Supplementary Information (SI) for Sustainable Energy & Fuels.

Supporting information

Saline Microalgae Cultivation for the Coproduction of Biofuel and Protein in the United States: An Integrated Assessment of Costs, Carbon, Water, and Land Impacts

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Contents

1. System Description and Key Assumptions

In this section, the system is described in detail in the following subsections. Table S1 summarizes the primary parameters and assumptions.

Table S1. Summary of key parameters and assumptions

Parameters	**Inputs/Assumptions**	**References**
CO_2 capture and transport		
Capture energy	0-1.06 MJ/kg CO_2 depending on source flue gas CO_2 concentration	[1]
Compression energy	0.11 kWh/kg CO_2	[1]
Algae cultivation and harvesting		
Individual pond size	10 acre	[2]
Individual algae farm size	1,000 to 38,500 acre (varies by individual sites; 3,900 acres on average)	[2]
CO_2 utilization efficiency	75%	[3]
Strain	High-protein *Tetraselmis* striata	[4]
Microfiltration + FO membrane power	0.0055 kWh/L	[5]
Deep well injection power	0.0012 kWh/L	[6]
Algae strain salinity tolerance	55 g/L	[4]
Pond depth	20 cm	[7]
Harvested density	0.5 g ash free dry weight (AFDW)/L	[4]
Biomass concentration after dewatering for fuel only production	20 wt% solids AFDW	[2]
Biomass concentration after dewatering for protein concentrate (PC) and fuel production	10 wt% solids AFDW	[2]
Salinity target in biomass product (achieved via washing in final dewatering stage)	15 g/L	[2]
Protein extraction and fuel conversion by HTL		
Total fuel yield (diesel, sustainable aviation fuel (SAF), and naphtha)	107 GGE/ metric ton AFDW for fuel only; 57 GGE/metric ton AFDW for fuel and protein coproduction	[2]
Diesel	14 GGE/metric ton AFDW for fuel only; 10 GGE/metric ton AFDW for fuel and protein coproduction	[2]
SAF	64 GGE/metric ton AFDW for fuel only; 34 GGE/metric ton AFDW for fuel and protein coproduction	[2]
Naphtha	24 GGE/metric ton AFDW for fuel only; 13 GGE/metric ton AFDW for fuel and protein coproduction	[2]
Protein product yield	0.34 metric ton/metric ton feedstock AFDW for fuel and protein coproduction	[2]

1.1 CO_2 Capture and Transport

The energy required for upstream point source CO_2 capture is influenced by two key factors: the CO_2 demand for microalgal cultivation, which varies by individual site, and the energy necessary to capture and transport 1 kg of CO_2, which depends on the CO_2 release concentration at the local point source. The CO_2 demand is calculated based on the carbon content in biomass and CO_2 utilization efficiency, with detailed calculations available below. The capture energy for 1 kg of CO_2 is derived from regression relationships between energy use and CO_2 concentrations based on data from Carnegie Mellon University's Integrated Environmental Control Model under a future technology scenario.[1] We consider various CO_2 sources for algae cultivation, and calculated capture energy per kg of CO_2 with different sources can be found in Table S2. In addition, a CO_2 compression energy of 0.11 kWh/kg CO_2 is added for all sources to achieve a pressure of 137 bar suitable for high-pressure pipeline transport.

The CO_2 demand for microalgal cultivation is calculated by the equation below:

$$D_{CO2} = \frac{B \times W_{CBio}}{E_{CO2} \times W_{CCO2}} \qquad \text{…Eq. S1}$$

where:

D_{CO2} = CO_2 demand (M ton/yr)
B = Ash-free dry weight (AFDW) biomass (M ton/yr)
W_{CBio} = Carbon fraction in biomass (0.48 – high protein algae, 0.55 - average)
E_{CO2} = CO_2 utilization efficiency (0.75)
W_{CCO2} = Carbon fraction in CO_2 (0.273).

Table S2. CO_2 capture cost and energy for current and future scenarios by source type.

Source	CO_2 Concentration (%)	CO_2 Compression Energy (kWh/metric ton CO_2)	Cost of Capture (\$/metric ton CO_2)	CO_2 Capture Energy (MJ/kg CO_2)

			Current	Future	Current	Future
Natural gas combined cycle	3.5	Electrical energy: 107	76	50.92	3.51	1.06
Pulverized coal	12		47	31.49	2.42	0.69
Integrated gasification combined cycle	40		29	19.43	1.42	0.45
Bioethanol	99		0	0	0.00	0.00
Refining	15		43	28.81	1.39	0.64
Hydrogen	45		28	18.76	1.39	0.43
Ammonia	45		28	18.76	1.78	0.43
Steel	22.5		37	24.79	1.72	0.55
Cement	25		35	23.45	0.00	0.53
Renewable natural gas processing	99		0	0	0	0

*Zero values artificially assigned for bioethanol and renewable natural gas; otherwise, values are sourced from [8-10]

1.2 Algae Cultivation and Harvesting

The algae biomass cultivation and harvesting system, which encompasses algae growth in open ponds, dewatering, and seasonal biomass storage, are shown in Figure S1. Inoculated biomass is cultivated in multiple 10-acre raceways to achieve a density of 0.5 g ash-free dry weight (AFDW)/L, using captured CO_2 along with added macro-nutrients urea and diammonium phosphate. Biomass is harvested at a rate that aligns with seasonal cultivation productivity, processing the biomass through settlers, membranes, and centrifuges for concentration from 0.05 wt% to 20 wt% AFDW for the fuel-only case, and to 10 wt% for the fuel and PC coproduction case. Exogenous ash is partially removed in a basket filter, while salt content is reduced by a washing step utilizing freshwater from forward osmosis (FO) desalination unit. Paddlewheel circulation ceases overnight, as this consumes substantial electricity in the algae farm. Seasonal storage of the dewatered biomass occurs during peak cultivation times, predominantly in summer and, to a lesser extent, in spring and fall, varying by location. This storage strategy employs a wet anaerobic method to minimize biomass degradation, thereby ensuring consistent throughput capacity for subsequent conversion processes.

Groundwater with a salinity of 40,000 mg/L total dissolved solids (TDS) or less is delivered to the cultivation ponds. The FO membrane unit is used to process the pond blowdown water, which is the volume of water removed and replaced to regulate the salinity of the pond media when it exceeds 55,000 mg/L, aligning with the strain's salinity tolerance (7). In addition, the FO membrane unit is employed to provide a freshwater wash stream prior to the final dewatering step to reduce salinity to 15,000 mg/L TDS for protein and fuel coproduction, safeguarding downstream conversion equipment and mitigating protein quality concerns. The replacement water comes from the freshwater fraction of the FO unit, while concentrate brine fraction from the FO unit is disposed of via deep well injection. More details on the FO system are provided in the Section 1.2.1 below.

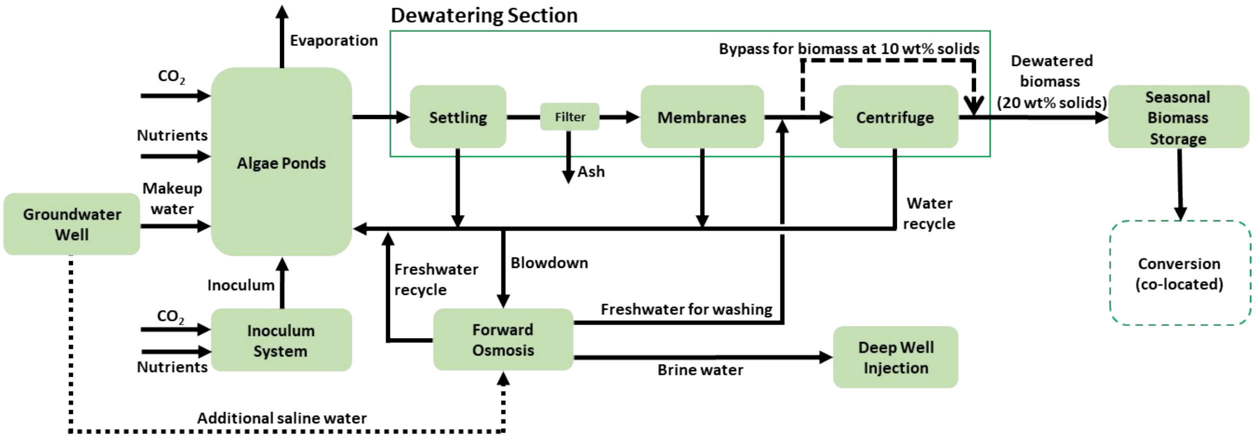

Figure S1. Flow diagram of algae biomass cultivation and harvesting

1.2.1 Forward osmosis and deep well injection

Desalination technologies are categorized into two types: thermal-based and membrane-based. Thermal-based technologies require more energy compared to membrane-based technologies due to the evaporation and condensation of water vapor. However, there are exceptions to this rule, such as membrane distillation and membrane crystallization technologies, which have relatively high specific energy consumption. In this study, the FO membrane desalination method is chosen due to its cost-effectiveness and lower energy consumption when compared to other brine treatment methods. Nevertheless, rigorous pretreatment is necessary to prevent scaling and fouling problems, and the FO membrane system may require energy-intensive draw solution regeneration.[6]

