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| Complete List of Authors: | Quiroz-Arita, Carlos; Sandia National Laboratories California,<br>Shinde, Somnath ; Sandia National Laboratories California<br>Kim, Sungwhan; Sandia National Laboratories California<br>Monroe, Eric; Sandia National Laboratories California<br>George, Anthe; Sandia National Laboratories California<br>Quinn, Jason; Colorado State University College of Engineering, Mechanical Engineering<br>Nagle, Nick; National Renewable Energy Laboratory, National Bioenergy Center<br>Knoshaug, Eric; NREL,<br>Kruger, Jacob; National Renewable Energy Laboratory,<br>Dong, Tao; National Renewable Energy Laboratory, National Bioenergy Center<br>Pienkos, Philip ; National Renewable Energy Laboratory,<br>Laurens, Lieve; National Renewable Energy Laboratory, National Bioenergy Center<br>Davis, Ryan; Sandia National Laboratories, 8624 |
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# Bioproducts from high-protein algal biomass: An economic and environmental sustainability review and risk analysis

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Carlos Quiroz-Arita [a†], Somnath Shinde [a], Sungwhan Kim [a], Eric Monroe [a], Anthe George [a], Jason Quinn [c], Nick J. Nagle [b], Eric P. Knoshaug [b], Jacob S. Kruger [b], Tao Dong [b], Philip T. Pienkos [b], Lieve M.L Laurens [b], Ryan W. Davis [a†]

High-protein algal biomass is an important bio-commodity that has the potential to provide a new source of sustainable protein products. Herein is a critical review that identifies 1) the most relevant sustainability findings related to the processing of proteinaceous algal biomass to higher value protein products and 2) the potential pathways to improve life cycle assessment (LCA) and techno-economic analysis (TEA) metrics, including life-cycle carbon dioxide equivalent ($CO_2$eq), life cycle energy, and minimum selling price (MSP) of these products. The critical review of the literature revealed a large variation in model input parameters relating to these metrics. Therefore, a Monte Carlo analysis was conducted to assess the risk associated with these input variations. To understand the uncertainties that propagate into high-protein algae to products' systems, we reviewed more than 20 state-of-the-art unit operations for algal biomass processing., including cell disruption, protein solubilization, protein precipitation and purification, and protein concentration. We evaluated displacement of proteinaceous products by algal-bioproducts, including ruminant feed, aquaculture feed, protein tablets, and biopolymers and biopolyesters, with prices in the market ranging from 1.9 to 120 \$ $kg^{-1}$ protein. This review realized that the MSP of ruminant and non-ruminant feed ranges from 0.65±0.56 to 2.9±1.1 \$ $kg^{-1}$ protein, and bioplastics' MSP ranges from 0.97 to 7.0 \$ $kg^{-1}$ protein. Regarding LCA metrics, there is limited research on life cycle energy in proteinaceous biomass concentration and bioproduct systems, reported at 32.7 MJ $kg_{protein}^{-1,}$ for animal feed displacement. Animal feed emissions in the literature report negative fluxes, representing environmental benefits, as low as -3.7 $kgCO_2eq$ $kg^{-1}$ protein and positive fluxes, i.e., global warming potential, as high as 12.8 $kgCO_2eq$ $kg^{-1}$ protein. There is limited research on bioplastics life cycle emissions reported at 0.6 $kgCO_2eq$ $kg^{-1}$ protein. In general, the studies to date of algae-derived protein bioproducts showed similar life cycle emissions to soybean meals, nylon, polymers, and polystyrenes. Our risk analysis realized that more than 50% of scenarios can result in negative-net life cycle $CO_2$eq emissions. This review and risk analysis assess and demonstrate the scenarios that improve economic and environmental sustainability metrics in high-protein algal bioproducts systems.

## Introduction

Algae and cyanobacteria are considered promising renewable resources, potentially replacing fossil fuels and bio-derived products while obtaining environmental benefits such as anthropogenic carbon capture and nutrients remediation from wastewater, thereby reducing life cycle $CO_2$eq emissions and energy associated with these sectors [1-8]. Some economic and environmental studies have considered algae- and cyanobacteria-derived protein as a co-product for animal feedstock, from biofuel production pathways including biodiesel, biogas, and renewable diesel [7, 9-19]; and more recently ethanol, bisabolane, heptadecane, and fusel alcohols [6, 20, 21]. However, there is a broad spectrum of opportunities with higher technological readiness for using high-protein algal bioproducts to displace commodities including food, [4, 22], soil biostimulants [23-25], and polymers [26-29] that can alleviate energy-intensive processes and $CO_2$eq emissions associated with meat, fertilizers, and virgin and recycled plastics manufacturing [30-32]. TEA and LCA studies of algae-derived bioproducts have diverse pathway possibilities, system boundaries, model inputs, and uncertainties that make them challenging to compare, requiring a comprehensive risk analysis of the studies to understand all the possible sustainability outcomes and strategies to obtain lower environmental impacts than traditional products.

[a.] *Sandia National Laboratories, 7011 East Ave, Livermore, CA 94550*
[b.] *National Renewable Energy Laboratory, 15013 Denver W Pkwy, Golden, CO 80401*
[c.] *Mechanical Engineering Department, Colorado State University, Fort Collins, CO 80523*
† Corresponding author:
Ryan W. Davis, Ph.D.
Principal Investigator for Advanced Algal Systems and Conversion Interface
Bioresource and Environmental Security Department
Sandia National Laboratories
7011 East Avenue, Livermore, CA 94550
E-mail: rwdavis@sandia.gov

Carlos Quiroz-Arita, Ph.D.
System Analysis and Sustainability Lead Author
Bioresource and Environmental Security Department
Sandia National Laboratories
7011 East Avenue, Livermore, CA 94550
E-mail: cquiroz@sandia.gov

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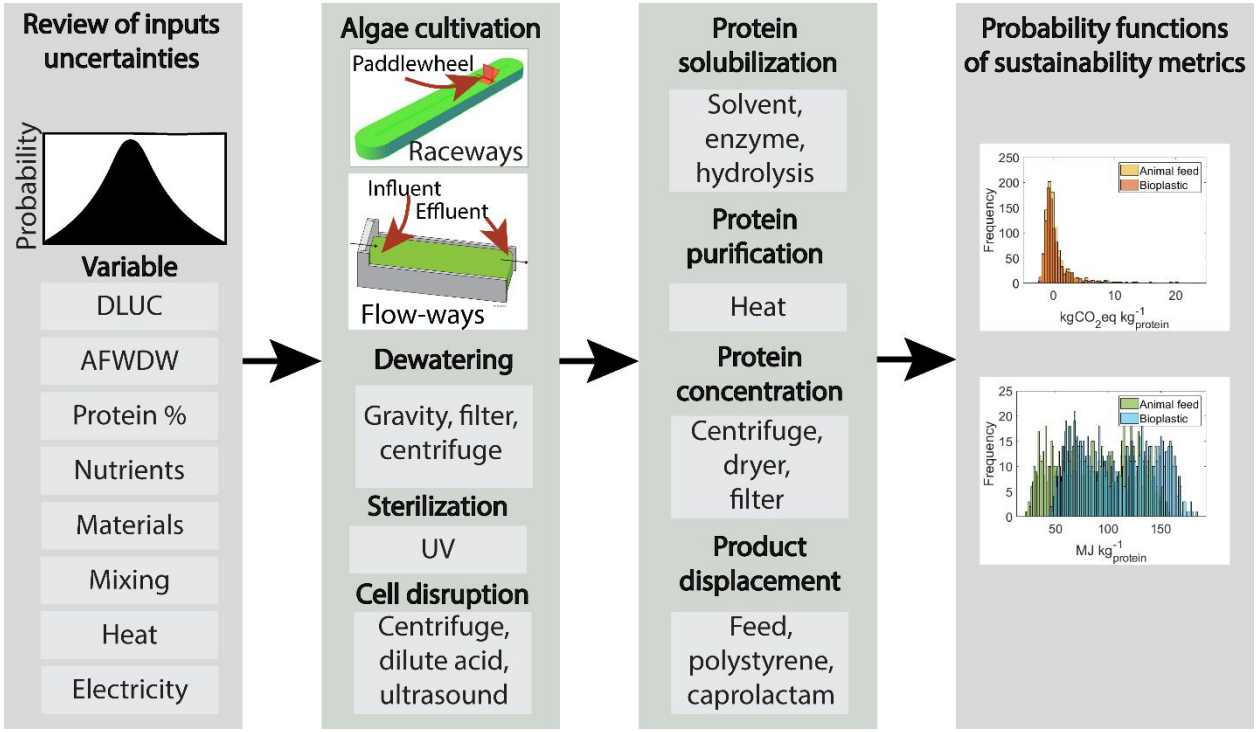


Figure 1. High-protein algae bioproducts system boundaries for environmental sustainability risk analysis. The first algae-derived animal feed system considers model input uncertainties for algae cultivation, biomass dewatering, biomass sterilization, cell disruption, protein purification, and concentration. The second algae-derived polymer system considers additional product preparation unit processes, including membrane separation, microwave processing, and jet milling. DLUC: Direct-land Used Change. AFDW: Ash-free dry weight biomass.

**Cultivation technologies and economics.**

The boundaries of the processes reviewed and used in the risk analysis of high-protein algal bioproduct sustainability are illustrated in Figure 1. The first unit process in sustainability studies of algae-derived biofuels and bioproducts systems includes cultivation through open-channel raceways, closed-photobioreactors (PBR), or attached-algae flow-ways (algal turf scrubbers) among several configurations [9, 15, 33-36]. Flow-ways are advantageous as the free-surface gravity currents propagate as in shallow open-channel flows [37], avoiding the need of external forces to accomplish mixing as opposed to PBR and raceways [38-44]. These systems have realized ash-free algae biomass productivities of 4 to 10 g $m^{-2}$ $d^{-1}$ over two years in the state of Texas in the United States (U.S.) and non-point nutrient removal at 300-500 mgN $m^{-2}$ $d^{-1}$ and 15-30 mgN $m^{-2}$ $d^{-1}$ [45]. There is little TEA and LCA research using flow-ways, where most recent studies report lower capital costs at 6.6 and 256 \$ $m^{-2}$, relative to PBR and raceways ranging from 1.1 to 2016 \$ $m^{-2}$ at different scales and geographical locations [9, 33-36, 46, 47]. However, flow-ways pumping energy requirements in one of these studies was reported at 17.5 W $m^{-2}$, surpassing the mixing energy requirements of PBR and raceways, that are published for industrial applications as high as 2 W $m^{-3}$. Recent research findings demonstrate well-mixed conditions in raceways inputs as low as 0.1 W $m^{-3}$ under pilot-scale environments [48]. Further studies are necessary to obtain specific energy requirements in the attached-algae flow-ways. The highest source of uncertainty in cultivation systems is the ash-free dry weight biomass (AFDW) productivity, for instance, in raceways a range of 2 to 50 g $m^{-2}$ $d^{-1}$ was determined in several studies, either experimentally or via modeling[10-18, 49]. Studies that assumed highest AFDW biomass productivity ignored factors that affect the growth and stability of algal biomass including predators [50, 51] and variability in dark- and photo-respiration [52, 53]. Another source of uncertainty ignored in most LCA studies of algal- and cyanobacterial cultivation sub-systems are the impacts due to direct-land used change (DLUC), that most authors assumed to be negligible in the U.S. barren land areas [54, 55]. However, geographical studies have demonstrated above-ground biomass (AGB) and soil organic carbon (SOC) that resulted in emissions that can be ≤20 $gCO_2eq$ $MJ^{-1}$ [55]. Economic, operating mixing energy, DLUC, and AFDW biomass productivities input uncertainties must be propagated in a sustainability risk assessment of algal-derived bioproducts.

