospatial productivity modeling[4] and the use of productivity targets associated with three possible biomass compositions: high protein, high carbohydrate, and high lipid.[16] In spite of recent developments, the persistent lack of fidelity in biochemical composition modeling coupled with a disconnect between research in biomass cultivation and downstream processing technologies has precluded a mechanistic understanding of how process-specific decisions (e.g., retention time in microalgal cultivation ponds) result in trade-offs among product yields, biomass and fuel selling prices, and overall optimizations among those metrics, as appropriate, for different conversion methods. Ultimately, the advancement of microalgal biofuels requires the integration of techno-economic analyses (TEAs) with a more mechanistic understanding of carbon storage and conversion processes to achieve increased fidelity in modeling the microalgae-to-biofuel system.

The objective of this work was to develop a unified modeling framework, which integrates unit process models (achieving mass and energy balances) with TEA models for microalgae biomass cultivation and conversion to analyze the overall system, to quantify the relative financial viability of RDB production through varying design decisions (e.g., conversion pathways, microalgae species, cultivation times), and then applying the results to inform potential research and development priorities, including longer-term goals for microalgae feedstock cultivation, and modeling as well as specific recommendations for whole biomass processing techniques or fractionation approaches to leverage specific biomass components to improve the economic viability of the overall system. To this end, a dynamic biological cultivation model was integrated with thermo-chemical/biological unit process models for downstream biorefineries and bridging the gap with biochemical composition (functional and storage biomass) as a unifying framework. Large-scale microalgae cultivation was modeled using the phototrophic process model (PPM)[17] to estimate cell growth and storage products as a function of cultivation system design and microalgal species. Produced biomass—with a given biochemical composition and resultant modeled production cost—was "purchased" by downstream biorefineries designed to produce RDB (diesel-range paraffinic products excluding biodiesel from transesterification processes given to the significant presence of oxygen not otherwise present in RDBs) and coproducts (naphtha and ethanol) using one of three candidate processes (Figure 1): HTL, CAP, or lipid-extraction for hydrotreating (LEH[18]). Product yields for each conversion process were predicted by coupling models of thermo-chemical (e.g., a multiphase component additivity (MCA) model for HTL[7]) and biological (i.e., fermentation and AD for CAP and LEH) processes with plant-scale simulations. Minimum product selling prices were quantified via discounted cash flow rate of return analysis (DCF) methods.[19−21] Variability in design, cultivation, and conversion parameters were accounted for through Monte Carlo simulations (uncertainty analysis[22]), and sensitivity analyses were conducted to elucidate key cost and fuel yield drivers and their relative impacts on the full microalgae−biofuel system. Environmental benefits and impacts associated with microalgae biofuel systems are excluded given that previous life cycle assessments in the literature provide extensive information relevant to the discussion, but outside the scope of this study.[23−25] Results were collectively interpreted to assess the influence of varying biomass cultivations on process-scale economic indicators to identify optimal cultivation−conversion operating regimes and

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to prioritize research and development goals in order to advance the financial viability of microalgae−biofuels.

2. MATERIALS AND METHODS

2.1. Overview of Integrated Cultivation−Conversion Systems. Integrated cultivation conversion systems consist of a biomass cultivation farm paired with a candidate downstream biorefinery (Figure 1). Microalgae are first grown under natural light to yield targeted biochemical compositions of pure cultures (green block, Figure 1). HTL (Process A, orange blocks, Figure 1) converts microalgal biomass to liquid biocrude oil using water under elevated temperatures and pressures (subcritical water).[6,7] The biocrude oil is then refined into fuel blendstocks (naphtha and RDB, Product A1) through a downstream hydrotreating step (catalytic hydrotreating using heterogeneous solid catalysts in the presence of H_2), the aqueous HTL stream was treated via catalytic hydrothermal gasification (CHG), and the gaseous stream was used to supplement natural gas for on-site hydrogen production.[19] For CAP (Process B, dark and light blue blocks, Figure 1), microalgal biomass is first pretreated with 1 wt % sulfuric acid and then fermented for ethanol (Product B1), which is separated from the fermented stillage. Lipids are extracted from the fermented stillage and hydrotreated to fuel blendstocks (Product B2), whereupon extracted residual stillage is diverted to AD.[20] LEH (Process C, light blue blocks, Figure 1) undergoes the same steps as in CAP except that no pretreatment or fermentation is conducted, and fuel blendstocks and electricity (Products C1 and C2) are the only products. Management of output streams including HTL solid, AD aqueous, and AD digestate, as well as other process options (e.g., fermentation of algal carbohydrates to nonfuel coproducts) were outside the boundary of this study. Details on cultivation modeling and each downstream process are provided in the following sections.