Desalination processes produce a significant amount of brine, and various brine disposal methods have been developed, including surface water discharge, sewer discharge, deep well injection (DWI), evaporation ponds, and land application. In this study, DWI is selected to dispose of saline water after the FO membrane system since it is inexpensive ($0.54-2.65/m3 of brine rejected) and has minimal impact on marine systems compared to surface water discharge. However, the DWI method requires an appropriate isolated aquifer structure, suitable geohydrology, and is not recommended for areas with high seismic activity. Sewer discharge method has been excluded in this study as it is rarely used in saline water desalination plants. Although evaporation ponds and land application do not affect marine systems, they are limited by weather conditions and irrigation needs and may pose a risk of soil and groundwater pollution. Evaporation ponds are only suitable for dry-weather areas, and land application is only recommended for small inland desalination plants due to seasonal irrigation needs and climate.[6]

In this study, it is assumed that blowdown undergoes pretreatment using microfiltration, followed by desalination using the FO membrane system. After treatment, approximately 82% of blowdown is recycled as freshwater, while the remaining concentrated saline water is disposed of by injecting it into deep wells. The throughput of an FO membrane system can vary based on various factors such as the type of membrane, the properties of the feed and draw solutions, operating conditions, and specific applications. Here, the throughput, retention time, lifespan, and life cycle inventory (LCI) for the FO membrane system are obtained from a previous study and are summarized in Table S3.[11] The LCI of microfiltration production is omitted from the study for simplification purposes since electricity usage is the main contributor for microfiltration systems according to literature.[12]

Table S3. Specification of the FO membrane system

Specification	Quantity	Units
Throughput	7	L/m^2 FO membrane
Retention time	1	day
Lifespan of FO membrane	5	years

1.3 Algae Conversion to Fuel and Protein Coproducts via HTL

This study investigates two downstream processing cases as shown in Figure S2: a fuel-only case and a fuel and protein coproduction case. The fuel-only case routes the algae slurry at 20 wt% solids content directly to HTL, producing biocrude, solids, aqueous, and gas outputs. For the fuel-only case, the biocrude is upgraded by hydrotreating and hydrocracking to produce diesel (17 wt%), sustainable aviation fuel (SAF) (60 wt%), and naphtha (23 wt%). The solid phase undergoes acid digestion for phosphorus recovery. The aqueous phase is recycled back to the algae farm directly from the HTL process. The HTL off-gas is used for heating and hydrogen generation. In the fuel and protein coproduction case, algal slurry at 10 wt% solids content undergoes high-pressure homogenization (HPH) and pH shift for protein extraction.[13] The protein-extracted algae are then sent to the HTL process, where the outputs are similarly processed as the fuel-only case, with aqueous outputs routed to an anaerobic digester to produce biogas for process heating. More detailed descriptions can be found in our previous study.[2]

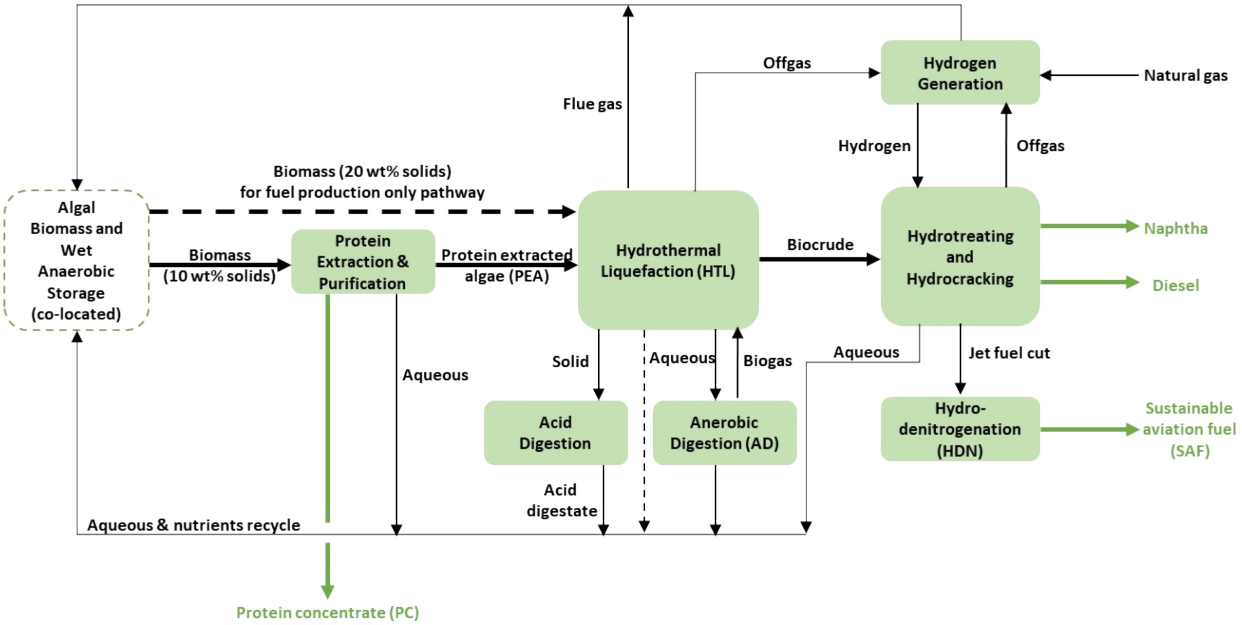

Figure S2. Flow diagram of saline microalgae protein extraction and HTL fuel conversion.

1.4 Potential bioproducts

In the three fuel and PC coproduction cases, it is assumed that the protein coproduct either directly substitutes for soybean and whey PC or is further processed to replace chicken meat. The selling prices (SPs) and market sizes for soybean PC, whey PC, and chicken meat are summarized in Table S4. The detailed processing procedure from PC to chicken meat alternatives (CMAs) can be found in Section 2.3.2 of the SI. Digestible protein is selected as the basis for estimating the replacement ratio of algae PC to substitute the three protein targets. Specifically, digestible protein is determined by multiplying the protein content by its digestibility coefficient. The detailed information about replacement ratios is Section 1.4.1 in the SI.

Table S4. SPs and market sizes associated with microalgae PC replacement targets

	Soybean PC[*]	Whey PC	Chicken meat
Price ($/kg product)	1.3	3.7	1.6
Price ($/kg protein)	2.0	6.1	5.0

References	14	14, 15	16
2030 forecast global market size (million metric ton [MMT]/year)	13	3.5	175
References	14, 17	18, 19	20, 21

*Soybean PC market size is based on protein ingredient market size.

1.4.1 Replacement Ratios by Using Digestible Protein

The functional unit for the protein coproduct and the target protein products is defined as 1 kg of digestible protein. The replacement ratios for the target protein products are calculated by dividing the digestible protein content of microalgae PC by the digestible protein content of the target protein products. Table S5 summarizes these replacement ratios, using digestible protein as the consistent functional unit.