Sustainability studies of high-protein algal bioproducts use lipid-extracted algae (LEA) and protein as the functional units [20, 56-59]. With increased interest in protein production from algal biomass, many compositional analyses on the protein content of different algal strains have been conducted and reported. In general, the protein content of algal biomass has a wide range from 5 to 70% of its dry weight, which depends on different strains, growth phase, and cultivation conditions [60-65]. Common values for nutrient-replete algae range from 35 to 50%. Despite the increasing interest in algal protein, the measurement of algal protein content also varies between different pretreatment and analytical methods. Hence, there have been many efforts to establish more reliable means to determine the protein content of algae [61-63, 66, 67]. For instance, various pretreatment methods have varied the protein content measured by Lowry method by as much as 20% [62]. Also, using Lowry method with bovine serum albumin (BSA) as protein standard is suggested instead of Bradford method with casein as protein standard due to higher similarity in the amino acid composition of BSA and algae [63]. Another standard approach to measure protein content is to measure total nitrogen content by either Kjeldahl method or elemental analysis and convert it to protein content by using nitrogen to protein conversion factor. However, using the nitrogen to protein conversion factor often leads to overestimating protein content since nucleic acids, amines, glucosamines, and cell wall materials also contain nitrogen [61]. Not only does the conversion factor overestimate protein content, but also the value varies between different strains, growth phases, and different total nitrogen measurement methods [62, 68, 69]. While the quantity of protein is being determined, the quality of protein is also considered essential and determined by the content, proportion, and availability of its amino acids [61]. The amino acid profile also varies between different strains and is often used to determine the actual amount of nutritious protein and its quality, as well as its potential as building blocks for the chemical industry [61, 64, 70]. Also, there are more direct methods to evaluate the quality of protein, including determination of the biological value, digestibility coefficient, net protein utilization, and protein efficiency ratio, which result in less favorable traits than animal proteins due to cellulosic cell wall [61]. Hence, effective pretreatment to disrupt the cell wall is suggested to maximize the accessibility of algal protein. These algae chemical composition studies demonstrated that reported protein content is another source of uncertainty that must be considered in the risk analysis of sustainability studies using this value as functional units.

**Protein solubilisation technologies and economics.**

Downstream of the cultivation unit process, proteinaceous algal biomass process selection in TEA has varied based on the targeted bioproduct including animal feed, human health, and polymers [20, 56-59]. TEA is a computational tool used to estimate the performance and cost of a facility during the design stage [15, 33, 71]. The primary unit processes for cell rupture for product recovery and conversion include, (i) cell disruption, (ii) protein solubilization, (iii) protein precipitation and purification, and (iv) protein concentration and hydrolysis [21, 72]. Chemical composition uncertainty in algae, particularly protein content, result in pretreatment and final product inconsistencies [73]. Uncertainties in the model inputs used for algae cultivation, protein content, pretreatment outcomes, and facility scales can be propagated into TEA of algal-derived bioproducts, resulting in probabilistic economic sustainability metrics such as MSP ($ $kg_{protein}^{-1}$). TEA instances for animal feed applications include *Nannocloropsis salina* with a protein content of 36.7%, reporting a MSP of 0.65±0.56 $ $kg_{protein}^{-1}$ [33]; *Staurosira* sp. with protein content ranging from 21 to 28% and a MSP of 2.9±1.1 $ $kg_{protein}^{-1}$ [74]; and benthic or periphytic polycultures assuming protein content of 30% and reporting a MSP of 0.73 $ $kg_{protein}^{-1}$ [20]. These authors assumed different Chemical Engineering Plant Cost Index (CEPCI) or conducted quotations at various times ranging from the years 2011 to 2015. Additionally, these three studies, for instance, obtained animal feed as a co-product from different biofuels pathways, including biodiesel, biocrude, and fusel alcohols, requiring different pretreatment processes, capital and operating expenses. TEA instances of polymer applications included *Scenedesmus acutus* with a protein content of 36% and an MSP of 0.97 $ $kg_{LEA}^{-1}$ [56], and a not specified strain algal biomass assuming 40% protein and a MSP of 7.0 $ $kg_{protein}^{-1}$ [57]. Both authors included different capital expenses, both considered cell disruption, one study assumed protein solubilization and the other protein concentration, and only one study considered product preparation processes including membrane, microwave, and jet milling. In general, to date TEA results showed promising MSP, considering that the markets for biomass feedstocks to displace animal feed are ranging from 1.4 to 25 $ $kg_{protein}^{-1}$ [33, 73-76]; human-food including Omega 3 and nutraceuticals ranging from 1.5 to 120 $ $kg^{-1}$ [74-76]; and thermoplastics, bioplastics, and polymers ranging from 1.9 to 20 $ $kg^{-1}$ [73, 77]. More TEA studies including fundamental biotechnology process experiments to reduce sources of uncertainty are necessary to assess the cost-effectiveness of these algal-derived bioproducts.

**Environmental sustainability metrics.**

Concerning environmental sustainability, model input uncertainties in algal cultivation, protein content, pretreatment, and facility scales similarly drive inconsistent metrics in LCA studies, including life cycle emissions ($kgCO_2eq\ kg_{protein}^{-1}$) and life cycle energy (MJ $kg_{protein}^{-1}$). LCA is a framework used to evaluate energy use, emissions and impact of direct, indirect, supply chain processes [78]. LCA research displacing animal feed studied *Scenedesmus* sp. and *Chlorella* sp. with 58.6% protein and estimated life cycle energy at 32.7 MJ $kg_{protein}^{-1}$ [79]; *Chlorella vulgaris* with 50% protein and life cycle emissions at 0.2 $kgCO_2eq\ kg_{protein}^{-1}$ [58]; macroalgae with not specified protein content and 1.0 $kgCO_2eq\ kg^{-1}$ [80]; *Spirulina platensis* with 60% protein, 12.8 $kgCO_2eq\ kg_{protein}^{-1}$, and 21.2 MJ $kg_{protein}^{-1}$ [59]; and benthic or periphytic polyculture with 30% protein and -3.0 $kgCO_2eq\ kg_{protein}^{-1}$ [20]. These authors assumed different processes and inputs materials and energy concerning cell disruption, protein solubilization and concentration. Human food research studied *Chlorella* sp. with 55% protein and estimated 118.6±116.8 $kgCO_2eq\ kg_{protein}^{-1}$, and *Spirulina* sp. with 55% protein and 137.2±83.6 $kgCO_2eq\ kg_{protein}^{-1}$. This study estimated significantly higher life cycle emissions than others because of energy-intensive cooling and heating requirements assumed in the systems. There is limited research concerning LCA studies of algal-derived polymers, where a study using *Scenedesmus acutus* assuming 36% protein content estimated 0.6 $kgCO_2eq\ kg_{protein}^{-1}$ [56, 81]. Given the limited research and available data, the environmental benefits of high-protein algal-derived bioproducts are not conclusive.

Other environmental aspects to consider in the sustainability of algal-derived bioproducts include water footprint, integration with wastewater remediation, and carbon capture and recycling. Many studies have considered non-competitive land availability in the arid-land areas of the U.S., but nutrients and water constrain the scalability of algae-derived biofuels and bioproducts [7, 9, 54, 55, 82]. Many authors studied the displacement of fertilizers and water required for cultivation of algae and cyanobacteria using municipal wastewater [8, 83-85], and higher nutrients availability from the sludge centrate produced in municipal wastewater facilities [5, 7, 86-88]. One of these studies demonstrated water quality improvement through cyanobacterial nutrient remediation in centrate and overall wastewater treatment process, and mitigation

of life cycle energy and carbon dioxide emissions in the operation of the facility [7]. Carbon capture using algae and cyanobacteria is one of the most attractive considerations that makes this technology promising to contribute not only to displace fossil fuel products but also to mitigate anthropogenic emissions and its climate change effects that are a common concern in today's society [1, 89-93]. Carbon dioxide capture rates from strains studied in the literature include *Anabaena* sp., *Nannochloropsis* sp., *Chlorella* sp., and *Scenedesmus* sp. at 0.70±0.65, 0.61±0.07, 0.14±0.73, and 0.05±0.02 $kgCO_2\ m^{-3}\ d^{-1}$ [1, 91]. The mechanisms driving this carbon capture variability are not fully understood in the literature. These $CO_2$ capture rates have inherent uncertainties that must be considered in risk analysis to assess the environmental benefits of algal bioproducts production.

**Summary.**

The results and discussion of this review are summarized into four aspects. First, we reviewed the U.S. Department of Energy's (DOE) reported algae biomass compositions using raceways, attached-algae flow-ways, closed-PBR, and wastewater treatment reactors (section 2). Secondly, we reviewed the state-of-the-art processes for cell rupture and product recovery or conversion, i.e., cell disruption, protein solubilization, protein precipitation and protein concentration (section 3). Thirdly, we overviewed the products from high-protein algae and conversion, including human nutrition, animal feed, biochemically derived bioproducts and fuels, and thermochemically derived fuels (section 4). Finally, we conducted a risk analysis using the materials and energy inputs reviewed in this study to predict the possible environmental sustainability outcomes in terms of life cycle energy and carbon dioxide emissions through a Monte Carlo method (section 5).

## Compositional analysis of biomass

Algal biomass composition is known to vary by species and is also highly responsive to cultivation conditions and nutrient environment of the cells [94, 95]. The stochiometry of the nutrient availability (C:N:P) in the cultivation environment is one of the primary drivers of the biomass elemental composition and under nutrient replete or sufficient conditions matches the considered optimal 106:16:1 (or more recently updated 163:22:1) ratio for growth purposes [96-98]. While this stochiometry is followed in algal biomass harvested from natural ocean-grown cells, it is possible to tune the ratio of C:N to potentially manipulate the stored energy content in algae. Beyond the elemental ratios, biomass composition affects processing and conversion options [99].A recent report compiled the biomass composition of a total of >1000 individual reported values and arrived at a median compositional profile of algae in rapid, exponential growth of 32.2% protein, 17.3% lipid, 15.0% carbohydrate, 17.3% ash, 5.7% RNA, 1.1% chlorophyll-a and 1.0% DNA as percent dry weight, with clear phylogenetic and species-dependent variability in the compositional profiles [94] . Some variability of the reported data is significant and can be attributed to the underlying analytical methodology, but there is overwhelming evidence that the protein content makes up at least a third of the biomass, and up to half for some species. Protein is usually present in algae as free amino acids, peptides, and protein complexes (with sugars and/or lipids) and, depending on their biochemical function, can range from highly hydrophobic to highly hydrophilic [69, 100]. These compositional profiles suggest that conversion approaches from the protein fraction can be more economically and environmentally viable. However, efforts to tune the remaining components, carbohydrate and lipids in the biomass can result in a shift of the respective energetic value of the biomass. Biomass composition is an important determinant in defining bioproduct options in a biorefinery that can realize better LCA and TEA metrics. However, there is limited information on exactly how biomass composition is affected by cultivation environment and physical configuration of reactors, and this section aims to provide a brief overview of what is known and what can be expected as cultivation is scaled and biomass is produced at larger quantities under minimally controlled conditions.

**Closed-PBR.**

Perhaps the closest to controlled algae cultivation is algae production in photobioreactors. While bioreactors are often considered to be more expensive to operate, these systems offer a most immediate opportunity to tune the biomass composition. In published work on biomass composition for multiple different species cultivated in photobioreactors, a detailed macromolecular and metabolic shift dynamic was described [95, 101-103]. The primary driver for a more energy dense compositional profile in photobioreactors can be associated with the higher light penetration and thus the physiological effects of light stress can cause a more rapid nutrient depletion phenotype [104, 105]. However, the same principles of nutrient-based biomass stochiometry can be found in biomass that is in the early, exponential growth phase of the cultivation cycle. While the protein content rapidly decreases upon light stress to favor the accumulation of carbohydrates and lipid in the biomass, the protein

composition remains remarkably consistent as indicated by the reported amino acid content of three different strains that were subjected to a two-week nutrient depleted environment [69, 95]. While the protein content decreased from 15-25% of the biomass to <10%, the respective amino acid profile remained mostly unchanged [95], indicating that the nutrient depletion stress metabolism mostly incorporates high-carbon containing macromolecules, leaving the protein composition unaltered.

**Open Ponds**

Typical biomass composition of open pond cultivation under fully nutrient replete conditions favors the accumulation of protein in the biomass, up to 40-50% of the total dry weight of the biomass [106, 107]. One study investigated the biomass composition in small-scale open-channel raceways on five geographical locations as part of an interlaboratory and nation-wide study [108, 109]. After an extensive harmonization effort between the different testbed sites, the biomass composition for each of the five testbed sites was measured on samples representing a full year of cultivation trials [106, 108, 109].