2.2. Cultivation Modeling for Biomass Production. Cultivation modeling was performed for three target species of microalgae—*Chlorella vulgaris*, *Scenedesmus acutus*, and *Nannochloropsis granulata*—using process configuration assumptions from Davis et al.[16] and biomass productivity and carbon storage modeling using the PPM (complete details in SI Section S1).[17] Consistent with Davis et al.,[16] a conceptual cultivation process employing pure cultures of microalgae was modeled for biomass growth in a sequence of four reactor systems with increasing volumes: closed photobioreactors (PBRs; smallest volume) followed by covered ponds, open fully lined ponds, and finally open unlined ponds (largest volumes); this entire system of reactors and ponds is the inoculation and basal biomass growth system. The inoculation and basal biomass growth system was designed to produce 190 000 ton AFDW·yr^{-1} of functional biomass (i.e., high protein biomass) under nutrient replete conditions across 5000 acres (wetted area) of ponds with an effluent (i.e., flow leaving the ponds) flow rate of 70 000 m^3·h^{-1}, which follows the TEA by Davis et al. supporting 5000 acres/190 000 ton AFDW·yr^{-1} production scales as a plausible baseline for microalgae−biofuels modeling (details in SI Section S1.b).[16] For the production of high protein biomass (i.e., functional biomass with no neutral lipids or stored starch), microalgae were harvested after growth to 0.5 g·L^{-1} (0.05%AFDW) in the basal biomass growth system and dewatered via trapezoidal settlers, hollow fiber membranes, and bowl centrifuges to a final concentration of 200 g·L^{-1} (20%AFDW solids) in the final product stream following assumptions detailed in Davis et al.[16] (details in SI Section S1.b). Functional biomass carbohydrate/lipid/protein content (in terms of % ash free dry weight, or % AFDW) assumptions were normalized to the highest observed protein content from past experiments with *C. vulgaris* (45/17/38%AFDW), *S. acutus* (53/14/34%AFDW; sum is >100% due to rounding), and *N. granulata* (16/26/59%AFDW; sum is >100% due to rounding).[5] Although these biochemical compositions reflect cultures that had already begun to transition to deplete conditions, initial storage products were adequately low such that each species demonstrated significant capacity for increased carbon storage under further nitrogen depleted cultivation.[5]

For production of biomass with increased lipid and carbohydrate content, biomass from the inoculation and basal biomass growth system entered an open unlined pond system upon nitrogen depletion from the medium; these additional ponds, called carbon-accumulation ponds, were designed to support carbon storage. Exposure to light under nitrogen-limited conditions has been demonstrated to be a reliable trigger for carbon storage in microalgae, and has been widely proposed for large-scale production of biomass rich in lipids and carbohydrates.[16] Carbon accumulation was dynamically modeled using the PPM, which included algal growth, endogenous respiration, and the storage and mobilization of carbohydrates and lipids.[17] Experimental data from the literature for the three modeled species, including biochemical composition over time,[5] were used for the calibration of maximum lipid and carbohydrate storage and the initial relative rates of lipid and carbohydrate storage within the PPM (details in SI Section S1.c). Initial areal productivities in carbon-accumulation ponds were assumed to be the same as unlined growth ponds (SI Table S2), but productivity decreased as cells approached their maximum carbon storage capacity.[17] Biomass was harvested and concentrated to 200 g·L^{-1} as described above.

2.3. HTL Modeling for Fuel Production and Product Selling Prices. Plant-scale process modeling for HTL of harvested biomass used the approach and process details reported in a TEA published by Jones et al.[19] as the reference to construct a TEA model in Microsoft Excel, without modifications to infrastructural design decisions (i.e., process flow diagrams, unit process selection, HTL biocrude upgrading, and treatment of HTL waste discharge),[19] but with modifications to characterize the process implications of the full range of microalgal biochemical compositions (complete details in SI Section S2). Fixed assumptions in the reference model were replaced by the MCA model[7] to predict variations in HTL product yields due to biomass composition (SI Table S8). All unit operations were scaled based on modeled process flows. Importantly, the post-HTL products were treated according to methods described in the reference model (i.e., catalytic hydrotreating and CHG for the biocrude and aqueous phase products, respectively[19]). Calculations based on this approach showed that mass balances between nonwater process inputs (i.e., dry microalgae biomass and methane) and final plant outputs were closed to within 98.9−100.5% for all biomass composition data points across the three modeled species. The structure of the Excel model was validated against previously published results from Aspen models[19] and was within 0.05% of reported values for $·gal diesel^{-1} and $·GGE^{-1} (GGE being gallon gasoline equivalent).

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2.4. CAP and LEH Modeling for Fuel Production. Plant-scale process modeling for CAP followed the general approach of HTL modeling in that a published TEA[20] was used as the reference model, with modifications to characterize the process implications of the full range of microalgal biochemical compositions, and updated to enable independent assumptions related to the extraction and processing of functional biomass, storage carbohydrates, and storage lipids (complete details in SI Section S3). For instance, storage carbohydrates were assumed to be fermentable and storage lipids were assumed to have higher conversion efficiencies to fuels than functional lipids. Additionally, a new model was developed for the AD unit to better account for the energetic content of residual biomass and nitrogen inhibition under high protein scenarios (see SI Section S3.c for details).[26] Calculations based on this approach showed that mass balances between nonwater process inputs and final plant outputs were close to within 97.1−101.5% for all composition data across the three modeled species. The structure of the CAP Excel model, with modifications from the reference model as described herein, was validated against previously published results from Aspen models[20] and was within 3% of reported values for $·gal diesel^{-1} and $·GGE^{-1}. In brief, LEH modeling followed the design of the CAP model, but with the removal of dilute acid pretreatment and fermentation steps (see SI Section S4 for details).

2.5. TEA for Biomass and Fuel Selling Prices. Minimum biomass selling price (MBSP, in $·ton AFDW^{-1}) was determined through DCF analysis following the general approach of the reference model for microalgal cultivation.[19−21] Although past work in the reference study fixed the total production of biomass (across three modeled compositions for each of three microalgae) at 190 000 ton AFDW·yr^{-1},[16] the approach used in our study was to fix the size of the inoculation and basal biomass growth system (to yield an average of 190 000 ton AFDW·yr^{-1} of functional biomass) and increase the carbon content of biomass (via lipid and carbohydrate storage) by adding carbon-accumulation pond area (subsequently increasing total biomass production above 190 000 ton AFDW·yr^{-1}). This approach of fixing the size of the inoculation and basal biomass growth system was designed to reduce the sensitivity of MBSP results to cost scaling assumptions of the infrastructure-intensive photobioreactors, covered ponds, and lined ponds. Seasonal variations in productivity were mitigated by drying and storage of a fraction of concentrated biomass (after dewatering) as accounted for in all downstream processes. Complete details regarding the microalgal cultivation system costing are available in SI Section S1.d.