Table S5. Replacement ratios[a] by using digestible protein as the matching functional unit

	Coproduct targets used in this study			Potential products from this study	
	Soybean PC	Whey PC 60	Chicken meat	Microalgae PC	Microalgae-based chicken meat alternatives[b]
Protein content	62%-65%	60%	32%	72%	28%
Protein digestibility	95-98%	100%	100%	55%-80%	55%-80%
Replacement ratio by using digestible protein	85%	84%	196%[b]		
References	22, 23	22	24	25-28	29

[a] Replacement ratio = (microalgal PC protein content × microalgal protein digestibility)/(target protein content × target protein digestibility)

[b] 1 unit of microalgae contains 72% protein. The meat alternative consists of 30% dried microalgae PC, 10% dried soybean PC, and 60% water. The protein that can be provided by 1 unit microalgae PC to meat alternative can be calculated by: 1×72%+(10%/30%)×62% = 0.93 unit of protein. The replacement ratio for chicken meat is estimated based on 0.93×avg(55%,80%)/(0.32×100%) = 196%.

2. Life cycle inventories (LCIs)

The LCIs for fuel-only and fuel and PC coproduction cases in site 1670 which has median GHG emissions can be found in the Table S6. The LCIs for the protein coproduct replacement targets—soybean PC, whey PC, and chicken meat—are detailed in sections 2.1, 2.2, and 2.3.1, respectively. The processing of algal protein coproduct into chicken meat alternative is discussed in section 2.3.2.

Table S6. LCI of the system with median GHG emissions

Item	**Units**	**Fuel + PC**	**Fuel only**
CO_2 capture, transport, and compression (per kg CO_2)			
Capture	MJ/kg CO_2	1.1	1.1
Transport	kWh/kg CO_2	0.11	0.11
Algae growth (per kg AFDW, with recycling)			
Resource consumption			
Electricity	kg /kg AFDW	0.60	0.61
CO_2	kg/kg AFDW	1.6	1.4
Urea	kg/kg AFDW	0.056	0.020
$(NH_4)_2HPO_4$	kg/kg AFDW	0.0095	0.011
FO membrane	kg/kg AFDW	0.0067	0.0066
Output			
Fresh Water in Biomass	kg/kg AFDW	9.0	4.0
Saline water blowdown	kg/kg AFDW	228	223
Conversion			
Biomass inputs			
Algae biomass	kg AFDW/MJ	0.14	0.076
Energy inputs			
Electricity Demand	kWh/MJ	0.092	0.0060
Natural Gas (Utility)	MJ/MJ	0.20	0.13
Natural Gas (H_2 Production)	MJ/MJ	0.15	0.21
Chemical and water demand			
Sulfuric Acid	kg/MJ	0.0014	0.0021
HT Catalyst (HTL)	kg/MJ	1.7E-05	1.8E-05
Hydrocracking catalyst (HTL)	kg/MJ	3.0E-07	3.0E-07
Membrane Flocculant	kg/MJ	0.0016	0
NaOH	kg/MJ	5.5E-05	2.3E-05
Water (Process demands)	gal/MJ	0.036	0.017
HCl	kg/MJ	1.1E-05	0
Output			
Renewable Diesel	MJ/MJ	0.17	0.18
Naphtha	MJ/MJ	0.22	0.23
SAF	MJ/MJ	0.60	0.60
Protein coproduct	kg/MJ	0.050	0

2.1 LCIs of soybean PC

The soybean PC is extracted from soybean. Soybean PC is the main product from soybean concentration and multiple co-products soybean hulls, soybean crude oil, and soybean molasses are generated in the production process. To assess the environmental impacts associated with soybean PC production, allocation methods have been utilized. However, it is important to note that these allocation methods can introduce uncertainties even if it is the main product. To address this, both mass and economic value allocation methods are employed to estimate environmental impacts.

The LCI of soybean (13% water) farming, harvesting, and transportation to soybean PC production plant is obtained from GREET model. The LCI of soybean PC extraction from soybean (13% water) is obtained from literature,[23] and the LCI data is allocated based on mass or price as shown in Table S7. Detailed LCI based on mass and price allocation can be found in Table S8. The transportation from collection stack to production plant is included in the GREET model for soybean production.

Table S7. Mass and price allocation for soybean PC

	Mass allocation	$ allocation
Soybean PC, 8% water	49.8%	75.4%
Soybean, hulls	6.83%	0.840%
Soybean, crude oil	16.6%	20.7%
Soybean, molasses	26.8%	3.10%

Table S8. LCI of soybean PC production based on mass and price allocation methods

Inputs	Mass allocation	$ allocation	Units
Soybean (water content 13%)	9.23E-01	1.40E+00	kg
Diesel, extraction	3.78E-01	5.72E-01	MJ
Electricity, extraction	2.77E-01	4.19E-01	kWh
Natural gas, extraction	6.64E-01	1.01E+00	MJ
Process water	2.31E-01	3.49E-01	kg
Output			
SPC, 8% water	1.00E+00	1.00E+00	kg

Waste water	1.53E-01	2.32E-01	kg

2.2 LCIs of whey PC

Commercial whey PC is produced by ultrafiltration, evaporation, and drying of liquid whey, with its LCI sourced from literature.[30] The LCI of whey PC can be found in Table S9. In this study, algal coproduct can be used to replace whey PC 60 (60% protein content) due to comparable protein content. Upstream LCI data associated with liquid whey, including milk and cheese production can be found in our previous study.[2] Liquid whey, a byproduct of cheese production, necessitates the use of allocation and system expansion methods to ascertain its environmental impacts. In life cycle assessment (LCA) studies, directly substituting one co-product with another, such as replacing whey PC with microalgae PC, is challenging due to the uncertainties of different methodological approaches. To capture the environmental credits from liquid whey production, both allocation and system expansion methods are employed. For assessing upstream liquid whey's environmental impacts, the GREET model and other literature sources are used.[31, 32] Kim et al. provided a comprehensive LCI for liquid whey on a dry mass basis using three allocation approaches: milk solids weight, plant-specific allocation from survey data, and revenue-based allocation when the first two are not viable.[31] Aguirre-Villegas et al. used economic value, solid content, and nutritional value for allocation when direct system subdivision isn't possible.[32] The system expansion method is another approach to attribute environmental credits to liquid whey production, exemplified by literature that substitutes the environmental impacts from liquid whey with those from barley production.[33] A detailed comparison of greenhouse gas (GHG) emissions from liquid whey production across different sources is presented in Table S10, and economic allocation has been selected to represent the LCA results from liquid whey production.

Table S9. LCI of whey PC production

Input	**Unit**	**WPC35**	**WPC60**	**WPC80**
Whey (6% dry weight)	kg/kg of whey PC	46	80	113
Electricity	kWh/kg of whey PC	0.49	0.56	0.55
Natural gas	MJ/kg of whey PC	3.6	3.8	3.8
Water consumption	kg/kg of whey PC	0.0	1.3	3.1

Table S10. GHG emission result comparison for 1 kg of liquid whey production

	GHG emissions	Units	Notes	Ref.
The production of barley (ready for use at the farm)	0.74	kg CO_2-eq./kg of barley dry mass	System expansion	33
Whey powder	0.82	kg CO_2-eq./kg whey powder	Economic allocation (cheese: 9.53 kg CO_2-eq./kg; 7% is allocated to whey powder)	34
Whey powder	N.A.	kg CO_2-eq./kg whey powder	Mass allocation (33% is allocated to whey powder)	34
Liquid whey	7.35	kg CO_2-eq./kg liquid whey (dry mass)	Process-based mixed allocation method and GREET	31
Liquid whey	8.51	kg CO_2-eq./kg liquid whey (dry mass)	LCI and solid content allocation and GREET	32
Liquid whey	5.28	kg CO_2-eq./kg liquid whey (dry mass)	LCI and nutritional value allocation and GREET	32
Liquid whey	2.04	kg CO_2-eq./kg liquid whey (dry mass)	LCI and economic value allocation and GREET	32

2.3 LCIs of chicken meat products and microalgae protein-based meat alternative

As shown in Figure S3, the system boundary of chicken meat and meat alternative production are confined to processing plants since it is assumed that chicken meat and meat alternative will go through similar processes after processing plants. It is noticeable that additional food quality control may be needed by using open runway pond as the cultivation media. These steps are omitted from this study for simplification purposes.