Table 1: Overview of seasonal biomass composition in small-scale open ponds as part of the year-over-year outdoor cultivation demonstrations at the AzCATI testbed site at Arizona State University in Mesa, AZ.

| Season | C | H | O | N | Ash | Protein | FAME lipids | Carbohydrates |
|---|---|---|---|---|---|---|---|---|
| Fall | 50.35 ± 1.49 | 7.56 ± 0.17 | 31.33 ± 1.61 | 9.36 ± 0.45 | 18.77 ± 10.35 | 36.5 ± 5.48 | 6.16 ± 1.29 | 18.72 ± 5.56 |
| Winter | 51.51 ± 1.11 | 7.56 ± 0.18 | 30.55 ± 1.14 | 8.97 ± 0.94 | 16.2 ± 1.13 | 36.02 ± 3.79 | 7.8 ± 0.76 | 26.39 ± 6.44 |
| Spring | 52.84 ± 1.24 | 7.73 ± 0.16 | 28.26 ± 1.62 | 9.78 ± 0.44 | 11.57 ± 4.7 | 41.34 ± 3.76 | 7.73 ± 1.2 | 23.37 ± 1.4 |
| Summer | 50.47 ± 3.04 | 7.52 ± 0.43 | 30.84 ± 3.84 | 9.77 ± 0.36 | 9.47 ± 1.54 | 42.27 ± 0.86 | 8.78 ± 0.74 | 22.29 ± 2.94 |

Notes: C, H, O, N data shown on an ash-free dry weight basis; other data shown on the basis of biomass dry weight (not ash-corrected), and all values are averages for at least two species and exceed between 9 and 25 datapoints, for mono-cultures of *Desmodesmus armatus*, *Desmodesmus intermedius* C046, *Scenedesmus obliquus* UTEX 393, *Monoraphidium minutum* 26B-AM, under fully nutrient replete conditions at the AzCATI testbed site at Arizona State University, in Mesa, AZ, as described before; protein determined via calculation with nitrogen-to-protein conversion factor of 4.78 [110], lipids quantified as fatty acids after in situ transesterification and carbohydrates through monomer quantification via optimized chromatography.[66,107,108,]

A recent analysis of biomass composition over a cultivation year (2018) was measured from cultures grown to support an annual state-of-technology demonstration of cultivation performance [111] . The average seasonal composition data for small scale open pond cultivation is shown in Table 1. The values shown indicate a high protein content, 36-42% of the biomass, and a minor increase in protein content in the Spring and Summer seasons, which is consistent with the increase (almost 2-fold) in growth rates and biomass productivity in those seasons.

In a nutrient depletion strategy in small-scale open ponds, specifically the rate of shifting the biomass composition towards a higher energy content, e.g. higher lipid and carbohydrate in the biomass, was reported [108] . The data observed is consistent with other literature on *Nannochloropsis* [112, 113] where a biochemical response was anticipated of increased lipid and reduced protein content of the cultures with respect to their composition upon media nitrogen depletion. The lipid content was increased from 10% to over 30% in the biomass samples investigated, which illustrates a significant impact of the physiological nutrient stress experiment and the possibility to rapidly impact the biomass compositional profile in outdoor ponds. The reported at least 2-fold reduction in protein content of the biomass over the course of a month cultivation, is consistent with physiological and metabolic rearrangements reported in the literature [112-114]. However, as was the case for multiple published reports on outdoor algae cultivation and compositional shifts in biomass composition, there is a significant impact on the productivity and established harvested biomass quality that needs to be carefully balanced against a more bioenergy-attractive composition.

**Attached-algae flow-ways**

Attached growth systems, where algae are tethered to an organic or inorganic substratum, can offer advantages over suspended algae cultivation systems in terms of managing the harvesting and water recycling operations, as well as the more consistent access of the algae communities to light [115]. Typically, attached-algae systems are applied to water treatment operations and focused on autotrophic and/or mixotrophic growth conditions [115-117]. However, because of the inherent mixed-culture algae community

 

dynamics [117] , there are challenges associated with controlling both the types of algae that appear, the stability of the community and the resulting harvested biomass quality. In most instances, the biomass will be high ash (>50% of the harvested material), with varying proportions of biogenic and abiogenic ash [118] . The high ash content of this biomass interferes with biological and chemical biomass processing. Recently, a new application of an alkaline pretreatment process was developed to remove most of the ash from algal biomass harvested from algal turf scrubber systems, yielding a primarily high-protein biomass feedstock [118] . The biomass composition has not been described in the literature in detail, because the organic component makeup is a function of the algae communities that were present in the attached algae growth systems. For example, polyunsaturated fatty acids for high-value applications may need species-specific cultivation, and similarly, high carbohydrate or lipid content traditionally required for bioenergy applications will be dependent on the algae species. The nitrogen and thus protein composition will vary, though as long as nutrients are not limiting, protein will be the dominant fraction of the organic content [117] .

**Wastewater treatment reactors**

The influence of wastewater as the basis for algae cultivation on biomass composition is not extensively documented in the literature. While there are many options for recycling nutrients by combining wastewater treatment with algae cultivation, there are some considerations in terms of the economics and biomass quality that need to be considered. There is potential to integrate algae production with wastewater treatment (WWT) to achieve economic and sustainability benefits. Because of the mixotrophic cultivation environment, a higher C:N ratio is expected in the biomass compositional profile and thus to lower in protein content. Algae cultivation on waste water can include the offsite reuse of a treated or untreated effluent [5, 7, 86-88, 119-122]. Algae have been grown on a wide variety of wastewaters, most prominently municipal, but also agricultural and industrial wastewaters and nitrogen, the largest predictor of protein content, is, among other nutrients, the limiting factor. However, some wastewaters contain inhibitors for algal growth, for example, high ammonia concentrations in animal waste and toxic compounds in industrial wastewaters, creating challenges to high-productivity algae cultivation, in addition to the often highly turbid nature of the water stream. While there are few reports on biomass composition from WWT systems, the biomass is generally assumed to have limited applications for bioproducts because of toxicity and other environmental safety hazards and thus methane from anaerobic digestion is one of the few energy products. Anaerobic digestion is understood to be agnostic to the material input and the yields are primarily driven by the C:N content of the feedstock.

## Cell rupture for product recovery/conversion

The recovery of proteins begins with solid-liquid separation unit operations including, the separation of cells and solubilized cell fractions (cell lysate/hydrolysate) [123, 124]. There are several options for solid-liquid separation of algal biomass/hydrolysate. The choice of method depends on whether the protein is intracellular or extracellular. This section focuses on the algal proteins which mostly are intracellular in nature and are released in to the medium during cell rupture and/or protein downstream processing. The dilute suspension of algal biomass (0.02- 0.05 % dry solids), the similarity of density of algal cells to the growth medium, the small size of micro-algal cells, and the negative surface charge on the algal cells pose different challenges in the downstream processing of algal biomass. As a first step in algal protein recovery, algae can be harvested using many methods, including sedimentation, flocculation, flotation, centrifugation, and filtration, or a combination of any of these [124]. Then, the harvested biomass is treated downstream to recover the algal-protein through mechanical, chemical and biological methods, including (i) cell disruption and protein solubilization, (ii) protein precipitation and purification, and (iii) protein concentration. The state-of-the-art techniques for cell rupture for protein recovery are reviewed in the following sections.

**Cell disruption and protein solubilization**

Table 2. Overview of various cell disruption methodologies for microalgal constituents/protein recovery.

| Cell disruption methods | Method | Algal strains | Target products | Product Recovery (%)* | Process conditions | Reference |
|---|---|---|---|---|---|---|
| Physical | Bead Milling | *T. suecica, C. vulgaris, N. oleoabundans* | Proteins | 43%~57% yield | 0.3, 0.4, 0.65 and 1 mm, 65% v/v, 25°C | 125 |
| | | *Nannochloropsis gaditana* | Proteins | >53% yield | 0.5mm, 25min 35°C | 126 |
| | Pulsed electric field | *Nannochloropsis* sp. | Proteins | 5.2% yield | 20 kV/cm, 1–4 ms, 13.3–53.1 kJ/kg | 127 |
| | | *Chlorella protothecoides* | Lipid | 55% (w/w dw biomass) | 6.2 MJ/m3 with 60ns EP | 128 |
| | | *Nannochloropsis gaditana* | Protein | 10% yield | 60g/L, 10 pulses (10.42 kWh/kg) | 126 |
| | High voltage electrical discharge | *Nannochloropsis* sp. | Proteins | 1.15% yield | 40 kV/cm, 1–4 ms, 13.3–53.1 kJ/kg | 127 |
| | | *Parachlorella kessleri* | Proteins | <15% yield | 40 kV/cm, 1–8 ms | 129 |
| | Ultrasonication (USN) | *Nannochloropsis* sp. | Proteins | 1.8% yield | 200 W, 1–8 mins, 12–96 kJ/k | 127 |
| | | *Arthospira platensis* | Proteins | 84% yield | 4 ± 1°C, 60mins | 130 |
| | High pressure homogenization (HPH) | *Nannochloropsis* sp. | Proteins | 91% yield | 150 MPa, 1–10 passes, 150–1500 kJ/kg | 127 |
| | | *Parachlorella kessleri* | Proteins | 72% yield | 1200bar, 10 passes | 129 |
| | | *Nannochloropsis gaditana* | Proteins | 50% yield | 1000 bar (0.32 kWh/kg), 1 pass | 126 |
| | High speed homogenization (HSH) | *Palmaira palmate* | Proteins | 40% yield | Not reported | 130 |
| | | *Nannochloropsis sp.* | Lipid, (EPA) | 83% lipid yield (94% EPA yield) | 5min, 8000rpm, 55°C | 131 |
| | Microwave assisted | *Arthospira platensis* | Proteins | 78% yield | 1000W (2450MHz), 3mins | 130 |
| | Osmotic stress | *Palmaira palmate* | Proteins | 39% yield | 4°C, 7hrs | 132 |
| Chemical | Alkali | *Arthospira platensis* | Proteins | 75% yield | 1M NaOH | 130 |
| | | *Palmaira palmate* | Proteins | 24% yield | 0.120M NaOH (0.1 g/100 ml NAC), 1:1.5 w/v, room temp. | 132 |
| | | *Scenedesmus almeriensis* | Proteins | 16.9% yield | 2M–40 °C–0.5 h | 133 |
| Biological | Enzymatic | *Arthospira platensis* | Proteins | 82% yield | pH 5, 50°C, 1% (w/w protein basis), 3hrs | 130 |
| | | *Palmaira palmate* | Proteins | 67% yield | Polysaccharidase & alkaline, 40°C, 24 hrs. | 132 |
| | | *Nannochloropsis gaditana* | Proteins | 35% yield | Proteases, pH8, 50°C,4 hrs, 5% (v/w) | 126 |

*The unit '% yield' under the section of 'Product Recover (%)' indicates the amount of the product released over the total amount of the product in the cell unless stated otherwise.

There has been considerable interest in developing effective protein extraction methods to improve bioavailability and utilization for food and feed applications [134]. Extraction of protein from algal biomass can be accomplished using two methods based on their mechanisms, mechanical and non-mechanical disruption. Mechanical methods involve disruption of cell walls by applying the various form of physical forces such as solid and liquid shear stress, energy transfer through wave, or electric field, to extract any intracellular substances, including proteins. Non-mechanical methods involve cell wall disruption through chemical or biological processes. Each type of cell disruption method has a different effect on the efficiency of downstream operation for protein recovery. Cell disruption efficiency can be driven by design and operating conditions, *i.e.,* chamber and agitator geometry, biomass concentration, agitator speed, suspension flow rate, bead filling ratio, bead types and geometry, solvent, pH, and pressure [125, 126, 129]. Algal cells' characteristics also have a role in cell disruption mechanisms. For instance, P. R. Postma illustrated the variability of protein release kinetics for different species. C. Safi *et al*. argued that the different product yields are highly related to intrinsic properties of algal cells such as size, shape, growth state, and cell wall thickness and composition [126]. Table 2 describes various disruption methods along with their effectiveness surveyed from other studies.