Minimum RDB selling price (MDSP, as $·gal RDB^{-1}) and minimum fuel selling price (MFSP, as $·total GGE^{-1}) were also determined with DCF. MDSP represents the potential sale price of RDB, with naphtha and ethanol (if produced) sold as a fuel coproduct at a fixed price. MFSP represents the potential sale price of all fuel products: RDB, naphtha, and ethanol (if produced); each was translated to GGE based on its energy content. Distinct from past work,[20] neither credits nor disposal costs were associated with output streams (i.e., nonfuel coproducts such as HTL solids or aqueous phase nutrients, AD digestate cake, CO_2 streams for CAP) in this study. While we acknowledge there is potential value in coproduct streams—for example, to offset nutrient and water consumption by recycling to upstream cultivation—it is highly uncertain how algal kinetics would be impacted by such recycles (e.g., whether inhibition would be observed[27]), and thus nutrient recycle using coproduct streams for any downstream process is outside the scope of this study. Finally, the modeled HTL, CAP, and LEH plants were designed to process a fixed annual average feedstock rate of 190 000 ton AFDW·yr^{-1} (i.e., the lowest productivity encountered from a single cultivation facility), with feedstock purchase prices set at the MBSP for a given biochemical composition (an individual cultivation facility could provide biomass to one or more biorefineries). The economies of scale of biorefineries are readily quantified,[19,20] and fixing their size in this study avoided bias in favor of microalgae with higher concentrations of storage products (which would have increased conversion plant size and, thus, cost efficiencies). Prices are reported on a 2011 U.S. Dollars (2011US$) dollar basis for easy cross-referencing with the baseline TEAs as referenced in this study.

2.6. Uncertainty and Sensitivity Analyses. Uncertainty analysis was performed through Monte Carlo simulation with Latin Hypercube Sampling (to reduce computation time)[28] and 1,000 trials for cultivation and each downstream process (HTL, CAP, and LEH). For the cultivation of biomass, uncertainty surrounding areal productivities (SI Table S2), PPM parameters (SI Table S5), and cost parameters (SI Table S6) was characterized using uniform or triangular probability distributions with minimum and maximum values extending 10% below and above the baseline value, respectively. For HTL, LEH, and CAP, process performance parameters (SI Tables S13, S14, S17, and S18), product characteristics (e.g., RDB heating value; SI Tables S13 and S18), and cost parameters (SI Tables S15 and S20−S22) were also characterized using uniform and triangular distributions based on literature data or, when data were lacking, by extending 10% below and above the baseline value. The sensitivity of MBSP, carbohydrate and lipid content of biomass as well as MDSP and MFSP to all uncertain inputs was determined via Spearman's rank correlation coefficients (i.e., Spearman's *rho*).

3. RESULTS AND DISCUSSION

3.1. Feedstock Production and MBSP. Biomass productivity varied with carbon-accumulation pond retention times, with the highest rates of carbon storage at the beginning of the carbon-accumulation process (SI Figure S1). Accumulation pond acreage varied linearly with retention time since the influent flow rate and pond depth (0.25 m) were held constant. Increases in retention time, and thus size, resulted in increased storage carbohydrates and storage lipids (along with slight losses in functional biomass due to endogenous respiration, see SI Figure S1), which also increased the total amount of biomass produced annually (given that total mass produced per year, as ton AFDW·yr^{-1}, was the sum of functional biomass, storage carbohydrates, and storage lipids).

Pseudomechanistic modeling of carbohydrate and lipid storage of the three microalgae with the PPM[17] revealed species-specific intrinsic kinetic and stoichiometric parameters (SI Tables S3−S5) that defined each alga's trajectory of biochemical compositions (SI Figure S1). After cell growth and nitrogen depletion in the inoculation and basal biomass growth system, storage product accumulation in the carbon-accumulation ponds was governed by three factors: (i) the physiological tendency of a species to store one product before the other (e.g., carbohydrates followed by lipids) or both in

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parallel; (ii) maximum specific rates of storage products (where "specific" rates are normalized to functional biomass concentration); and (iii) maximum specific storage capacity for each storage product. Sequential storage of carbohydrates followed by lipids was observed for all three species (e.g., Figure 2A for *C. vulgaris*), resulting in maximum carbohydrate content (as %AFDW) for retention times between 3.1 and 3.3 days (SI Figure S1). Lipid content reached 95% of their maximum for HRTs between 9.3−11.4 days, with storage rates diminishing as the maximum specific storage capacity was approached (SI Figure S1). Beyond these limited biomass composition scenarios, unique, continuous pathways of potential compositions were generated for each microalga (SI Figure S2) to develop a deeper understanding of the systems-scale implications of cultivation design decisions.

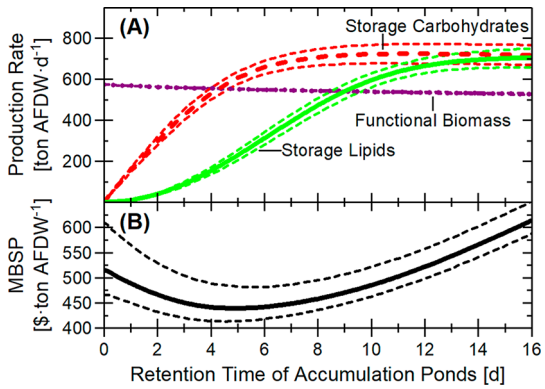

Figure 2. Average annual (A) production rates and (B) MBSP (prices in 2011US$) for *C. vulgaris* as a function of retention time of the carbon-accumulation ponds. Biomass is under nitrogen-deplete conditions, leading to a slow net loss (via endogenous respiration) of functional biomass (functional proteins, lipids, and carbohydrates) and accumulation of storage carbohydrates and storage lipids. Uncertainty analysis results are shown as 5th and 95th percentiles (dashed lines). Results for *S. acutus* and *N. granulata* and method details are provided in the SI.