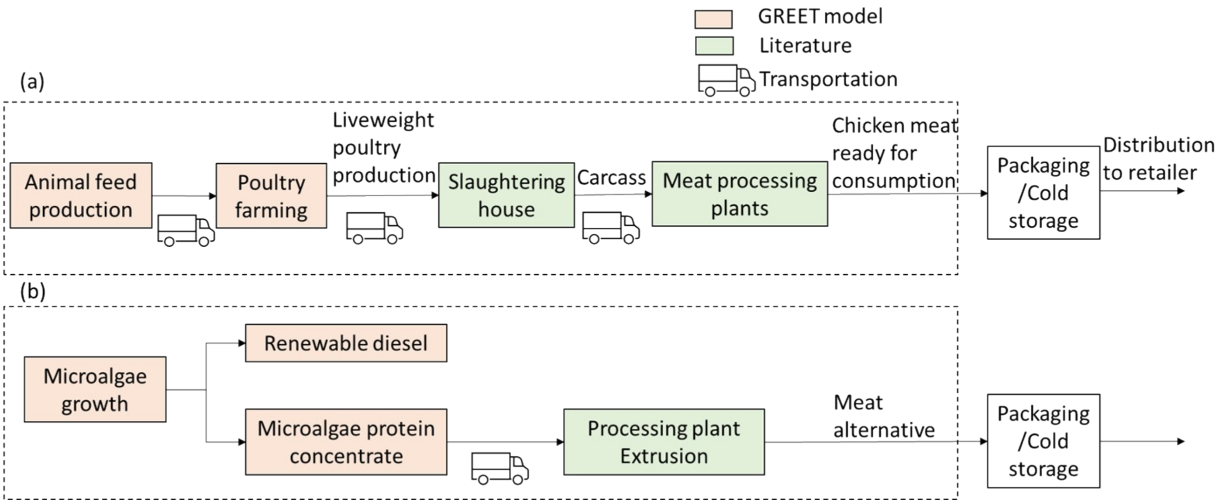

Figure S3. Flow diagram of chicken meat and microalgae-based chicken meat alternative production from cradle to production plant gate (boundary confined within dashed lines)

2.3.1 LCI of Chicken Meat

The microalgae PC co-product can be further processed to replace meat, with chicken considered as the replacement in this study. While beef, pork, and other types of meat could also be potential targets for replacement, they are not investigated here. Chicken meat substitution is selected as an example to demonstrate the potential for GHG emission and cost reduction. The system boundaries for meat alternative and chicken are both from raw material extraction to production plant gate since it is assumed that meat alternative and chicken meat will go through similar processes after production. As shown in Figure S3(a), the LCIs of animal feed production and live poultry farming illustrated in pink color are obtained from GREET model. The LCIs of poultry slaughtering and chicken meat processing shown in green are obtained from literature,[35] and detailed inventories for poultry slaughtering and chicken meat processing, and associated upstream data can be found in Tables S11-S13. The LCI of poultry slaughtering and processing are based on Serbia conditions, and it is assumed that similar processes are used to produce chicken meat in the U.S. due to lack of detailed LCI data for the U.S. Truck is assumed to be used in poultry and poultry product transportation, and the average transportation distance from farm to slaughtering house and from slaughtering house to processing plant are set to be 0.03 kg-km and 0.01 kg-km, respectively. It is noticeable that the transportation energy for carrying carcasses from slaughtering house to processing plant may be higher than the calculated value due to the use of refrigerator and the energy consumption for meat refrigerating is omitted in this study for simplification purposes.

Table S11. LCIs of poultry slaughtering and chicken meat processing

Inputs	Slaughtering house	Meat processing plant	Unit	Notes
Live weight chicken	1.4		kg	
Water	9.0	6.5	kg	
NaOH	0.001	0.016	kg	
NaClO	0.0003	0.004	kg	See sodium hypochlorite (NaClO) section below
Electricity	0.64	1.4	kg	
Diesel	0.33		MJ	
LPG	0.051		MJ	
Natural gas	0.29		MJ	
PVC film E	0.002	0.008	Kg	Omitted from the study for comparison purposes
Polypropylene PP		0.026	Kg	Omitted from the study for comparison purposes
Paper	0.001	0.036	kg	Omitted from the study for comparison purposes
Transport	0.03	0.01	kg-km	
Outputs				
Chicken meat	1	1	kg	
Wastewater with biowastes	0.008	0.007	m^3	See “wastewater treatment” section below

Sodium hypochlorite (NaClO)

Sodium hypochlorite (NaClO) is prepared by reacting dilute caustic soda solution with liquid or gaseous chorine as shown in the stoichiometry below. The reaction is exothermic and after the reaction, the mixture is cooled. The yield is assumed to be close to 100% of theoretical value and the pH adjustment is omitted from the inventory. Detailed LCI can be found in Table S12.

Cl_2+2NaOH -> NaClO + NaCl + H_2O

Table S12. LCI of NaClO

Inputs	Quantity (kg)
Cl_2	0.95
NaOH	1.07
Outputs	Quantity (kg)
NaClO	1.00
NaCl	0.79
H2O	0.24

Wastewater treatment

Slaughterhouse wastes contain high fat and protein content, and different methods have been used to treat the wastes, such as composting, aerobic and anaerobic digestion, alkaline hydrolysis, rendering, and incineration.[36, 37] Here, it is assumed that the waste from slaughterhouse are co-treated with wastewater in municipal wastewater treatment plants. The LCI of municipal wastewater treatment is obtained from literature and the primary unit processes include wastewater collection, screening and grit removal, primary sedimentation, sludge thickening and dewatering, sludge incineration, aeration, secondary clarifiers, and primary disinfection and sodium hypochlorite.[38]

Polyacrylamide (PAM) is used in sludge thickening and dewatering, and the LCI is not available in GREET model. At the industrial scale, water soluble PAM is mainly synthesized by a free radical polymerization process.[39] Free-radical polymerization is a process to produce hydrogels by using low-weight monomers in the presence of a cross-linking agent, and for PAM production, water is often considered a solvent choice.[40] Here, the water media used for radical polymerization is omitted due to lack of data. The energy consumption for radical polymerization of PAM is estimated from the energy consumption for polymerizing superabsorbent polymers.[41] The input material for producing PAM is acrylamide monomer, and nowadays, acrylamide monomer is mainly produced from acrylonitrile by a biological process using enzyme, which has high conversion efficiency and selectivity. As a result, the production of acrylamide is based on the stoichiometry of the reaction listed below, and detailed LCI of PAM is shown in Table S13.

$CH_2{=}CHCN$ (acrylonitrile) + $H_2O \rightarrow CH_2{=}CHC(O)NH$ (acrylamide)

Table S13. LCI of PAM production

Inputs	Quantity	Unit
Acrylonitrile	0.75	kg
Water	0.25	kg
Electricity	4.4	kWh
Outputs		
PAM	1	kg

The environmental impacts of municipal wastewater treatment (1 m^3) are calculated by using GREET model, and GHG emissions and total energy consumption are compared with the results from literature for data quality purposes.[38]

Table S14. LCA result comparison between GREET model and Cashman et al. (2014)

	GREET model	Cashman et al.
Total energy (Btu/m^3)	5,555	7,383
GHG emission (g-CO$_2$/m^3)	395 (excl. biogenic CO$_2$)	470 (excl. biogenic CO$_2$); 960 (incl. biogenic CO2)

2.3.2 LCI of Microalgae Protein-Based Meat Alternatives

Microalgae have higher protein content than meat, and comparable nutrients, such as key amino acid and vitamins. The protein digestibility and absorption of microalgae protein is slightly lower than beef and beef products, and comparable with soybean, wheat gluten, and wheat cereal protein.[42] Plant-based meat alternatives are produced by extrusion, and there are two major extrusion technologies: high moisture extrudates (HME) and low moisture texturized vegetable protein (TVP). The main difference between these two technologies are the moisture content inside the extrusion barrel.[43] In this study, HME technology is selected to represent the process to produce meat alternatives and it undergoes the same process as chicken meat after production plants as shown in Figure S3. The water and energy consumption for processing dried microalgae PC is adjusted from processing dried soybean PC to meat alternative. This adjustment is based on their similar water and energy consumption, as communicated with the expert from DIL-eV.[44] Notably, dried microalgae PC can be only included in meat alternative up to 30% when combined with soybean PC due to its properties, and the moisture content in HME is ~60%. To simplify the model, it is assumed that 30% of chicken meat alternative is from dried microalgae and the rest of 10% is from dried soybean PC, which is recommended by the expert form DIL-eV. [44] Other ingredients, such as seasonings, are not included due to a lack of data. The detailed LCI for producing 1 kg of microalgae-based meat alternative can be found in Table S15.