**Mechanical methods.** Algal cell harvesting can be achieved using centrifugation because of the high solids content of the algal culture. Considering that the analysis presented in this review focuses on photoautotrophic growth for biobased commodities, a significant challenge in commercialization of algal proteins is reduction of the operational energy required for its production and, in particular, the energy used in cell harvesting and protein extraction and concentration. Significant improvements continue to be made in several aspects of centrifuge design and construction. The use of disc stack centrifuge to achieve a combined cell harvesting, cell disruption, and biomolecule separation process is a promising technique [135]. Energy consumption found in centrifuge systems and their sustainability implications is reviewed in section 5. Mechanical disruption methods use various types of physical forces to break down cells into smaller particle size, resulting in larger surface area as well as smaller degree of polymerization and crystallinity [136]. Mechanical methods have been widely used for various applications as they are not specific to the type of biomass or target product [137]. One physical force instance is using shear stress, applied by a solid component such as bead milling and high-speed homogenization, or by a shear flow such as in high-pressure homogenization. Bead milling was able to achieve 85% to 99.9% of cell disruption efficiency along with 40-60% protein extraction yield regardless of strain as long as proper operating conditions were applied [125, 126, 138]. High-speed homogenization (HSH) consists of a stator-rotor assembly and creates high shear stress (20,000 to 100,000 $s^{-1}$) in the region between the rotor and the stator by causing hydrodynamic cavitation [137, 139]. Although HSH has been widely used in industries of food, cosmetics, and chemicals, there has been not much work done with cellulosic biomass such as algae and often is considered inefficient for cell disruption because of its high energy demand [131, 139]. Recently, high lipid extraction yield (83-95%) from *Nannochloropsis* sp. using a high shear mixer (HSM) was achieved at its optimized operating condition, which led to the most economical process among other two-step wet extraction methods [131]. High-pressure homogenization (HPH) utilizes hydraulic shear force generated when slurry pumped through an orifice collide against a valve set with high pressure (150 – 1500 bar). HPH has been widely used in industries due to its various advantages such as simple operations, scalability, and thermal stability. HPH was found very effective for algal cell disruption as several studies show high protein extraction yield (70-90%) with multiple passes [127, 129]. The technique was also able to rupture algal strains with very rigid cell walls, such as *Nannochloropsis* [126, 140]. The ultrasonication method is another hydraulic cavitation driven extraction method. The major advantages of the ultrasonication technique are fast processing time, mild temperatures, and low solvent consumption while producing higher purity final product [134, 136]. However, these authors highlited the scalablity limitations of ultrasonication techniques due to its long hydraulic residence times and energy intensive process. Although ultrasonication is barely found in large scale applications, it is one of the most effective techniques for cell disruption on small scale [141]. Studies reported that cell disruption efficiency by ultrasonication is species-specific, with a wide range of protein extraction yield from 1.8% to 84% for various algal species [127, 130, 141]. Pulse electric field (PEF) has been used in small scale cell disruption method as it allows selective extraction of intracellular contents of algae [127]. However, it has a limitation for scalability due to conductivity of the growth media and electrode gap [134]. A study comparing various extraction methods using *Nannochloropsis gaditana* in terms of protein yield and energy input found that PEF method was the least favorable method for protein yields, achieving only 5-10%,

and consumed the highest amount of energy [126, 127]. Microwave irradiation alters the electric field to heat a dielectric material, which enhances the penetration of the solvent into the matrix to disrupt the cell wall and release the intracellular substances [130]. Microwave irradiation is the most commonly used method due to its high disruption efficiency with less energy input [130, 134, 136, 137]. These studies reported that protein yield from *Arthospira platensis* using microwave was successfully achieved at around 80%. Although microwave is one of the most promising extraction methods, the increase in temperature during the process might limit the application for proteins extraction due to their thermal sensitivity. The reader should note that these research contributions were considered under laboratory scale conditions, that can suffer a dramatic effect in the energy balance under industrial scale environments.

**Chemical and enzymatic methods.** Organic solvents such as Trichloroacetic acid (TCA)/acetone and phenol have been used to extract protein from algal biomass. Such methods are often accompanied by an additional mechanical process such as HSH, ultrasonication, and microwave, as well as a catalyst such as mineral acids (hydrochloric acid, sulfuric acid, phosphoric acid), bases (lime, sodium hydroxide, ammonia), and certain salts [131, 142, 143]. Although organic solvents can be recycled efficiently by distillation, it is often expensive and the recovery process is also considered an energy-intensive process, and requires additional operating cost [136]. Another commonly used chemical method is driven by acid/base (alkaline) hydrolysis reactions. Alkaline hydrolysis is a widely studied chemical method for protein recovery as it is considered more effective than acid at mild temperatures [144]. One study showed that 16.9% of protein yield was achieved by alkaline hydrolysis from biomass collected from pig manure. Acid pretreatment is a practice commonly used to solubilize hemicellulose and increase cellulose hydrolysis [145-147] in lignocellulosic biomass processing. One advantage of acid pretreatment is that its energy requirement (*e.g.*, 2.5 J $kg^{-1}$) is significantly lower than enzymatic hydrolysis, ranging from 5.2 to 10.5 MJ $kg^{-1}$ [148]. One demonstrated caveat, however, is that although acid pretreatment is effective for carbohydrate hydrolysis, protease enzymes are required for effective protein hydolysis (*i.e.*, acid alone hydrolyses ~40% of protein); in contrast, enzymatic hydrolysis was effective hydrolyzing ~70% of algal proteins [149]. Furthermore, since chemical methods are likely considered to be used with mechanical methods, acid hydrolysis has a significant drawback from industrial employment due to its toxic and corrosive characteristics, mainly when operating at high temperature, which causes higher operational and maintenance cost. One of the major disadvantages associated with alkaline hydrolysis is difficulty in recovery of used alkalis, which exacerbates the disadvantage of its high initial cost.

Enzymatic hydrolysis is one of the most common disruption methods, and is highly effective with significantly lower energy input compared to mechanical methods [137]. One of the major advantages of enzymatic hydrolysis, among other methods, is that it can be specific to type of biomass and type of product [137, 150]. However, the requirement for high enzyme to substrate concentration ratios may be prohibitive at industrial scale [132]. One study reported that protein yield from *P. palmata* with xylanase and cellulase was ten-fold higher than that from a mechanical method [151]. Also, enzymatic hydrolysis resulted in one of the highest protein yields of over 80% from *A. platensis* among other mechanical and chemical methods [130]. Although the technique is highly effective, it is often considered to be used as a pretreatment process coupled to other mechanical or chemical methods. A study reported that protein yield from *P. palmata* was increased by 63% when combining enzymatic hydrolysis with alkaline extraction [152]. Alkaline treatment followed by enzymatic treatment increased lipid yield to 90% from *Nannochloropsis* sp. and HPH followed by enzymatic treatment carried out increased lipid yield to 92.6% from *Neochloris oleoabundans* [153, 154]. Comparison of mixed alcohols production from acid pretreated and enzymatically hydrolyzed algae biomass for three days of residence time in a microaerobic fermentation system resulted in 100% and 38% higher fuel yields, respectively, than baseline conditions (*e.g.*, no pretreatment)[145]. These results suggest that acid/enzymatic pretreatment can be a suitable co-processing technique for generating feed and fermentative products (e.g., alcohols) when utilization of both the carbohydrate and protein fractions simultaneously is advantagous. Downstream applications such as bioplastics manufacturing, on the other hand, will require longer oligomers and intact proteins. However, the residence time in pretreatment can affect the level of hydrolysis that acid pretreatment and enzymatic hydrolysis can achieve, and therefore raise opportunities for energy consumption and bioproduct optimization. These studies demonstrate an energy consumption and product recovery tradeoff between acid/base and enzymatic hydrolysis that must be considered in sustainability assessments.

**Protein concentration**

Downstream processing for protein concentration begins with solid-liquid separation to achieve clarified extract using filtration and/or centrifugation of the extract to remove cell debris and particulate material [155]. Next steps in downstream processing involve protein concentration (by water removal), fractional purification by removal of other polymeric materials (such as nucleic acids and polysaccharides), and, then partial purification of proteins by removal of the unwanted proteins. A substantial reduction in volume and protein loads is achieved at this stage which reduces the operational cost for subsequent protein purification steps. Based on the desired application of the protein product, it is then subjected to one or more advanced chromatographic purification techniques that achieves the desired protein purity and separates the protein from the remaining unwanted proteins. Each unit operation in protein separation, concentration and purification process may affect the native protein structure and desired protein/s activity. Therefore, process and unit operations needs to be carefully designed for maximal yield and minimal to none protein denaturation, modification, and degradation. Protein downstream processing contributes significantly to overall cost of the bioproduct and hence the minimum number of downstream unit operations with speedy operation and high yield considerably determines the overall efficiency of any protein purification process. This is achieved through process design where unit operations in a multi-step process complement one another in their requirements and selectivities. Proteins should be purified only to the extent required for the final purpose, and in some cases (e.g., thermochemical conversion to fuels) may not be required at all. Protein concentration and hydrolysis methods can include filtration, centrifugation, drying, acidic or alkaline, and enzymatic, e.g. protease [21]. This section reviews the most common operations for protein concentration, chemical precipitation and membrane filtration. The sustainability implications of these protein concentration unit processes are discussed in section 5.

**Chemical precipitation.** Proteins precipitation, a widely used technique for protein concentration, is achieved by a variety of agents such as neutral salt (ammonium sulphate), weakly polar solvents (ethanol), acid/alkali (sulfuric acid), hydrophilic uncharged organic polymers (polyethylene glycol), polyelectrolytes (polyacrylic acid, polyethyleneimine) and metal ions (calcium). Ammonium sulphate is the most commonly used protein precipitating agent for lab scale operations, however its use for large-scale operations is limited due to the need for pH control and waste disposal. Recently, algal protein extraction and precipitation was achieved through a pH shift. For instance, a combined alkaline extraction and acid precipitation was used on wet brown seaweed biomass *Saccharina latissima* showed 34 % of the total protein extraction at pH 12. This alkaline protein extract was then subjected to lower pH to 4, where the protein precipitation is obtained, with highest yield of 34.5 % at pH 2 [156]. Such 'pH shift method' has been proven to achieve partial separation and concentration of microalgal proteins [157, 158]. A pH shift method achieved nearly 16 % of the total *Saccharina* proteins recovery. This yield can be satisfactory for algal protein recovery but leaves significant room for improvement when compared to protein extraction from conventional terrestrial sources, such as soy.

**Filtration.** Membrane separations are based on the selective separation of different components according to their molecular weight, wherein a semi-permeable membrane separates a fluid into two distinct fractions, permeate and retentate, by selectively permitting some compounds to pass through it. Dairy industry has been widely using membrane separation technologies in the cheese-making process to recover whey proteins from milk [134, 159]. Membrane technologies, being non-thermal and more sustainable, are industrially favorable unit operation for algal protein concentration and partial purification [134, 159].

Algal protein separation and concentration can be achieved through a combination of membrane technologies. Pressure-driven membrane separations are classified in to microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) based on membrane pore size and molecular weight cut-offs. Microfiltration (MF) is often used in a dairy industry for non-thermal decontamination and separation of microorganisms such as bacterial contamination, without compromising the overall organoleptic properties of milk [160]. Similarly, for algal protein separation, MF finds a use in removal of algae cell debris and bacteria with a molecular weight greater than 200 kDa. Algal proteins and other micromolecules can then be concentrated using UF membrane with molecular weight cut-off range of 1 and 200 kDa (based on desired protein/s of interest). The resultant monovalent salts present in the protein concentrate results in high ionic strength which can be removed by NF membrane, followed by further protein concentration by reverse osmosis (RO) [134, 159]. Combined with a cell disruption technique/s, membrane separation is a promising alternative for algal protein enrichment. In

most cases, cell disruption is necessary to increase algal protein extraction [134, 161]. 100% of native phycoerythrin protein from *Grateloupia turuturu* was retained using UF from homogenized cell mass [134, 162].

Membrane technologies are proven to be efficient for industrial algal biorefinery processes enabling a selective separation of heavy metals and complete valorization of the total algal biomass [134, 163]. Membrane technologies also finds an application in algal biomass harvesting and seaweed components isolation. Efficient algal biomass harvest recovery (70%–89%) was achieved by tangential flow microfiltration (MF) [164]. Membrane separations in combination with other techniques have also been used for recovery of algal polysaccharide components. *Sargassum pallidum* polysaccharides were separated using UF in conjunction with supercritical $CO_2$ extraction and ultrasound [134, 165]. *U. fasciata* polysaccharides with antioxidant properties were separated using UF in combination with hot water extraction [134]. Two-stage UF process in combination with high-pressure homogenization enabled *Tetraselmis suecica* starch polysachharide retention using with a 100-kDa membrane followed by algal protein enrichment with a 10 kDa membrane [134, 166].

#### Protein purification

The extent of protein purification is driven by its ultimate application of bioproducts. Advanced protein purification techniques such as chromatographic are required to achieve high-resolution purity of protein for applications such as therapeutic proteins and industrial enzymes while moderate efforts and traditional protein purification techniques are sufficient to achieve desired protein purification for other miscellaneous bioproducts. This section highlights the protein purification techniques used mainly for algal bioproducts for human food, animal feed, and other biochemical applications. Purification of proteins can be accomplished through physical and chemical operations using heat, acid/alkaline, and solvents [21]. The initial separation of protein form its crude extract/cell hydrolysate can be achieved by three-phase partitioning (TPP) with high recovery yields. This simple, rapid and easy to scale-up technique is an attractive tool for one-step purification of proteins from crude extract and/or cell hydrolysate [167]. TPP with combination of ammonium sulfate and t-butanol can efficiently isolate, concentrate and purify proteins in the middle phase and separate non-polar fractions in the upper solvent phase and polar fractions in the lower aqueous phase. Proteins in crude extract are selectively precipitated from crude extract and form middle protein concentrate layer at the interface of the aqueous and organic phases. Combination of co-solvent precipitation, kosmotropic, osmotic electrostatic forces, salting out effect, isoionic precipitation, protein hydration shifts, and conformation tightening contributes to protein precipitation at the interface of aqueous and organic phases [168]. There is also an evidence of an enhanced enzyme activity through TPP protein separation and partial purification technique [169-172]. Important process optimization parameters for TPP technique includes ammonium sulfate concentration, choice of solvent, incubation time, pH, solid load, and slurry to solvent ratio. TPP concentration and purification approach resulted in 78.1 % w/w protein concentration in the middle protein concentrate phase [173, 174]. The sustainability implications of physical purification methods (e.g., heat) are discussed in section 5.