Increasing retention times results in a trade-off between (i) increasing the storage of lipids and carbohydrates and (ii) decreasing annual average areal productivity and increasing capital and operating costs from larger carbon-accumulation ponds (SI Figure S1). The rates of carbohydrate and lipid storage are the highest when cells have no existing storage products, and rates of storage gradually decrease as the maximum storage capacity is approached (Figure 2A).[17] Thus, decreasing areal productivity (in units of $g \cdot m^{-2} \cdot d^{-1}$) stems from both storage product accumulation and endogenous respiration,[17] which leads to diminishing gains in biomass production with increasing retention times. Altogether, these factors are reflected in the MBSP which, in the case of *C. vulgaris*, reaches a minimum value with a retention time of 5 days (Figure 2B). After this point, the reduced kinetics of carbon storage (evident from the changes in gradients of storage product curves shown in Figure 2A) leads to additional production costs outweighing increased total biomass (functional biomass + storage lipids + storage carbohydrates) production. MBSP results for *S. acutus* and *N. granulata* reinforce this observation, although the species-specific accumulation rates determine different lowest MBSP points at different retention times (SI Figure S1). MBSP results (Figure 2B and SI Figure S1D−F) agree with previous analysis conducted by Davis et al.,[16] with the MBSPs of three compositions for each of the three species from the reference model (491−585 $·ton $AFDW^{-1}$ for the same three species, which did not evaluate kinetic trade-offs against the compositional profiles) falling within the broader range of compositions and MBSP values in this study (439−707 $·ton $AFDW^{-1}$). Individual MBSP values were also similar, with the high carbohydrate scenario for *S. acutus* (31%AFDW lipids, 14%AFDW proteins, 55%AFDW carbohydrates) estimated at 491 $·ton $AFDW^{-1}$ in Davis et al. and at 532 $·ton $AFDW^{-1}$ in this study. MBSP trajectories could therefore provide a more dynamic range of "at the gate" cultivation results to supplement future integrated process modeling studies.[3,12] To understand the full implications of cultivation decisions, however, cultivation modeling must be linked to downstream conversion processing in TEAs that similarly account for differences in biochemical composition.

3.2. Linking Cultivation to Downstream Processing via Biochemical Composition. By including independent (i.e., distinct) state variables for functional biomass, storage lipids, and storage carbohydrates, cultivation modeling can yield bulk biochemical composition over retention time (Figure 3A). Leveraging recent mechanistic studies that have characterized the implications of biochemical composition on downstream aqueous conversion processes (CAP, HTL, and LEH), performance of these conversion processes could be modeled across the compositional trajectories and MBSPs of each microalga to determine resulting product yields and final fuel prices.[19,20] Results illustrate that RDB yield, total GGE yield, MDSP, and MFSP results all vary with biochemical composition (Figure 3B−E for *C. vulgaris*; all other results are shown in SI Figures S3 and S4). In the case of HTL, the whole biomass is processed to yield biocrude oil along with solid, liquid, and gaseous waste streams that are highly dependent on lipid, carbohydrate, and protein content of the feedstock.[6,7] Specifically, based on the MCA model previously developed by Li et al.,[7] the highest yields of biocrude oil are 85% and 45% from lipid and protein components, respectively, whereas carbohydrates are only expected to achieve biocrude oil yields of 20% (SI Table S8).[7] Between the two lipid-extraction pathways, CAP surpasses LEH on all aspects of process performance via an additional ethanol coproduct stream derived from the fermentation of biomass sugars,[8,20] which supports the CAP fractionation approaches aiming to maximize valorization/utilization of microalgae biomass components beyond only lipids.[3,12] Based on these results, all further discussion is focused on HTL and CAP.

For both HTL and CAP—two conversion pathways with fundamentally different mechanisms for production of fuel products—results presented here support biomass composition as a key determinant of integrated process performance regardless of choice of downstream biorefinery and microalgal species, albeit at differing magnitudes for both processes as further discussed in Section 3.3. Consistent with past reports that have explored the impact of a subset of biochemical compositions on conversion performance and fuels and bioproduct yields,[6,7,10,20,29] these results further reinforce the notion that biomass composition will be a major factor in the final selling price of biofuels from any method of microalgal biomass conversion (aqueous or otherwise) and should be a foundation upon which process analysis is conducted (experimental or modeling).