Table S15. LCI for processing dried microalgae PC to chicken meat alternative

Input	Quantity	Unit
Dried microalgae PC	0.3	kg
Dried soybean PC	0.1	kg
Water	23.4	kg
Electricity consumption	0.29	kWh
Output		

Meat alternative	1	kg
Biowastes	0.033	kg

3. Correction Factor R

The correction factor R is used to measure the hardness to desalinate saline water with different salinity to freshwater, and this factor has been incorporated into WSF calculation to quantify quality-based WSF. Detailed explanation of saline water-based WSF can be found in Section 2.2.7 in the main context, and the relationship between correction factor R and salinity is shown in Figure S4.

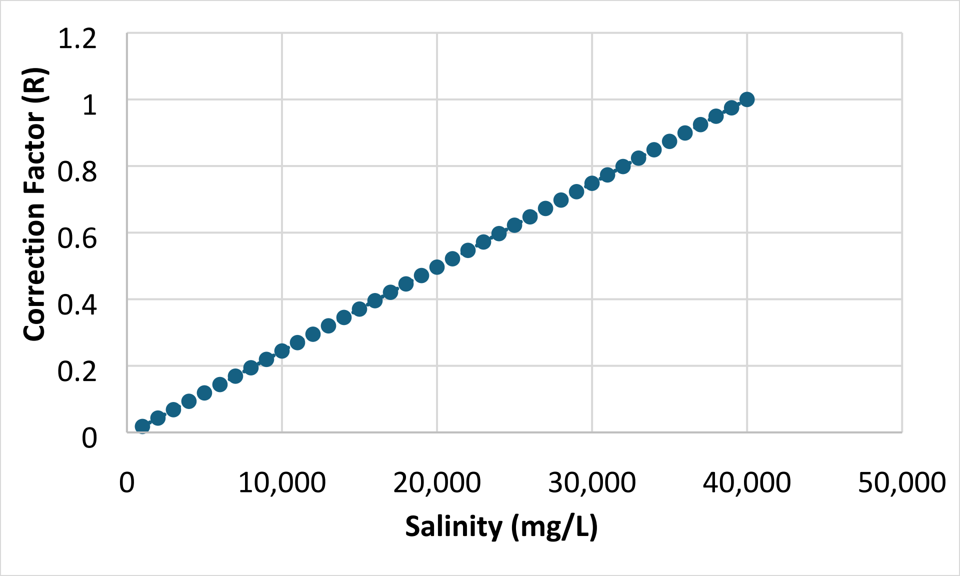

Figure S4. Relationship between correction factor R and salinity

4. Direct Land Use Change and Indirect Land Use Change

The equation below shows the carbon emission calculation for direct land use change (DLUC) of microalgal biorefineries.

$$DLUC = EF_{DLUC} \times Facility\ Size \times EA\ /\ LT\ /FP_{Annual}$$

$$EF_{DLUC} = \sum_{i}^{All\ land\ uses} p_i \times EF_{i2o} \qquad \text{…Eq. S3}$$

DLUC – Annual land use change emissions per unit of fuel (g CO_2-eq./MJ of fuel);
EF_{DLUC} – Direct land use change emissions factor (g CO_2/ha);
Facility Size – (ha);
EA – Energy allocation (%). Only DLUC emissions from the fuel only case are calculated and EA = 100%;
LT – plant lifetime (years). LT of microalgae plant = 30 years;
FP_{Annual} – Annual fuel production (MJ/year);
P_i – percentage of original land use i;
Ef_{i2o} – Carbon emission factor by changing from original land use i to open pond.

Figures S5 illustrates that direct land use change (DLUC) emissions vary between -1.83 g CO_2-eq./MJ and 9.33 g CO_2-eq./MJ, with an average of 1 g CO_2-eq./MJ. In addition, the probabillity of original land use for the selected sites are shown in Figure S6.

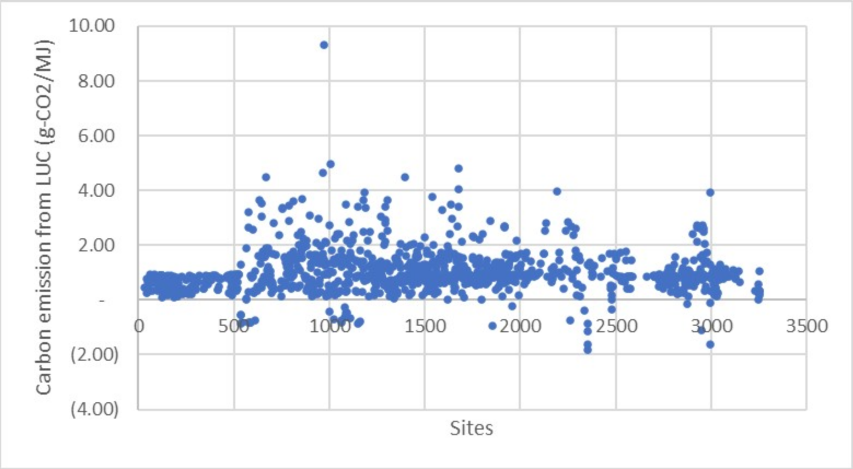

Figure S5. Direct land use change carbon emissions

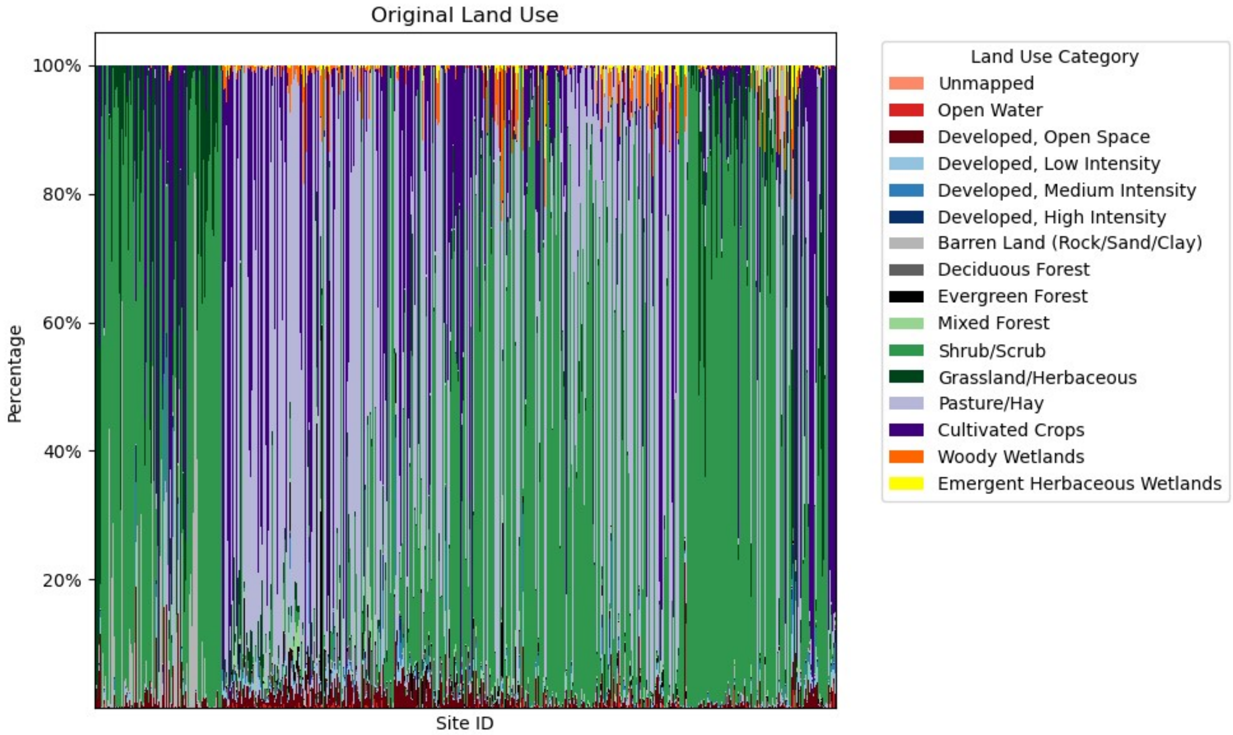

Figure S6. Probability of original land use in each site

Table S16 summarizes the emissions from land use change (LUC) of soybean, milk, and chicken meat production in different sources. The summary aims to provide the LUC emissions from conventional target protein products. While some reviewed studies explicitly attribute emissions to ILUC or DLUC, others do not specify. Emissions attributed to DLUC in some studies may be ILUC in others. For example, if crops replaced by microalgae are cultivated in Brazil, DLUC emissions in Brazil in ILUC in the U.S.