## Products from high protein biomass

Many studies have highlighted the potential of algae-derived biopolymers and biopolyesters to displace highly resource-intense products and obtain sustainability benefits [4, 22-27, 29, 76, 175-177]. Some strains have gained more interest because of their high-protein content that can potentially displace products for human food, animal feed, and biochemical applications. Some instances of high-protein algal strains include *Arthrospira platensis* (*Spirulina*), *Dunaliella* sp., *Porphyridium cruentum*, *Chlorella vulgaris*, *Nannochloropsis* sp., and *Scenedesmus* sp. with protein contents from 43 to 77%, 27 to 57%, 27 to 57%, 38 to 53%, 18 to 47%, and 31 to 56%, respectively, that in general showed lower lipid content and higher quantities of structural biopolymers such as proteins and carbohydrates [22]. In light of the sustainability implications, algal-bioproducts are briefly overviewed in the following sections.

#### Human nutrition

Algae-based protein could have favorable nutritional characteristics for human or animal nutrition ingredients or formulations; however additional research, quality, and performance assessments will be needed prior to implementation at scale [69, 178, 179, 4, 178, 180-184]. In general, the quality of algal protein for human or animal consumption depends on its amino acid composition and whether some amino acids are limiting, palatability and digestibility of the proteins, amount of non-proteinaceous nitrogen, and presence of any deleterious components. Typical amino acid compositions of several leading algal strains indicates high amino acid scores for human

nutrition, up to, and sometimes exceeding the designation of a "complete protein source" before correction for digestibility [185-187]. The reported amino acid compositions and associated characteristics support the conclusion that high nutritional value can be obtained from algal biomass-derived protein.

Related work on producing protein products for human nutrition from terrestrial biomass-based biorefineries may also be applicable to algal biorefineries [185-187]. In general, the application of algal protein for human or animal nutrition depends on its amino acid composition, in particular the limiting amino acid(s) but also palatability of the material. Often overlooked parameters for nutritional applications are the digestibility of the protein present in the residual biomass after processing, the amount and nature of non-protein nitrogen, and the potential for the presence of other anti-nutrients in the biomass. These are compounds present in food that may interfere with the absorbance of other nutrients, such as phytates that can chelate metals such as iron, calcium, and zinc.

Integrating food or feed uses as a route to valorize residual materials after processing, will need to be thoroughly evaluated to ensure these processed residues can become an acceptable nutritional resource. For example, the presence of heavy metals from flue gasses, flocculating agents used for dewatering, lipid extraction solvents, or acid residuals from algae pretreatment may reduce the residual biomass quality for nutritional applications.

Furthermore, the costs of drying or otherwise stabilizing the resulting protein needs to be included in technical and economical assessments of enabling feed operations. One attempt to value post-extracted algal residue has been proposed where comparisons to soybean meal are used as a comparison [188].

Feeding trials of algal residues have been carried out for ruminant cows [189] and for aquaculture [190-194], with some successes indicating that at least partial displacement of traditional feed applications can be achieved with residual algae protein materials.

In summary, algae-derived protein are anticipated to help displace other energy-intensive protein sources for human consumption, including meat [175]. Human food applications have the advantage that solvents are not required for extraction, which can reduce costs and indirect materials and energy to improve product sustainability [22].

**Animal feed**

**Ruminant.** Ruminant animal feed is a low-value product alternative for high-protein algae and cyanobacteria [175]. Nutritional quality of protein can be estimated using the biological value (BV), a measure of nitrogen retained for growth or maintenance; the digestibility coefficient (DC); and the net protein utilization (NPU), a measure that incorporates both BV and DC values of the assimilated amino acids [4, 183, 195]. Becker suggests that ruminant animal feed does not require cell disruption , as ruminants can digest algae cell walls [4]. This application can be advantageous to eliminate materials (enzymes) or mechanical cell rupture processes that can increase energy requirements and emissions.

**Aquaculture.** Other synergistic benefits can be obtained in integrated algae and aquaculture systems [195, 196]. For instance, algae was reported to control the pathogenic bacteria *Vibrio harveyi* by disrupting its sensing communication, and the co-digestion of algae and bacteria resulted in healthier *Artemia* culture because of better nitrogen assimilation [175]. Previous studies estimated that 30% of the global algae production is used for this animal feed application [4].

**Soil biostimulant**

Algae and cyanobacteria can be used as soil inoculants, biofertilizers, pesticides, and algal blooms prevention in an engineered algae cultivation setting. One application includes soil inoculation with cyanobacteria to enhanced seed germination, plant growth, grain yield, and crop nutritional value [24]. Additionally, Renuka and coauthors reported that green algae and cyanobacterial excretion of extracellular polymeric substrates (EPS) increase soil organic carbon, prevent soil erosion, and improve soil structure [24]. Other instances include *Bacillus* and *Pseudomona*s used to control soilborne plant diseases, and cyanobacteria used to fix atmospheric nitrogen to enhance nitrogen assimilation in plants [25]. Cultivation of nitrogen- and phosphorous-enriched algal and cyanobacterial using wastewater, and downstream biomass application as fertilizers can be used to mitigate global concerns such as the need to close the nutrient loop to prevent eutrophication, algal blooms, and oxygen depletion [7, 23].

**Functional Proteins and Enzymes**

High-protein algal biomass has the potential to be used for the production of industrial enzymes of interest as a value-added co-product. New industrial enzymes are always of interest in terms of efficiency, substrate range, resistance to feedback inhibition, and thermo-, cryo-, and/or salt tolerance. The sustainable production of industrial enzymes may be an option, given that algae can be cultivated on salt or waste water sources with $CO_2$

capture. Some thoughts around algae-specific enzymes of most interest include: carbonic anhydrases, cellulases, hydrogenases, laccase, lipases, nitrilases, nitrogenases, peroxidases, phosphatases, phytases, proteases, and thiolases, among others [197-199]. However, the final purity and function and activity of the produced enzymes is paramount for any success in this space.

**Pigments**

High-protein algal biomass is known to contain large amounts of chlorophyll and carotenoids that can be co-extracted with lipids but also separated from lipids to create a novel, high-value product stream. Chlorophyll and its derivatives have been used as natural antioxidants and nutraceuticals due to their health benefits [200]. Extracted chlorophyll can be used as value-added co-product; however, because chlorophyll contamination in the extracted crude oil can deactivate the catalyst in biodiesel production[201] and also degrade the quality of biodiesel [202], it is critical to remove chlorophyll from lipid products. Different applications have been demonstrated to separate pigments and in particular chlorophyll from the rest of the lipid fraction. For example, bleaching earth or clay can be used to adsorb chlorophyll [203-205]. Similarly, activated carbon, montmorillonite clay, and silica gel removed chlorophyll and other impurities from extracted lipids prior to catalytic hydrotreating [206-209]. An alternative process is based on an adaptation of acid catalyzed de-alkylation method of algal oils to remove up to 99% of chlorolphyll. In this process, acids, such as phosphoric acid or sulfuric acid cleave the phytol side chain from the porphyrin structure, which is not soluble in oil and can be washed out of the oil. The resulting precipitation of pheophorbide allows for easy separation from the oil. The hydrophobic phytol is soluble in the algal oil and can be a valuable component of the biofuel product after hydrogenation. Moreover, phosphoric acid discoloration treatment is compatible with conventional oil refining practices, since phosphoric acid is routinely added for oil degumming [210].

Similar to chlorophyll, most carotenoids are naturally associated with photosynthetic pigment-protein complexes [211] and lipid bodies [212]. A considerable amount of carotenoids can be co-extracted with lipids and separated based on chromatography applications or supercritical carbon dioxide technology [213, 214]. The purified carotenoids have value as nutraceuticals and anti-oxidant additives to animal feed [215, 216].

**Conversion products: Biochemical**

**Biomaterials.** Biomaterials, such as bioplastics, foams, adhesives and biocomposites, can be derived from the high-protein biomass and residues from algae, especially after processing for fuels and products derived from lipids and carbohydrates. [99, 217-220] Often, the material properties are highly dependent on the associated composition of the non-protein portion of the material, e.g. remaining ash, and for example for bioplastics applications, will require plasticizers to achieve equivalent mechanical properties [221-223]. Most research on protein-based plastics has used waste terrestrial feedstocks[224], and little published research has utilized algal proteins as a feedstock, with the exception of a recent report describing a process to produce polyurethanes using algal proteins [225]. The process was tested on the amino acid glycine initially, then subsequently on algal protein hydrolysates after chromatography separations generating pure amino acids and peptide feedstocks [226]. This peptide mixture was reacted with 1,2-diaminoethane to convert the carboxylic acids to amides then reacted with ethylene carbonate to produce urethane polyol feedstock. Similar reaction mechanisms have been described for the production of polyurethane foams from other protein-rich feedstocks. For example, polyurethane foams can be produced from soy-derived protein isolates after alkaline pretreatment to solubilize and fractionate the protein fraction [227]. A similar alkaline (NaOH) pretreatment was used to create effective adhesives from protein extracted from *Spirulina platensis* and *Chlamydomonas reinhardtii* [228-230]. More recently, a new pathway was described to produce a novel hyperbranched poly-ester urethane from alanine, without the use of isocyanates with excellent polymer properties [231], indicating a plethora of opportunities for high-quality biomaterials from a protein-rich algal biomass feedstock.

Commercial bioplastics can include polyhydroxyalkanoate (PHA) with applications for packaging, biomedicine and 3D printing, and biocreams for cosmetics [29]. PHA biopolyesters are biodegradable materials that have plastic-like properties, therefore, they can be used as a feedstock to penetrate the plastic market [76, 177]. Promising PHA-accumulating cyanobacteria strains include *Aulosira fertilissima, Nostoc muscorum, Spirulina maxima, Spirulina platensis, Synechococcus* sp. MA19, and *Synechocystis* sp. PCC 6803. For *Nostoc muscorum*, a mass fraction of PHA in dry cell biomass of up to 43 % was observed, after specifically culturing for increases associated with cultivation condition manipulation, such as mixotrophy, chemoheterotrophy and gas limitation [232, 233]. PHA serves as storage materials for carbon and energy; therefore, it can provide sustainability benefits to contribute effectively to the production of sustainable and biodegradable bioplastics [177].

As an alternative to bioenergy products, algae have also been explored for use in papermaking as the primary fiber source from the storage carbohydrate macromolecular

 

pool of the biomass or as filler materials, which includes a use for the protein fraction [234].

#### 4.6.2 Carboxylic acids and fusel alcohols.

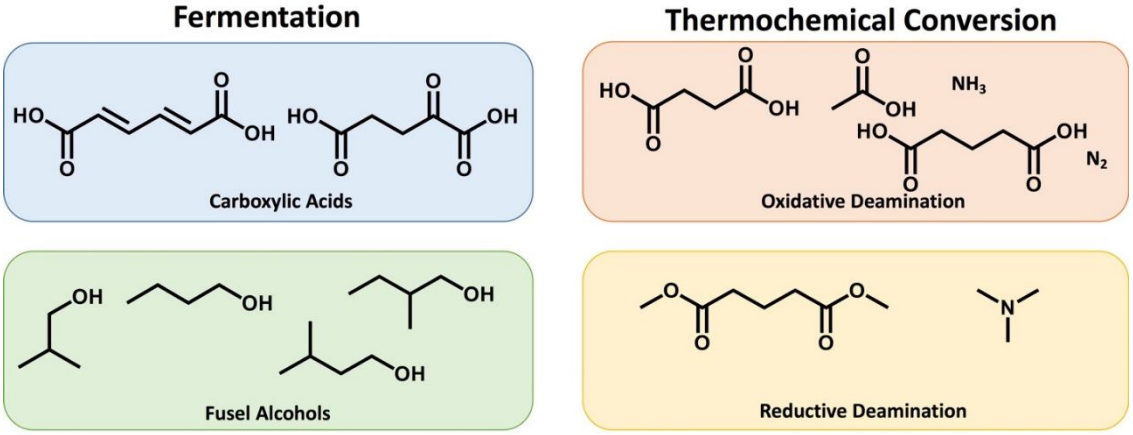


Figure 2. Overview of molecular structure of biochemical and thermochemical protein-derived small molecule conversion products as described in the text.