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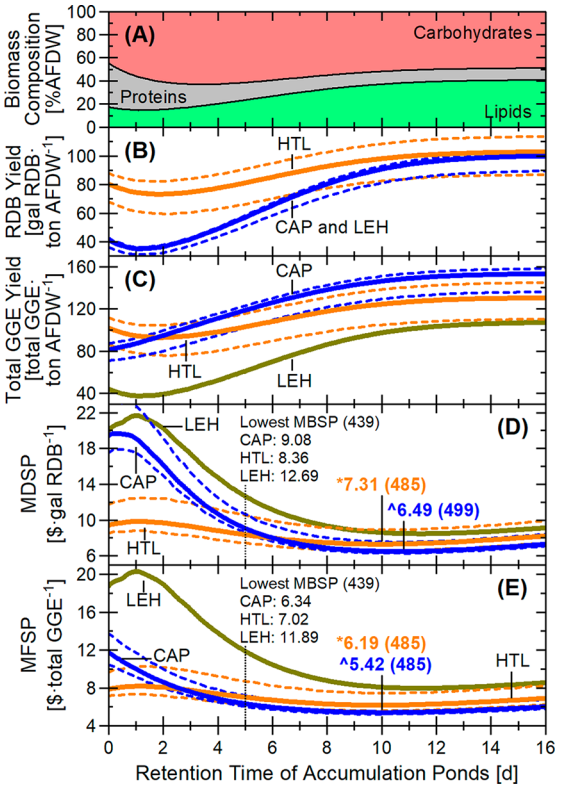

Figure 3. Average annual (A) biomass composition (beginning as only functional components at time 0), (B) RDB yield, (C) total GGE yield, (D) MDSP, and (E) MFSP for *C. vulgaris* as a function of retention time of the carbon-accumulation ponds. HTL, CAP, and LEH biorefineries were sized to process an average of 190 000 ton AFDW·yr^{-1} of biomass. CAP and LEH exhibited identical RDB yields. Uncertainty analysis results for HTL and CAP are shown as 5th and 95th percentiles (dashed lines); uncertainty for LEH was not performed. Results corresponding to the lowest predicted MBSP of *C. vulgaris* (439 $·ton AFDW^{-1}) are indicated with the dotted line and labeled in (D) and (E). Lowest predicted MDSP and MFSP results are labeled (orange and with * for HTL; blue and with ^ for CAP; not indicated for LEH), with the corresponding MBSP shown next to it in parentheses. All prices are in 2011US$. Results for *S. acutus* and *N. granulata* and method details are provided in SI Section S5.

3.3. Integrated Modeling for System Optimization.

Independent optimization of cultivation (e.g., minimizing MBSP) and downstream processing (e.g., minimizing MFSP for a feedstock with a specific biochemical composition) is a barrier to the advancement of the microalgae-to-biofuel system as a whole. This fact is illustrated by the lack of alignment between biochemical compositions with the lowest MBSPs and biochemical compositions that yield the lowest MDSPs and MFSPs (Figure 3). Optimal MDSPs and MFSPs for each process and microalgae species were instead found at compositions with lipid accumulation beyond those with the lowest MBSP (Figure 3B–E for *C. vulgaris*, SI Figures S3 and S4 for *S. acutus* and *N. granulata*, respectively). MBSP still remains the dominant contributor to fuel selling prices (52.4–87.8% of MDSP across both CAP and HTL of all compositions and species analyzed; SI Figure S5), which is in agreement with previous studies (74% of MDSP for HTL;[19] 70% of MFSP for CAP[20]). However, results suggest that designing cultivation systems to supply biomass at the lowest MBSPs (e.g., as a primary cost reduction measure) is unlikely to optimize fuel prices for current conversion technologies given the resultant compositions were suboptimal for both CAP and HTL; the development of conversion processes capable of improved protein valorization may reduce this tension between MBSP and MDSP/MFSP. Overall, the trends in optimal fuel prices are relatively consistent across processing technologies and microalgae (Figure 3D–E), with minimum MDSPs and MFSPs with storage polymers at 86–99% and 82–89% of species' carbohydrate and lipid storage capacity, respectively (Figure 3, SI Figure S1).

In addition to optimal biochemical compositions for each microalga in each conversion process, another key finding is that each conversion pathway (HTL vs CAP) has feedstock compositional spaces in which it outperforms the alternative process based on key metrics (RDB yield, total GGE yield, MDSP, and MFSP; Figure 4). Specifically, HTL outperforms

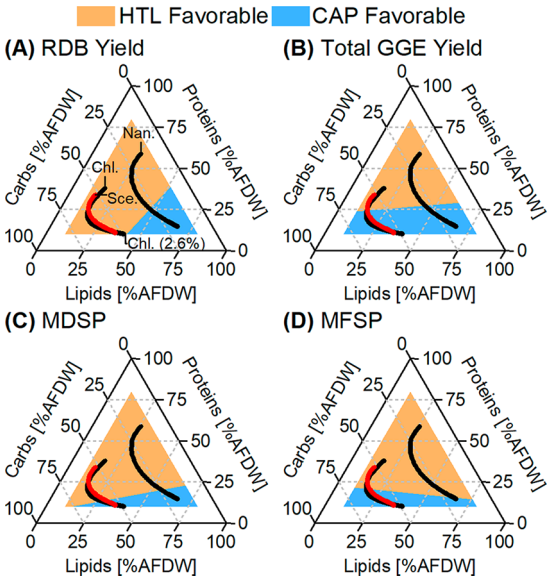

Figure 4. Comparisons between integrated process modeling of HTL and CAP for (A) RDB yield, (B) total GGE yield, (C) MDSP, and (D) MFSP. Compositional regions favoring one conversion process over the other are highlighted in orange (HTL favorable) or blue (CAP favorable), within 10–80%AFDW of each component as a rough range of compositions that may ultimately be achievable through cultivation. The coordinates (%AFDW lipid, carbohydrate, protein) of all points on ternary plots sum up to 100%AFDW exactly. Compositional trajectories for each of the three microalgae included in this study—*C. vulgaris* (Chl.), *S. acutus* (Sce.; differentiated with red lines), and *N. granulata* (Nan.)—are labeled in (A) and shown in (A–D). Numerical results for MDSP and MFSP, along with uncertainty analysis results, are available in SI Figures S2–S5).