Emissions from ILUC can significantly affect the final results. To exemplify potential ILUC carbon emissions from microalgal biorefineries, we assume the original cropland use is for soybean cultivation. As per Table S16, soybean LUC carbon emissions could vary from 455 to 46,100 kg/ha. In our study, GHG emissions from microalgae cultivation and harvesting range from 50,600 to 117,000 kg/ha. The example represents the larger values of ILUC carbon emissions, and the use of croplands for algae cultivation should be avoided since one of the advantages of algae farms is their ability to grow on marginal land. Quantifying the actual variations is challenging with diverse original crops and hay/pasture productions in different locations.

Table S16. Summary of land use change (LUC) emissions from selected literature

	References	LUC emissions (kg CO_2-eq./kg product)	LUC emissions (kg CO_2-eq./hectare)	Region
Soybean production	Castanheira and Freire (2013)[45] (DLUC)[a]	0.06-17.80	455 - 46,120	Latin America
	Esteves et al. (2016)[46]	0.75-4.27	1,973 - 11,154	Brazil
	U.S. EPA (2010)[47], (ILUC)[a]	0.24	821	United States
	Searchinger and Heimlich (2008)[48]	0.78 - 2.12	2,669 - 7,277	United States
Milk production	Audsley et al. (2009); Flysjö et al. (2012)[49, 50]	0.66-0.83[b]	1430	United Kingdom
Chicken meat production	Schmidt et al. (2011); Flysjö et al. (2012)[50, 51]	1.38-2.11[b]	5,470 - 5,850	Global
	Caro et al. (2018)[52] (DLUC)[a]	0.51	34,800	Brazil
	Ponsioen and Blonk (2012)[53] (DLUC)[a]	2.04	4,900-12,200	Global

[a] If the studies specifically indicate the emissions are from DLUC or ILUC, it is listed in the parenthesis.

[b] Cows coproduce milk and meat, and the carbon emissions from LUC for milk production are calculated based on system expansion method.

5. Nutrient and energy consumption comparison between saline algae and soybean cultivation (per kg AFDW)

Based on the biorefinery-level GHG emission results, conventional soybean PC production generates far fewer GHG emissions than microalgae-based PC. The material and energy consumption for the cultivation of soybeans and microalgae are compared in Table S17, where it is shown that significantly less energy and fewer nutrients are required for the production of 1 kg of AFDW soybean.

Table S17. Energy and nutrients comparison between algae and soybean cultivation

	Algae cultivation	Soybean cultivation	Units per kg AFDW
Electricity	0.60	0.019	kWh
Natural gas	1.8	0.57	MJ
Carbon	0.44	0	kg
Nitrogen	0.054	0.0023	kg
Phosphorus	0.0044	0.0040	kg

6. MSP by accounting protein selling credits and GHG emissions by using economic allocation

To facilitate the comparison with the results from other studies, MSP by accounting protein selling credits and GHG emissions by using economic allocation method are shown in Figures below.

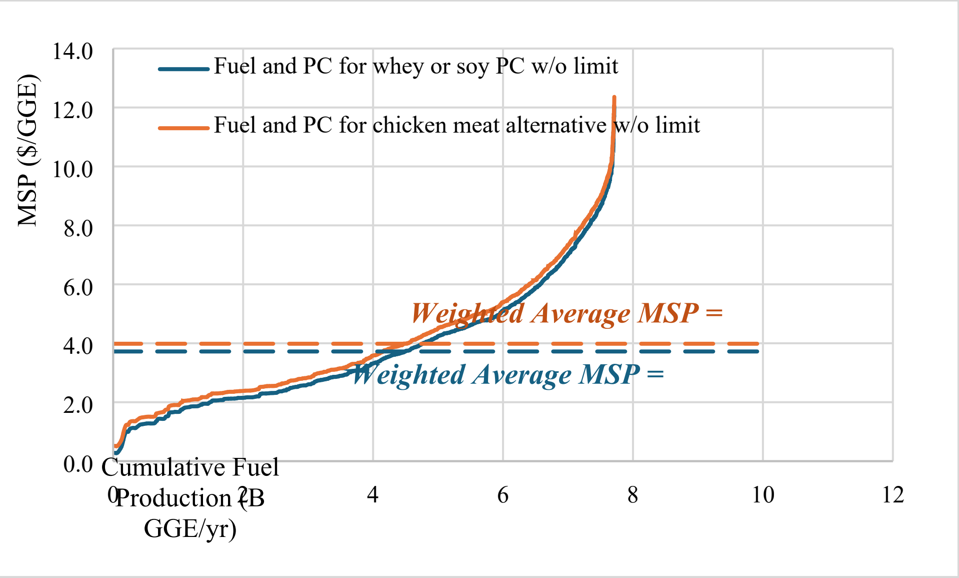

Figure S7. MSP by accounting protein selling price

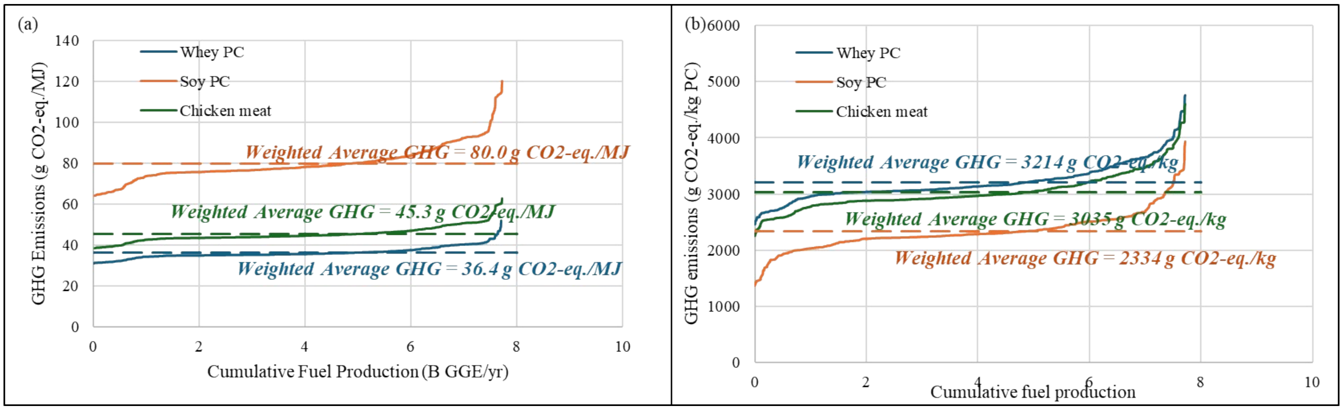

Figure S8. GHG emissions for (a) 1 MJ of fuel and (b) 1 kg of PC by using economic allocation method

7. MSP and GHG emissions breakdown

MSP and GHG emissions breakdown for fuel and PC scenario and fuel only scenario are shown in Figures S9 and S10.Algae growth and CO2 capture and transport contribute to the largest MSP and GHG emissions.