Carboxylic acids can be produced from feedstocks rich in amino acids and proteins. These have been reviewed from carbohydrates[235] and waste proteins [236] as feedstocks for microbial conversion to sustainable fuels and chemicals (Figure 2). Recently, several microorganisms, including *Pseudomonas putida*, *Corynebacterium glutamicum*, *Escherichia coli*, and *Saccharomyces cerevisiae* have been engineered for muconate production [237-242]. Muconic acid is the basis for several platform chemicals, including adipic acid, a monomer for nylon 6,6 production [243, 244], which can be catalytically upgraded and deoxygenated to a hydrocarbon fuel intermediate [245]. In addition to carboxylic acids, further reduced products, including fusel alcohols [246] have recently been demonstrated from high-protein *Microchloropsis salina* biomass using an engineered strain of *E. coli* [247]. For utilization of high-protein algal biomass, *P. putida* is especially promising. As a rhizospheric bacterium, *Pseudomonas putida* is well known to possess an extremely diverse metabolism, even preferring alternative carbon sources over glucose [248-250], as well as being genetically malleable allowing for the production of a wide range of natural and non-natural chemicals from a diversity of feedstocks [250]. Pseudomonads are well-known proteolytic milk-spoilage organisms and secrete proteases in response to peptides and proteins [251-254]. *P. putida* has been shown to rapidly consume free amino acids and oligo peptides and actively regulates amino acid metabolism through complex changes in the functioning of the TCA cycle depending on growth stage (early, mid, or late exponential) [255]. Amino acids entering the TCA cycle are converted to intermediates providing energy, reducing equivalents, and carbon backbones for the synthesis of other amino acids while the overflow can be directed through the engineered pathway for muconic production, using an engineered *P. putida* [256].

A processing strategy that has been described in the literature to selectively deaminate amino acids to carboxylic acids and then convert the remaining carbon backbones to fuels and chemicals allows for biological deamination and recycling of ammonia for fertilizer applications[257]. Metabolic engineering strategies that can be used to channel microbial products to higher alcohols, such as n-butanol and isobutanol, have been reviewed recently.[258] Particularly interesting is the transformation of *E. coli* to be able to convert proteins to higher (C3-C8) "fusel" alcohols[259]. One of the challenges with this approach includes channeling the diverse set of amino acids to fewer products and redesigning the cell's nitrogen flux to favor deamination.[260] Biological or enzymatic deamination has also recently begun to gain traction for lab-scale and potential commercial production of α-keto acids [261, 262]. Several types of enzymes have activity for this reaction, including amino acid oxidases, dehydrogenases, aminotransferases, and deaminases.[263-266] Like many of the deamination products described above, α-keto acids are versatile molecules in foods, feeds, pharmaceuticals, and chemical synthesis [267].

**Conversion products: Thermochemical**

**Hydrothermal liquefaction (HTL).** The presence of up to 80% of water in the wet harvested algal slurry can be a barrier to cost-effective high-temperature treatment such as pyrolysis and gasification [268]. HTL is an alternative thermal process that can directly convert wet biomass into an organic bio-oil under subcritical water condition, and is ideally suited for a high-protein biomass feedstock [269]. The resulting biocrude oil can be converted into biofuel through catalytic upgrading. HTL may facilitate cost reduction in biorefining as HTL bypasses the energy intensive step of drying biomass and simplifies downstream processing. Directly converting wet microalgal biomass into fuel range compounds via HTL has gained significant momentum in the past 5 years [270-273].

It has been realized that the biochemical composition could impact the bio-oil yield, and several research groups have demonstrated relationship between the algal biochemical composition and bio-oil yields using predictive models [272, 274, 275]. Generally, lipids contribute most to the bio-oil yield followed by protein, while carbohydrate contribute the least to bio-oil. Thus, HTL can be a favored conversion pathway to convert high protein algal biomass into bio-oil for biofuel production. However, high protein algal biomass contains proportionally high nitrogen, some of which is converted to organonitrogen compounds in the oil phase. This nitrogen can be challenging to remove, but must be eliminated prior to fuel blending.

**Selective Deamination.** Several approaches to selectively deaminate amino acids have been developed, though mainly for analytical purposes. Each of these routes produces carboxylic acids of various chemistry, which can subsequently be separated as high value products or intermediates, or converted to fuels (Figure 2). It is worth noting that while the deamination is selective when applied to individual amino acids, application to whole algae protein will produce a mixture of products that depends on the composition of the protein feedstock. For some applications, such as conversion to fuels, obtaining a mixture of carboxylates is not a detriment (e.g., the mixed acids may be converted to a mixture of hydrocarbons suitable for fuel by ketonization, condensation, and HDO) [245]. Some of these techniques release the nitrogen as $NH_3$, which can be recovered by ion exchange on an acidic resin and recycled for algae cultivation, while others release nitrogen as $N_2$ or methylamines, which, in the case of $N_2$, presents a significant sustainability penalty to return biologically fixed nitrogen to the atmosphere.

Stadtman showed with very dilute (50 mM) solutions under physiological conditions (40 °C, aqueous) that $H_2O_2$ was capable of oxidizing amino acids to a small mixture of products corresponding to the parent amino acid [276, 277]. In particular, a carboxylic acid one carbon shorter than the parent amino acid was formed as an end product, while the corresponding α -keto acid and aldehyde were reaction intermediates. Under these conditions, conversion of leucine was ~50% after 90 min. Most other amino acids reacted at a similar rate, though proline, tyrosine, glycine, tryptophan, methionine, and aspartate reacted at roughly one-third to one-tenth the rate of leucine [276]. Additionally, other amino acids give less clean product spectra, though the primary products were $NH_3$, $CO_2$ and carbonyl-containing compounds in each case. The oxidation is accelerated by the presence of $HCO_3^-$, iron chelators such as ADP (though these compounds become inhibitory at concentrations higher than that of $Fe^{2+}$), and metal ions, especially $Fe^{2+}$, which activates $H_2O_2$ via well-known Fenton chemistry.

It has been known for more than a century that nitrous acid, $HNO_2$ (or HONO) readily deaminates primary amino acids to a carboxylic acid, $H_2O$, and $N_2$ at reaction times under 30 min and temperatures under 40 °C [278]. Additionally, HONO is usually generated in situ by adding sodium nitrite, $NaNO_2$, to a solution of the strong acid. Thus, this oxidation approach may integrate well with upstream operations employing acid for cell lysis or protein hydrolysis. The advantages are that the reaction is high-yielding at mild conditions and short reaction times, that this approach maintains carbon in the resulting carboxylic acid (oxidation with $H_2O_2$ loses one carbon), and the byproducts ($H_2O$ and $N_2$) are environmentally benign. The disadvantages are that $NaNO_2$ is a relatively expensive oxidant, and that this approach requires stoichiometric consumption of $NaNO_2$ and strong acid (though it could also be seen as a method of neutralization for upstream unit operations). Additionally, it is known that secondary and tertiary amines are not fully converted to $N_2$ (at least, not at typical reaction conditions), instead being converted to a nitroso group (-N=O) or a complex mixture of products [279]. As with HTL, this residual nitrogen would likely be detrimental to fuel and combustion properties, but could be removed by an acidic ion exchange resin prior to downstream catalytic upgrading. This approach, in combination with $H_2O_2$, has recently been employed to increase the digestibility of sewage sludge [280].

**Reductive deamination.** De Schouwer et al. showed that two amino acids, aspartic acid and glutamic acid, could be N-methylated by formaldehyde over a Pd/C catalyst in methanol solvent in yields above 90%, and the subsequent N,N-dimethyl amino acid analog could be deaminated to trimethylamine and a dicarboxylic acid dimethyl ester corresponding to the parent amino acid in yields above 80% using a $Pt/TiO_2$ catalyst under an $H_2$ atmosphere in methanol. The deamination was complete, and the most of the remaining carbon could be recovered as glutaric acid derivatives [281]. Water was also explored as a solvent, but but glutarate yield and selectivities were lower. Several other metal/support combinations were also explored for the second reaction step, but were found to be less selective for the dimethyl diester. Amino acids other than aspartic and glutamic acid were not explored, so it is unknown how efficiently acids with more complex side chains, such as lysine or proline, would undergo deamination. For primary amines, however, this appears to be a high-yielding and carbon-efficient approach to generating carboxylic acids.

## Sustainability assessment

### Goals and scope

This part of the study presents a review of models' uncertainty inputs and risk analysis to simulate scenarios with a probability of sustainability outcomes in high-protein algal bioproducts using a Monte Carlo method. In developing the goals and scope of this study, first, we defined the system boundaries from unit processes found in the literature, including algae cultivation, biomass dewatering, biomass sterilization, cell disruption, protein solubilization, protein purification, protein concentration, and product displacement. Second, we summarized model input uncertainties reviewed from biotechnology processes, and TEA and LCA studies of algal-derived bioproducts. Lastly, we propagated model input uncertainties in a Monte Carlo simulation to quantify the probability outcomes of environmental sustainability metrics, including life cycle energy and life cycle emissions. The functional unit used in the sustainability risk analysis is one kilogram of algal-protein ($kg_{protein}$) to displace products such as animal feed and polymers. The risk analysis for environmental sustainability of high-protein algae-derived -animal feed and –polymers was simulated in OpenLCA [282] by constructing two different systems, and using open source databases and the non-distributed U.S. electricity mix for the year 2019 [283].

### System analysis and model input uncertainties

Table 3. Capital costs input uncertainty for various algae strains and high-protein algae bioproducts processes.

| Sub-system | Parameter/unit process | Units | Costs year | Value | | | Reference |
|---|---|---|---|---|---|---|---|
| | | | | Mean | Lower | Upper | |
| Cultivation | Raceway* | $ $m^{-2}$ | 2007,2014 | 782±1082 | 1.1 | 2016 | 34, 36, 46 |
| | Flow-way | $ $m^{-2}$ | 2014,2003 | - | 6.6 | 15.2 | 34, 35 |
| | Closed-PBR | $ $m^{-2}$ | 2013,2007 | - | 29.6 | 831.2 | 9, 33, 46 |
| Dewatering | Gravity thickening | $ $m^{-2}$ | - | 17392 | - | - | 57 |
| | Filtration | $ $m^{-3}hr^{-1}$ | 2016 | 5575 | - | - | 56, 81 |
| | Centrifuge | $ $m^{-2}$ | 2013 | 16.9 | - | - | 33 |
| Sterilization | UV | $ $m^{-3}hr^{-1}$ | 2016 | 19 | - | - | 56, 81 |
| Disruption | Centrifuge | $ $m^{-3}$ $hr^{-1}$ | 2003 | 41,455 | | | 284 |
| | Centrifuge | $ $m^{-2}$ | 2013 | 16.4 | - | - | 33 |
| | Centrifuge | $ $kg_{biomas}^{-1}$ | - | 0.017±0.007 | 0.009 | 0.021 | 74 |
| | Centrifuge | $ $m^{-3}$ | 2014 | 120 | - | - | 36 |
| | Dilute acid | $ $kg_{biomas}^{-1}$ | 2011 | 0.25 | 0.19 | 0.29 | 20 |
| | Dilute acid | $ $kg_{biomas}^{-1}$ $hr^{-1}$ | 2016 | 174 | - | - | 285 |
| | Dilute acid: Settler(S), mixer(M) | S: $ $m^{-2}$ | - | 17391 | - | - | 57 |
| | | M: $ $m^{-3}$ | - | 0.3 | - | - | |
| | Ultrasound | $ $m^{-3}$ | 2014 | 60 | - | - | 36 |
| Solubilization | Solvent (hexane) | $ $kg_{protein}^{-1}$ | - | 0.15±0.06 | 0.08 | 0.18 | 74 |
| | Solvent (hexane) | $ $kg_{protein}^{-1}$ $hr^{-1}$ | 2016 | 884 | - | - | 56, 81 |
| | Solvent (hexane) | $ $m^{-3}$ | 2014 | 20 | - | - | 36 |
| | Enzyme | $ $kg_{protein}^{-1}$ | 2011 | 0.11 | 0.09 | 0.13 | 20 |
| Concentration | Filter,centrifuge,dryer | $ $kg_{protein}^{-1}$ | 2011 | 0.37 | 0.32 | 0.38 | 20 |
| | Centrifuge(C), drum drying(D) | C: $ $m^{-3}hr^{-1}$ | - | 146549 | - | - | 57 |
| | | D: $ $m^{-2}$ | - | 15,833 | - | - | |
| Product preparation (bioplastic | Membrane | $ $m^{-3}$ $hr^{-1}$ | 2016 | 2.9 | - | - | 56, 81 |
| | Microwave, jet milling | $ $kg_{biomas}^{-1}$ $hr^{-1}$ | 2016 | 967.9 | - | - | |

*Capital costs include $CO_2$ spargers or mixers, paddlewheels, land, liners, and various construction costs.