CAP for higher protein biomass, largely due to the conversion of proteinaceous biomass into biocrude fuel-intermediates by HTL (>40% of proteins are converted to biocrude;[7] SI Table S3); this additional yield, however, is accompanied by increased biocrude nitrogen content[6,7] which may create challenges for hydrotreating for fuel production (e.g., increased nitrogen content in HTL RDB which may require additional hydro-denitrogenation steps[30]). In CAP, the protein fraction is managed as residual biomass and valorized via AD, which achieves markedly lower levels of energy production (by way of biogas) per gram of proteinaceous material.[20,22] Conversely, CAP outperforms HTL for biomass with higher concentrations of storage polymer due to the mechanisms of fuel production in CAP including (i) a less energy intensive pathway for the

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conversion of lipids to RDB and naphtha through solvent extraction followed by catalytic hydrotreating (as compared to subcritical water reformation followed by catalytic hydrotreating in HTL[19]); and (ii) targeted valorization of carbohydrates through an ethanol fermentation pathway (as compared to lower biocrude yield from carbohydrates through HTL; around 17% of carbohydrates converted to biocrude; SI Table S3[7]). Furthermore, the biochemical fractionation approach pursued by CAP also allows for the production of valuable coproducts beyond fuels from each of the product streams.

In addition to biochemical compositions in which one conversion technology out-performs another (CAP favorable vs HTL favorable sections of Figure 4), it is also important to consider the magnitude of those differences. For instance, in areas where HTL is more favorable than CAP (i.e., higher protein compositions), the percentage difference between the two technologies (for MDSP and MFSP) is much larger than in areas where CAP is more favorable than HTL (Figure 3D,E). This observation is, in part, driven by the more rapid changes in biochemical composition in the first few days of carbon accumulation, as opposed to slower changes in composition after longer periods of carbon accumulation (Figures 2A and 3A). HTL can be considered as a viable alternative to CAP, with the minimum MFSP for CAP and HTL, respectively, at 5.42 and 6.19 $·total GGE^{-1} for *C. vulgaris*, 6.09 and 6.93 $·total GGE^{-1} for *S. acutus*, and 4.68 and 4.67 $·total GGE^{-1} for *N. granulata* (results for minimum MDSP follow the same trends; SI Figures S3 and S4). Beyond TEA results, it is important to note that other factors not mechanistically modeled in this analysis may also play a role in system design. For instance, stochastic or qualitative factors should also be considered, including culture stability (e.g., resistance to crashes) in the accumulation ponds at longer retention times[31] and challenges in scaling up either downstream process.[3] On-going and future research may circumvent these challenges (e.g., metabolic engineering of microalgae to achieve target compositions without the need for long-term nutrient starvation),[32] the value of which can only be quantified through integrated modeling platforms linking cultivation and downstream processing (as developed in this study). Altogether, these results demonstrate that neither downstream conversion process is inherently more favorable than the other, and that both are likely to be viable within biomass compositional regions more suited to the principal mechanisms of the conversion technology and whether or not coproducts may be included in the overall process integration (Figure 4).

3.4. Prioritization of Research and Development Pathways. The analysis presented here has demonstrated an overarching modeling approach aiming to more rigorously track variations in microalgae species, biomass compositions, cultivation kinetics, and conversion pathways to improve the technical robustness of microalgal biofuel system models in order to highlight paths to improve the economic viability of these processes. As we seek to further advance such systems, the relative importance of individual factors can be characterized via sensitivity analysis. Final projections of MFSP and MDSP are sensitive to both cultivation and downstream processing parameters that influence biochemical composition and the conversion efficiencies of storage products to biofuels, even more so than static process farm/plant design decisions not directly connected to biomass compositions (SI Figures S6 and S7). For cultivation, the relative importance of individual growth and carbon storage parameters (kinetic, which relates to areal productivity, and stoichiometric) varied as a function of retention times (SI Figure S6). In particular, maximum specific storage rates (i.e., kinetics) were of greater importance at short retention times and maximum specific storage capacities (i.e., stoichiometric) were of greater importance at longer retention times (SI Figure S6, right column). Future work targeting increased areal productivities in scalable cultivation systems should include monitoring of biochemical composition over 24 h cycles to reduce the uncertainty around growth and carbon storage kinetics: a necessary step in improving the accuracy of MBSP projections, as well as adjust N and P loading to match the specific needs of the individual cultivated species (N and P were 2.3−3.6% and 0.89−1.4% of MBSP, respectively).

For conversion processes, MFSP and MDSP for HTL are particularly sensitive to biocrude yields from carbohydrates (5th−95th percentiles of Spearman's *rho* values of −0.77 to −0.14 for MFSP and −0.77 to −0.13 for MDSP) and lipids (5th−95th percentiles, −0.78 to −0.23 and −0.79 to −0.24 for MFSP and MDSP, respectively; SI Figure S7). Sensitivity of HTL yields to lipid content can be expected due to the (approximately) linear response of biocrude yields with increasing lipid content as found in previous work.[6] Sensitivity to carbohydrate content, however, is likely the result of the rapid rate of carbohydrate storage, which helped drive down MBSP and, in the case of CAP, increased ethanol yields. Given the limited energetic benefit of carbohydrates to HTL products,[6,7] alternative pretreatment or prefractionation methods should be explored to better valorize the carbohydrates prior to HTL conversion (e.g., hybrid CAP-HTL processes) to take better advantage of this early lipid and carbohydrate storage. The MFSP from CAP was most sensitive to fermentable carbohydrates hydrolysis efficiency (5th−95th percentiles of Spearman's *rho* values of −0.63 to −0.10; SI Figure S7). For carbohydrate hydrolysis, higher yields may be achieved by exploring alternative acids and other potential catalysts, as well as alternative conditions (acid loading, temperature, retention times). A previously unreported design parameter to which CAP MFSP and MDSP were sensitive was the C:N design ratio for AD (5th−95th percentiles of Spearman's *rho* values of 0.10−0.51 for MFSP and 0.11 to 0.51 for MDSP). Maintaining consistent digester feedstock and loading could help the microbial community acclimate to the waste stream and maintain stable digester performance at the lower C:N limit. MDSP of CAP was also sensitive to RDB density and neutral lipid extraction efficiency. The former was expected given RDB liquid density would influence the volumetric production of RDB after conversion from mass unit inputs of biomass, but this finding also supports the need for further efforts to better characterize CAP RDB physical properties.