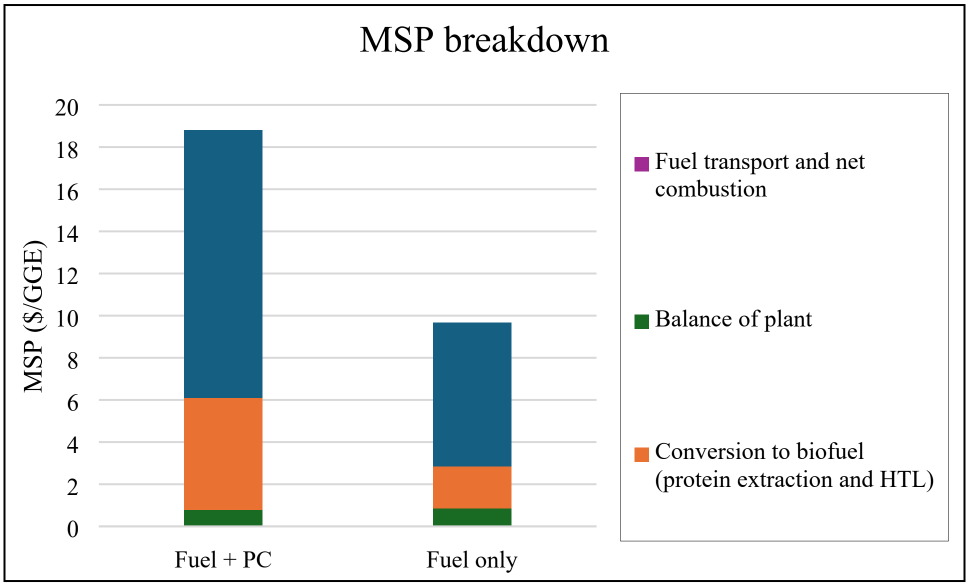

Figure S9. MSP breakdown by life cycle stages

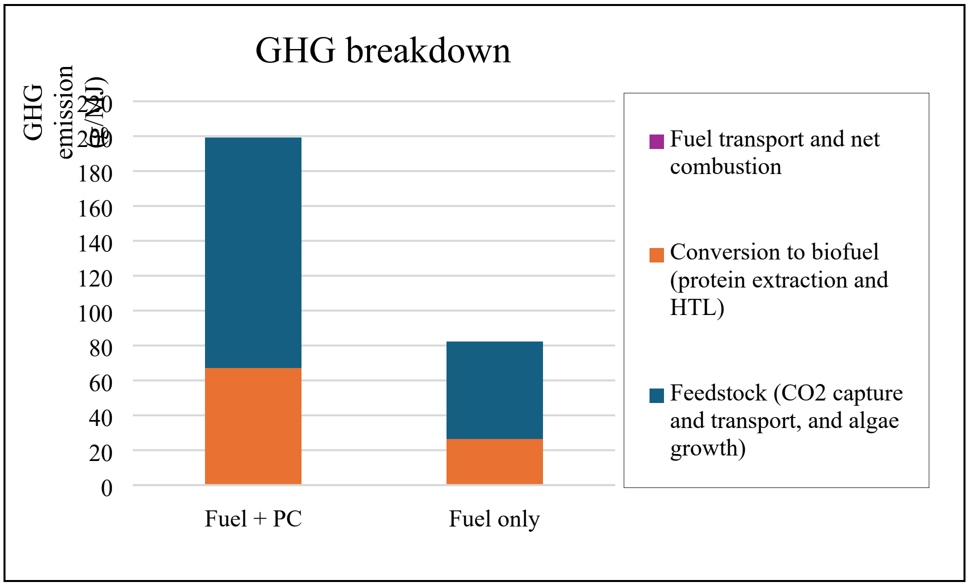

Figure S10. GHG emissions breakdown by life cycle stages

8. Benchmarking MSP and GHG emissions with Other Studies

For validation purposes, the MSP and GHG emission results obtained from the literature are compared with those from this study, and the comparison is summarized in Table S18.

Table S18. Comparison and validation with other studies

References	Functional Unit	MFSP or GHG Emissions	Coproduct Handling	Conversion to Comparable Units	Our Study Results
Batan et al.[54]	per L biodiesel	\$3.69/L from fuel only; \$-0.47/L from fuel and aquaculture feed and naphtha as coproducts.	N/A	\$15.0/GGE from fuel only; -\$1.9/GGE from fuel and aquaculture feed and naphtha as coproducts	\$6.7-13.1/GGE from fuel only; \$0.28-12/GGE from fuel and PC for soybean or whey PC; -\$2.0-9.8/GGE from fuel and PC for chicken meat
Beal et al.[55]	per ha and per L	\$2.64/L from fuel only; \$0.86/L from fuel and algal meal feed as a coproduct (Case 8)	System expansion	\$10.7 from fuel only; \$3.5/GGE from fuel and algal meal feed as a coproduct (Case 8)	\$0.28-\$12/GGE from fuel and PC production for soybean PC

Gnansounou and Raman[56]	per km travel	260 g/km with biofuel and coproduct for soybean protein used as animal feed	System expansion	130 g CO_2-eq./(1 MJ biodiesel + 0.051 kg protein)	109-219 g CO_2-eq./ [1 MJ fuel + 0.05 kg PC]

*Low heating value (LHV) of conventional diesel is 128,450 Btu/gal and biodiesel is ~119,624 Btu/gal

* Fuel economy given in Gnansounou and Raman is 0.053 kg biodiesel/km; protein production is 1.94 kg protein/kg algae biodiesel

9. GHG emissions from four different cases

GHG emissions from four different cases using the current U.S. electricity mix, electricity at half the carbon intensity compared to the current condition, and carbon-neutral electricity are depicted in Figure S11. Sites where GHG emissions exceed those from conventional fuel and PC production are excluded from the analysis since reducing GHG emissions from biorefinery systems is a key research objective. Figure S11(b) represents the biorefinery-level GHG emissions from fuel and PC production for soybean PC or whey PC. The yellow and purple lines in Figure S11(b) represent GHG emissions for fuel and PC production for whey PC only, as all soybean PC cases are removed from the analysis due to their higher GHG emissions compared to conventional fuel and soybean PC production with current U.S. electricity mix and electricity at half the carbon intensity compared to the current condition. Microalgal fuel and PC production for soybean PC can achieve lower biorefinery-level GHG emissions than conventional fuel and soybean PC production at specific sites using carbon-neutral electricity. Since the market size of protein ingredient (soybean PC) is larger than whey PC, the green line in Figure S11(b) reflects the biorefinery-level GHG emissions within protein ingredient market. The benchmarks of biorefinery-level GHG emissions and market pricing for all cases are listed in Table S19.

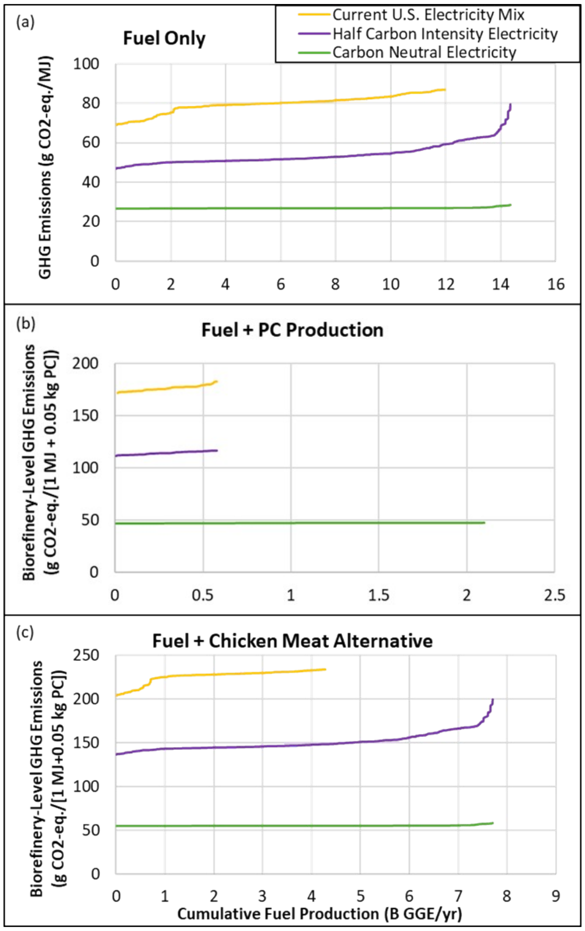

Figure S11. Biorefinery-level GHG emissions from (a) fuel only case, (b) fuel and PC coproduction for soybean PC and whey PC case, and (c) fuel and PC coproduction for chicken meat, capped by GHG emissions from conventional fuel and PC production

Table S19. Biorefinery-level GHG emission and market pricing benchmarks for conventional fuel and PC production

	GHG emission benchmarks (g CO_2-eq./MJ)			Market pricing benchmarks ($/GGE)		
	Current U.S. Electricity Mix	Half Carbon Intensity Electricity Mix	Carbon Neutral Electricity	Current U.S. Electricity Mix	Half Carbon Intensity Electricity Mix	Carbon Neutral Electricity

Fuel	87	85	85	2.6	2.8	2.8
Fuel + PC for substituting soybean PC	116	111	104	9.2	9.4	9.4
Fuel + Whey PC	695	613	425	20.7	20.9	20.9
Fuel + Chicken meat alternative	233	207	156	21.8	22.0	22.0

10. Relationship Between Total Dissolved Solids (TDS) and minimum selling price and GHG Emissions

Figure S13 shows the relationship between TDS and minimum selling price (MSP) and GHG emissions in different cases. No significant correlation has been found between TDS and MSP and between TDS and GHG emissions. However, it is shown that GHG emissions have a stronger relationship with TDS than MSP, since electricity consumption is one of the main drivers of GHG emissions, and desalination electricity consumption is correlated with salinity.