Table 4. LCA input uncertainty for various algae strains and high-protein algae bioproducts processes

| Sub-system | Parameter/unit process | Units | Value | | | Reference |
|---|---|---|---|---|---|---|
| | | | Mean | Lower | Upper | |
| Cultivation | AFDW algae productivity | g $m^{-2}$ $d^{-1}$ | 23±12 | 2.0 | 50.0 | 15, 49 |
| | DLUC: AGB | Tons $Ha^{-1}$ | 4.9 | 0 | 644 | 55 |
| | DLUC: SOC | Tons $Ha^{-1}$ | 8.6 | 0 | 85 | 55 |
| | Carbon capture | mg $l^{-1}$ $d^{-1}$ | 1125±544 | 564 | 1880 | 1 |
| | N | mg $l^{-1}$ | 14.3±10.7 | 4.6 | 25.8 | 49 |
| | Nitrogen depletion | days | 10 | - | - | 49 |
| | $P_2O_5$ | mg $l^{-1}$ | 8.2±5.2 | 2.5 | 12.6 | 49 |
| | Padelwheel mixing | W $m^{-3}$ | 1.0±1.0 | 0.1 | 2.1 | 48 |
| | Flow-way pumping | W $m^{-2}$ | 17.5 | - | - | 34 |
| | PBR mixing | W $m^{-3}$ | 1.1±0.7 | 0.4 | 1.9 | 48 |
| | Protein content | % | 51±13 | 20 | 71 | 4 |
| | Protein density | g $l^{-1}$ | 1345±76 | 1220 | 1430 | 286-292 |
| Dewatering | Gravity thickening | MJ $m^{-3}$ | 0.14±0.17 | $2\times10^{-4}$ | 0.36 | 286, 293, 294 |
| | Belt filter press | MJ $m^{-3}$ | 1.07±1.85 | $2\times10^{-3}$ | 3.2 | 56, 81, 294 |
| | Centrifuge | MJ $m^{-3}$ | 1.85±2.24 | $2\times10^{-2}$ | 4.68 | 286, 294, 295 |
| Sterilization | UV | MJ $m^{-3}$ | 0.07±0.09 | $1\times10^{-5}$ | 0.18 | 56, 81, 294 |
| Disruption | Centrifuge | MJ $m^{-3}$ | 20.0±20.0 | 1.1 | 54.0 | 284, 296, 297 |
| | Centrifuge | MJ $kg_{biomas}^{-1}$ | 3.7±4.0 | 0.2 | 8.0 | 74 |
| | Dilute acid | MJ $kg_{biomas}^{-1}$ | 16.0±22.6 | $7\times10^{-4}$ | 32.0* | 56, 81, 298 |
| | Dilute acid ($H_2SO_4$) | kg $m^{-3}$ $hr^{-1}$ | 5.4 | - | - | 20 |
| | Osmotic shock | MJ $m^{-3}$ | - | 1.8 | 2.3 | 294 |
| | Ultrasound | MJ $m^{-3}$ | 13.0 | - | - | 299 |
| Solubilization | Solvent (hexane) | MJ $kg_{protein}^{-1}$ | - | 0.01** | 1.3 | 56, 58, 81 |
| | Solvent (hexane) | $g_{C6H14}g_{biomass}^{-1}$ | 5.9 | - | - | 56, 81 |
| | Enzyme | $g_{enzyme}$ $l^{-1}$ | 0.01 | - | - | 20 |
| | Mesophilic anaerobic digestion with thermal hydrolysis | MJ $m^{-3}$ | - | 0.05 | 0.07 | 294 |
| Purification | Heat | MJ $kg_{protein}^{-1}$ | 9.1±5.0 | 4.2 | 14.2 | 74 |
| Concentration | Centrifuge | MJ $m^{-3}$ | - | 0.018 | 0.047 | 294 |
| | Drying | MJ $kg_{protein}^{-1}$ | 129.6±82.8 | 46.8 | 212.4 | 296 |
| | Filtration | MJ $kg_{protein}^{-1}$ | 1.7±1.5 | 0.7 | 3.4 | 59, 294 |
| Product preparation (bioplastic) | Membrane | MJ $m^{-3}$ | | 0.7 | 1.1 | 56, 81, 294, 300, 301 |
| | Microwave | W $kg^{-1}$ | 84.3±143.5 | 0.5 | 250 | |
| | Jet milling | MJ $kg_{LEA}^{-1}$ | 1.7 | - | - | |
| | Injection molding | MJ $kg^{-1}$ | - | 1.5 | 8 | |

*GREET reference considers life cycle energy. ** Units are MJ $kg_{flow}^{-1}$

ARTICLE

From the spectrum of cultivation, and cell rupture for product recovery and conversion processes reviewed in sections two and three, we summarized this list based on the most relevant biotechnology, and TEA and LCA data reported in the literature with implications in high-protein algal-derived bioproducts' sustainability assessment. For instance, most studies considered algae cultivation in raceways, flow-ways, and closed-PBR. Biomass dewatering studies included gravity, filters, and centrifuges, and biomass sterilization assumed ultraviolet (UV). Cell disruption studies to recover metabolites investigated centrifuge, dilute acid, and ultrasound. Protein solubilization and purification research considered solvent, enzyme, hydrolysis, and heat. For protein concentration, most sustainability studies assumed centrifuges, dryers, and filters. Displacement of polymers and polystyrene, considered additional unit processes such as membrane separation, microwave, and jet milling to manufacture bioplastics. Table 3 includes the review of biotechnology process costs, and Table 4 the direct materials and energy requirements, in high-protein algal-derived bioproducts studies. In the following sections, we present the results of this review and discuss the implications in sustainability studies.

**Sustainability studies neglected DLUC, carbon capture, wastewater remediation, and protein content uncertainty.** The reviewed processes start with algal cultivation, including raceways, closed-PBR, and flow-ways. The TEA review suggests lower capital costs required in flow-ways relative to raceways and photobioreactors. For instance, Hoffman, J. and Pizarro, C. reported area-specific flow-ways costs ranging from 6.6 to 15.2 \$ $m^{-2}$, and [9, 33, 34, 36, 46] reported area-specific raceway and PBR costs ranging from 1.1 to 2016 \$ $m^{-2}$. CEPCI years and quotation dates, and scales of operation units are barely reported in TEA studies, which limits the normalization of these studies to perform an analysis that considers process scale-up.

The environmental sustainability risk analysis of this review considered AFDW productivity uncertainty from the most researched biotechnology, open-channel raceways, because of long-term operations under different seasons available in the literature [10-18, 49]. This source of uncertainty allowed us to explore not only common AFDW productivities from 10 to 20 g $m^{-2}$ $d^{-1}$, but also pessimistic scenarios reported at Arizona during Summer 2015 with photo-respiration effects resulting in lower productivities at 2 g $m^{-2}$ $d^{-1}$, and long-term optimistic goals assumed in some LCA studies from 20 to 50 g $m^{-2}$ $d^{-1}$. To account for DLUC impacts during the construction stage in U.S. barren land areas, we incorporated SOC and AGB emissions reported by [55]. DLUC has been neglected in most algae-derived biofuels and bioproducts studies but must be considered in a comprehensive LCA.

Additional sources of uncertainties in our risk analysis of algae cultivation include carbon capture rates [1], nitrogen and phosphorous uptake rates [49], and mixing energy through paddle wheels [48]. Carbon capture uncertainty allowed us to consider a spectrum of probabilist scenarios that can provide environmental benefits to target negative fluxes and net carbon neutral algae-derived bioproduct pathways. Nitrogen and phosphorous uptake rates uncertainties provided us the range of indirect material supply (fertilizers) considered in the literature of algae-bioproduct LCA. Although displacement of fertilizers is possible using wastewater [8, 83-85], but mainly providing optimum no –growth limiting and –inhibiting nutrient concentrations from sludge centrate [7], this scenario was neglected in the risk analysis to have a consistent comparison with most LCA studies in the literature. Mixing energy uncertainty through paddlewheels provided scenarios under the most common operating conditions in industrial raceways, 1 to 2 W $m^{-3}$, and recent research findings demonstrating well-mixed conditions at low mixing energy requirements, 0.1 W $m^{-3}$ [48].

Lastly, our risk analysis of algae-derived bioproduct included protein content uncertainty reported in the literature from 20 to 71% by [4], and protein density reported from 1220 to 1430 g $l^{-1}$ [286-292]. Our review shows that protein content is an aleatory uncertainty as it varies with different strains, growth phase, cultivation conditions, and pretreatment and analytical method [60-67]. Most studies in the literature assumed a fixed or constant protein content, obtaining unrealistic results in the reported metrics of sustainability. The protein content is highly sensitive in sustainability studies as protein is the functional unit that drives product magnitude and, therefore, metrics of sustainability performance.

**Studies showed a discrepancy in algal biomass dewatering, sterilization, and cell disruption costs and energy consumptions.** The most common processes studied in sustainability assessments considered gravity thickening, belt filter press, and centrifuge for algal biomass dewatering process; UV for biomass sterilization; and centrifuge, dilute acid, and ultrasound for cell disruption. Among the algal biomass dewatering processes, centrifuge shows the lowest capital expenses, for instance, with area-specific facility costs of 16.7 \$ $m^{-2}$ reported by [33] for a 2013 CEPCI. Table 3, however, demonstrates a high-uncertainty in the direct energy consumptions through a centrifuge, with two-orders of magnitude differences between the studies.

Beckstrom, B. D. proposed biomass sterilization using UV for bioplastics applications at 19 \$ $m^{-3}$ $hr^{-1}$ for 2016 costs that should be considered for any algal bioproduct for human or animal consumption to obey with health and safety regulations. However,

energy consumptions reported by [56], $1\times10^{-5}$ MJ $m^{-3}$, are about four orders of magnitude lower than UV energy consumption commonly used in wastewater treatment facilities [294]. These differences illustrate the need for more costs and energy research associated with this sterilization process. For cell disruption processes, the centrifuge is the most researched unit, but studies again show a discrepancy in the costs found in Table 3 and energy consumptions ranging from 1.1 to 54 MJ $m^{-3}$. For instance, [284] illustrated how different scales, operational modes, suspended solids, and performances could impact the volume-specific energy consumption. Sustainability studies barely describe these specifications in the devices used in the processes, that can lead to higher errors in their results. Tables 1 and 2 show other cell disruption costs and energy consumptions units including, dilute acid, osmotic shock, and ultrasound that can potentially reduce costs and energy consumptions in the recovery of high-protein algal metabolites.

**Solvents for protein solubilization and centrifuge for protein concentration are the most common unit process for animal feed bioproducts.** Sustainability research is illustrated in Tables 3 and 4 for protein solubilization and concentration. Given the limited data reported in the literature, to date, studies show enzymatic hydrolysis and solvents as the preferred methods for protein solubilization. For instance, [20] reported costs ranging from 0.019 to 0.13 \$ $kg_{protein}^{-1}$ using enzymes, and [74] reported costs ranging from 0.08 to 0.18 \$ $kg_{protein}^{-1}$ using hexane. However, thermal hydrolysis shows lower energy requirements, as high as 0.07 MJ $m^{-3}$ reported by [294] in wastewater reactors, relative to hexane as high as 1.3 MJ $kg_{protein}^{-1}$ (1,749 MJ $m_{protein}^{-3}$ assuming a protein density of 1345 g $l^{-1}$) reported by [58]. Heating, filtration, centrifugation, and drying are the most common unit processes researched for protein purification and concentration, but limited research is available in the sustainability assessment of high-protein algal bioproducts.