This work has demonstrated the potential viability of HTL and CAP conversion processes or, more broadly, both whole biomass processing and fractionation pathways for microalgal biomass conversion to biofuels. This finding supports continued investment in research and development of such multiple competitive conversion pathways to reduce long-term risk and provide industry with a portfolio of technological solutions that can be tailored to locality- and market-specific scenarios. Specific recommendations for whole biomass processing techniques (e.g., HTL) include a need to reduce

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uncertainty surrounding conversion parameters of individual compounds (e.g., stored carbohydrates), which can be achieved by expanding the library of compositions and microalgae species used for model calibration and validation while also developing predictions for biocrude oil and hydrotreated RDB product quality. Future work transitioning away from smaller-scale experimental samples toward pilot-scale or continuous-flow demonstrations would also provide higher fidelity prediction models for integrated system design, as well as expanding the scope of study to better understand the influence of nutrient recycle using coproduct streams on overall process economics. For biomass with higher concentrations of storage products, fractionation approaches (i.e., CAP) could potentially provide a platform for more economical biofuels through a portfolio of valuable products from the separated fractions as reported in emerging literature, such as fermentation of sugars to produce succinic acid instead of ethanol, isolating algal sterols (e.g., for conversion to surfactants), or upgrading of a portion of lipids to polyurethanes.[10,33] Moving toward higher-value coproducts beyond fuels could expand the range of biochemical compositions within which the CAP pathway achieves the lowest fuel selling prices, and may be a critical consideration in the pursuit of fuel costs on the order of 3 $·total GGE^{-1} or lower (which neither conversion scenario demonstrated here when focused exclusively on fuels and lower-value CHP coproducts). Another key opportunity for fractionation-type processes would be the development of improved methods for the valorization of the protein fraction of biomass—one possible approach for this would be the integration of HTL-type thermochemical sidestream processes for conversion of the proteinaceous fraction to biocrude oil, thereby replacing AD as the primary treatment for protein residuals.

Ultimately, cultivating biomass compositions to achieve the lowest MBSP or to maximize lipid content has been shown through this analysis to lead to suboptimal fuel production costs, demonstrating the clear need to leverage integrated modeling platforms to advance microalgae biofuel systems as a whole. Through a combination of rigorous experimentation and integrated process modeling, multiple microalgal conversion platforms may continue to be advanced and contribute to a portfolio of economically viable biofuels.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03663.

Additional method details and supporting results (PDF)

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Notes

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■ REFERENCES

(1) National Research Council. *Transitions to Alternative Vehicles and Fuels*; The National Academies Press: Washington, DC, 2013.

(2) U.S. Department of Energy. *National Algal Biofuels Technology Roadmap*, DOE/EE-0332; U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program Office: Washington, DC, 2010.

(3) U.S. Department of Energy. *National Algal Biofuels Technology Review*, DOE/EE-1409; U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office: Washington, DC, 2016.

(4) Moody, J. W.; McGinty, C. M.; Quinn, J. C. Global Evaluation of Biofuel Potential from Microalgae. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (23), 8691−8696.

(5) 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.

(6) 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.

(7) 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.

(8) 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.

(9) 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.

(10) Laurens, L. M. L.; Markham, J.; Templeton, D. W.; Christensen, E. D.; Wychen, S. V.; Vadelius, E. W.; Chen-Glasser, M.; Dong, T.; Davis, R.; Pienkos, P. T. Development of Algae Biorefinery Concepts for Biofuels and Bioproducts; a Perspective on