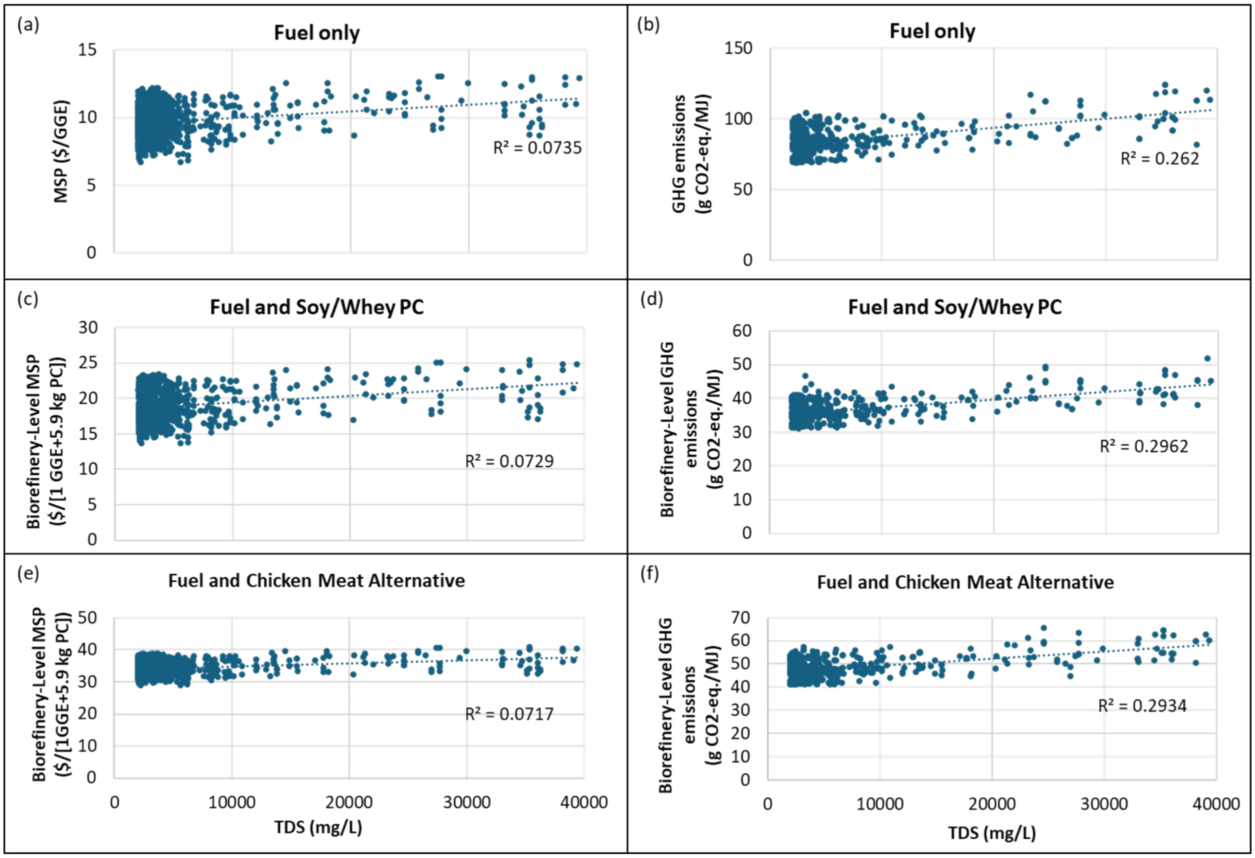

Figure S12. Relationships between TDS and MSP and GHG emissions in different cases (a) and (b) fuel only, (c) and (d) fuel and PC production for soybean or whey PC, and (e) and (f) fuel and PC production for fuel and PC production for chicken meat alternative.

11. Spatially Explicit TDS, freshwater characterization factor, biomass productivity, and biomass yield

As mentioned in the main text, three main regions are investigated in the study: Southwest, South Central, and Western. There is no indication that any particular region is better for TDS or biomass yield. However, the Southeast has the lowest freshwater characterization factor and highest biomass productivity according to Figure S14.

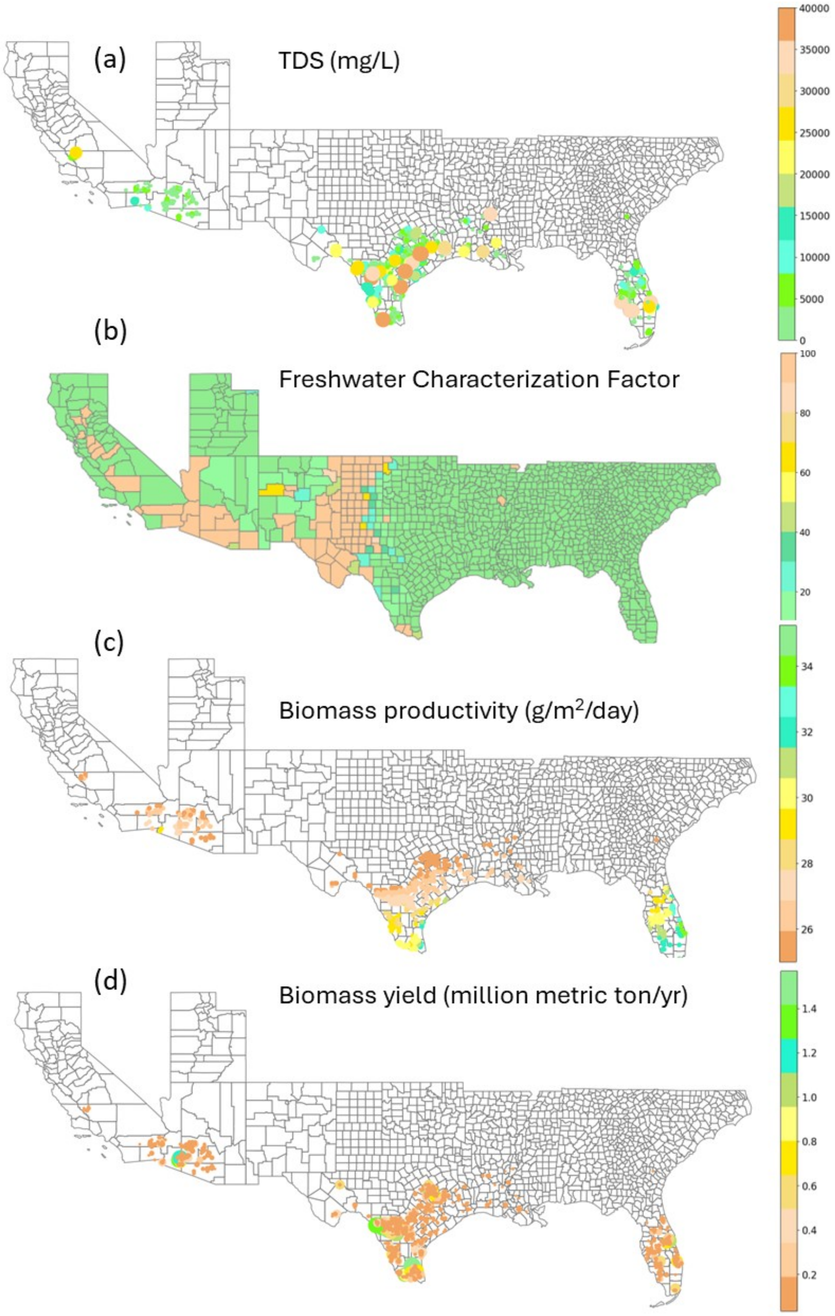

Figure S13. Spatially explicit (a) TDS (mg/L), (b) freshwater characterization factor, (c) biomass productivity (g/m^2/day), and (d) biomass yield (million metric ton/yr)

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