DOI: 10.1021/acs.est.8b03663
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Process-Compatible Products and Their Impact on Cost-Reduction. *Energy Environ. Sci.* **2017**, *10* (8), 1716−1738.
(11) U.S. Department of Energy. *Bioenergy Technologies Office Multi-Year Program Plan: March 2016*, DOE/EE-1385; U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office: Washington, DC, 2016.
(12) National Research Council. *Sustainable Development of Algal Biofuels in the United States*, 10.17226/13437; The National Academies Press: Washington, DC, 2012.
(13) Gerber, L. N.; Tester, J. W.; Beal, C. M.; Huntley, M. E.; Sills, D. L. Target Cultivation and Financing Parameters for Sustainable Production of Fuel and Feed from Microalgae. *Environ. Sci. Technol.* **2016**, *50* (7), 3333−3341.
(14) Béchet, Q.; Shilton, A.; Guieysse, B. Maximizing Productivity and Reducing Environmental Impacts of Full-Scale Algal Production through Optimization of Open Pond Depth and Hydraulic Retention Time. *Environ. Sci. Technol.* **2016**, *50* (7), 4102−4110.
(15) Davis, R. E.; Fishman, D. B.; Frank, E. D.; Johnson, M. C.; Jones, S. B.; Kinchin, C. M.; Skaggs, R. L.; Venteris, E. R.; Wigmosta, M. S. Integrated Evaluation of Cost, Emissions, and Resource Potential for Algal Biofuels at the National Scale. *Environ. Sci. Technol.* **2014**, *48* (10), 6035−6042.
(16) Davis, R.; Markham, J.; Kinchin, C.; Grundl, N.; Tan, E. C.; Humbird, D. *Process Design and Economics for the Production of Algal Biomass: Algal Biomass Production in Open Pond*, Technical Report NREL/TP-5100-64772; National Renewable Energy Laboratory: Golden, CO, 2016.
(17) Guest, J. S.; van Loosdrecht, M. C. M.; Skerlos, S. J.; Love, N. G. Lumped Pathway Metabolic Model of Organic Carbon Accumulation and Mobilization by the Alga Chlamydomonas Reinhardtii. *Environ. Sci. Technol.* **2013**, *47* (7), 3258−3267.
(18) Davis, R.; Aden, A.; Pienkos, P. T. Techno-Economic Analysis of Autotrophic Microalgae for Fuel Production. *Appl. Energy* **2011**, *88* (10), 3524−3531.
(19) Jones, S.; Zhu, Y.; Anderson, D.; Hallen, R.; Elliott, D.; Schmidt, A.; Albrecht, K.; Hart, T.; Butcher, M.; 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*, Technical Report PNNL-23227; Pacific Northwest National Laboratory, Richland, WA; National Renewable Energy Laboratory, Golden, CO, 2014.
(20) Davis, R.; Kinchin, C.; Markham, J.; Tan, E. C. D.; Laurens, 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*, Technical Report NREL/TP-5100-62368; National Renewable Energy Laboratory: Golden, CO, 2014.
(21) Tao, L.; Markham, J. N.; Haq, Z.; Biddy, M. J. Techno-Economic Analysis for Upgrading the Biomass-Derived Ethanol-to-Jet Blendstocks. *Green Chem.* **2016**, *19* (4), 1082−1101.
(22) Shoener, B. D.; Zhong, C.; Greiner, A. D.; Khunjar, W. O.; Hong, P.-Y.; Guest, J. S. Design of Anaerobic Membrane Bioreactors for the Valorization of Dilute Organic Carbon Waste Streams. *Energy Environ. Sci.* **2016**, *9* (3), 1102−1112.
(23) 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.
(24) Sills, D. L.; Paramita, V.; Franke, M. J.; Johnson, M. C.; Akabas, T. M.; Greene, C. H.; Tester, J. W. Quantitative Uncertainty Analysis of Life Cycle Assessment for Algal Biofuel Production. *Environ. Sci. Technol.* **2013**, *47* (2), 687−694.
(25) Pérez-López, P.; Montazeri, M.; Feijoo, G.; Moreira, M. T.; Eckelman, M. J. Integrating Uncertainties to the Combined Environmental and Economic Assessment of Algal Biorefineries: A Monte Carlo Approach. *Sci. Total Environ.* **2018**, *626*, 762−775.
(26) Cook, S. M.; Skerlos, S. J.; Raskin, L.; Love, N. G. A Stability Assessment Tool for Anaerobic Codigestion. *Water Res.* **2017**, *112*, 19−28.
(27) Loftus, S. E.; Johnson, Z. I. Cross-Study Analysis of Factors Affecting Algae Cultivation in Recycled Medium for Biofuel Production. *Algal Res.* **2017**, *24*, 154−166.
(28) Mckay, M. D.; Beckman, R. J.; Conover, W. J. A Comparison of Three Methods for Selecting Values of Input Variables in the Analysis of Output From a Computer Code. *Technometrics* **2000**, *42* (1), 55−61.
(29) Laurens, L. M. L.; Slaby, E. F.; Clapper, G. M.; Howell, S.; Scott, D. Algal Biomass for Biofuels and Bioproducts: Overview of Boundary Conditions and Regulatory Landscape to Define Future Algal Biorefineries. *Ind. Biotechnol.* **2015**, *11* (4), 221−228.
(30) Biller, P.; Sharma, B. K.; Kunwar, B.; Ross, A. B. Hydroprocessing of Bio-Crude from Continuous Hydrothermal Liquefaction of Microalgae. *Fuel* **2015**, *159*, 197−205.
(31) White, R. L.; Ryan, R. A. Long-Term Cultivation of Algae in Open-Raceway Ponds: Lessons from the Field. *Ind. Biotechnol.* **2015**, *11* (4), 213−220.
(32) Ajjawi, I.; Verruto, J.; Aqui, M.; Soriaga, L. B.; Coppersmith, J.; Kwok, K.; Peach, L.; Orchard, E.; Kalb, R.; Xu, W.; Carlson, T. J.; Francis, K.; Konigsfeld, K.; Bartalis, J.; Schultz, A.; Lambert, W.; Schwartz, A. S.; Brown, R.; Moellering, E. R. Lipid Production in *Nannochloropsis Gaditana* Is Doubled by Decreasing Expression of a Single Transcriptional Regulator. *Nat. Biotechnol.* **2017**, *35* (7), 647.
(33) Knoshaug, E.; Mohagheghi, A.; Nagle, N.; Stickel, J.; Dong, T.; Karp, E.; Kruger, J.; Brandner, D.; Manker, L.; Rorrer, N.; Hyman, D.; Christensen, E.; Pienkos, P. Demonstration of Parallel Algal Processing: Production of Renewable Diesel Blendstock and a High-Value Chemical Intermediate. *Green Chem.* **2018**, *20* (2), 457−468.

DOI: 10.1021/acs.est.8b03663
Environ. Sci. Technol. XXXX, XXX, XXX−XXX