**Membrane separation, microwave, and jet milling were proposed for algae-derived bioplastics applications.** Bochenski, T. and Beckstrom, B. D. researched the sustainability of elastomers and bioplastics from high-protein algal bioproducts. [57] reported a MSP of 7.0 \$ $kg_{protein}^{-1}$ to produce polymers (elastomers), and [56] reported 0.97 \$ $kg_{LEA}^{-1}$ to manufacture bioplastics. [57] Assumed a non-specified strain algal biomass with 40% protein content, with downstream processing including high-pressure homogenization, dilute acid using hydrochloric acid, and protein concentration through centrifuge and drum drying. Product preparation unit processes to manufacture elastomers were excluded from the boundaries. [56] used *Scenedesmus acutus* and assumed 36% protein content, with downstream processes including dewatering, cell disruption using dilute acid, protein solubilization using hexane and heat, and product preparation units including membrane separation, microwave, and jet milling. There are limited algal-derived polymers costs and energy consumption data in the literature. [56] energy consumptions were found to be three orders of magnitude lower than reported by [300] for general industrial applications. [56] assumed 1.7 MJ $kg_{LEA}^{-1}$ for bioplastic jet milling, however, [301] researched injection molding energy consumptions for the plastics industry ranging from 1.5 to 8 MJ $kg^{-1}$. Sustainability results of to date high-protein algal-derived polymers are not conclusive given that these product preparation uncertainties were ignored.

**Risk analysis**

To assess the risk associated with high-protein algal-derived bioproducts input variations, we used a Monte Carlo methodology to simulate scenarios with probabilities of sustainability metrics performance. The two sustainability metrics and impacts considered in the risk analysis are the net life-cycle energy and net life cycle $CO_2$eq emissions that we elicited under model input uncertainties reviewed in the literature and summarized in section 7.2 and Table 4. Given the epistemic nature of these uncertainties [302], we assumed a uniform distribution of the reviewed model inputs for the Monte Carlo risk analysis.

The production of bioproducts in a low energy-intensive fashion is the primary goal of any potential technology to displace fossil fuels, and its derive bioproducts. Therefore, we selected life cycle energy ( $E$) as the first metric of interest in the risk analysis:

$$E\left(\text{MJ kg}_{\text{protein}}{}^{-1}\right) = \frac{E_{consumed} - E_{displaced}}{P} \quad \text{Eq 1.}$$

The net $E$ was computed by subtracting the direct and indirect materials and energy consumption in the system ($E_{consumed}$), and the energy requirements displaced from conventional products such as soybean and polymers ($E_{displaced}$). $E$ is normalized in this risk analysis by the protein (P, kilograms) produced in the system, and considering the uncertainty of the protein content presented in Table 4.

Technologies that reduce or contribute to negative net lifecycle greenhouse gas (GHG) emissions (environmental benefits) are part of the primary goal of bioproducts. GHG is defined by the Intergovernmental Panel on Climate Change (IPCC) as the carbon dioxide equivalent emissions because of the direct and indirect amount of fuel or energy consumed in the system [303]:

$$GHG\left(\text{CO}_2\text{eq kg}_{\text{protein}}{}^{-1}\right) = \frac{FC \cdot EF_{fuel} \cdot P_{technology} - \text{CO}_2\text{eq}_{displaced}}{P} \quad \text{Eq 2.}$$

The first term results in the $CO_2$eq emissions produced by the systems that include the fuel or energy consumed ($FC$), the emission factor based on the type of fuel or energy technology ( $EF_{fuel}$), and the penetration or fraction of the energy source of given energy technology ($P_{technology}$). The second term, $\text{CO}_2\text{eq}_{displaced}$, consider photosynthetic carbon capture and indirect emissions displacement because of fossil fuel products replaced by algae-derived bioproducts. $GHG$ is normalized in this risk analysis by the protein (P, kilograms) produced in the system, and considering the uncertainty of the protein content presented in Table 4. The

following sections present the risk analysis results using the Monte Carlo method, including life cycle $E$ and $GHG$.

**Probabilistic results of algae-derived animal feed and bioplastics show that 67% of scenarios will demand life cycle energy consumptions equal to or lower than Nylon 6.**

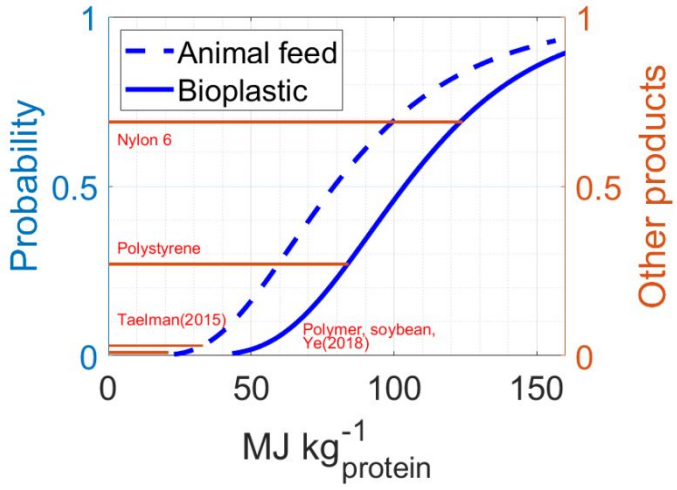


Figure 3. Lognormal probabilistic distribution of high-protein algal-derived animal feed (dashed-line) and polymer (continuous-line) life cycle energy. Algae-derived animal feed life-cycle energy probabilistic distribution was compared against metrics reported in the literature, including algae-derived animal feed and soybean [59, 79, 298]. Algae-derived bioplastics life-cycle energy probabilistic distribution was compared against products reported in the literature, including nylon 6, polystyrene, and polymer [298].

To analyze the life cycle energy probabilistic results from the risk analysis, we conducted a fit against normal, exponential, lognormal, and stable distributions. Figure 3 illustrates the lognormal probability distribution of the life cycle energy of algal-derived animal feed and polymers. The algae-derived animal feed mean and standard deviation of logarithmic values are 4.370 and 0.349, with a standard error of 0.01. The algae-derived bioplastic mean and standard deviation of logarithmic values are 4.641 and 0.349, with a standard error of 0.01. Algae-derived animal feed mean and variance are 88.1 and 1864 MJ $kg_{protein}^{-1}$. Algae-derived bioplastic mean and variance are 110.2 and 1578 MJ $kg_{protein}^{-1}$. As expected, mean life cycle energy values of algae-derived animal feed are lower than bioplastics because algae-derived bioplastics require additional unit processes for product preparation, including membrane separation, microwave, and injection molding.

Life-cycle energy probability distributions were compared against metrics of sustainability reported in the literature for the algae-derived animal feed itself, soybean, and conventional nylon 6, polystyrene, and polymer. Algae derived protein tablets researched by [59] resulted in life cycle energy values of 21.2 MJ $kg_{protein}^{-1}$, and algae animal feed investigated by [79] resulted in 32.7 MJ $kg_{protein}^{-1}$. These values are similar to those reported for soybean meal by [298]. Based on the risk analysis, life cycle energy values of ≤37 MJ $kg_{protein}^{-1}$ in algae-derived animal feed are likely in 5% of the scenarios by considering model input uncertainty. Meaning that these low metrics of sustainability are possible under the most optimistic conditions, considering for instance high AFDW biomass productivity and protein content, and low energy consumption in downstream cell rupture for product recovery and conversion processes.

Algae-derived bioplastics LCA studies neglected life cycle energy calculations. Therefore, we compared the probabilistic distribution of our risk analysis against conventional plastic products, including nylon 6, polystyrene, and polymer. Life cycle energies of these products reported by [298] are 124 MJ $kg_{nylon}^{-1}$, 84 MJ $kg_{polystyrene}^{-1}$, and 21 MJ $kg_{polymer}^{-1}$. Based on our probabilistic distribution of algae-derived bioplastics, 69% of the scenarios resulted in life cycle energies that are equal or lower than nylon 6; 27% of the scenarios resulted in values equal to or lower than polystyrene; and a negligible fraction of the scenarios are equal to polymers. These probabilistic results suggest that algae-derived bioplastics would obtain the best environmental benefits from a life-cycle energy consumption perspective by displacing products such as nylon 6 and polystyrene under the most optimistic conditions for cultivation, protein content, low intense energy in downstream extraction and conversion processes.

**Probabilistic results of algae-derived animal feed and bioplastics show that more than 50% of scenarios can result in negative-net life cycle CO2eq emissions.**

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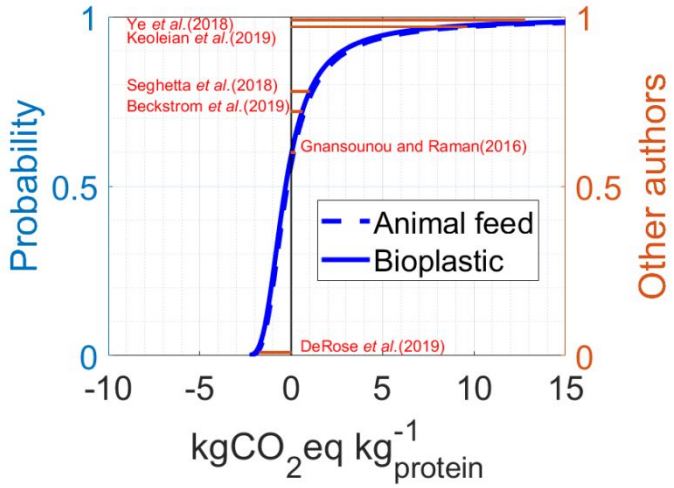


Figure 4. Stable probabilistic distribution of high-protein algal-derived animal feed (dashed-line) and polymer (continuous-line) life cycle $CO_2$eq emissions. Life cycle $CO_2$eq probabilistic distribution is compared against metrics reported in the literature including algae-derived animal feed [20, 58, 59, 80, 298] and algae-derived bioplastic [56].

To analyze the life cycle $CO_2$eq emissions probabilistic results from the risk analysis, we conducted a fit against normal, exponential, lognormal, and stable distributions. Figure 4 illustrates the stable probability distribution of the life cycle $CO_2$eq of algal-derived animal feed and polymers. The algae-derived animal feed first and second shape and scale and location parameters are 1.149, 1.989, 0.702, and -0.542 with standard errors of 0.033, 0.010, 0.023, and 0.038. The algae-derived bioplastic first and second shape and scale and location parameters are 1.188, 1.0, 0.699, and -0.621, with non-detectable standard errors. Algae-derived animal feed and bioplastic means are 2.36 and 1.68 MJ $kg_{protein}^{-1}$. Despite higher energy requirements to manufacture algae-derived bioplastics, these showed lower mean life cycle $CO_2$eq values than animal feed because of the environmental benefits to displace fossil fuel emissions required in the manufacturing of conventional plastics.

Life cycle $CO_2$eq emissions probability distributions were compared against metrics of sustainability reported in the literature for algae-derived animal feed and bioplastic. Algae-derived protein tablets researched by [59] reported 12.8 $kgCO_2eq\ kg_{protein}^{-1}$, the highest value in the literature. Based on our risk analysis, 99% of the algae-derived animal feed scenarios would have values equal to or lower than reported by [59]. Algae-derived animal feed studies of [80] and [58] and reported life cycle emissions values at 1.0 $kgCO_2eq\ kg_{protein}^{-1}$ and 0.2 $kgCO_2eq\ kg_{protein}^{-1}$. 80% of the algae-derived animal feed scenarios would have values equal or lower than reported by [80] and [58]. Likewise, [298] reported soybean meal emissions at 0.48 $kgCO_2eq\ kg_{protein}^{-1}$. Concerning algae-derived bioplastic, [56] reported emissions at 0.6 $kgCO_2eq\ kg_{protein}^{-1}$. Our probabilistic risk analysis showed that 80% of the algae-derived bioplastics would result in values equal to or lower than 0.6 $kgCO_2eq\ kg_{protein}^{-1}$. [20] reported the only negative net emissions in the literature at -3.0 $kgCO_2eq\ kg_{protein}^{-1}$, suggesting that more optimistic conditions were assumed. Our risk analysis, however, reveals that more than 50% of the algae-derived bioplastic scenarios are likely to produce negative net $CO_2$eq emissions. Additional upgrades to the system, including integration with wastewater nutrients remediation using algae or cyanobacteria, could provide additional environmental benefits through water quality improvement with low energy consumption than conventional nutrient removal processes, and displacement of indirect energy requirements of fertilizers. These results suggest that both algae-derived animal feed and bioplastics can contribute to reduce and displace $CO_2$eq emissions through carbon capture and utilization in the form of high-protein bioproducts to provide environmental sustainability benefits.

**Conclusions**

Literature review and risk analysis using a Monte Carlo method demonstrated the sustainability assessment model inputs' uncertainties and the scenarios that provide environmental benefits in high-protein algal bioproducts systems. Our review reveals that most TEA and LCA studies neglected uncertainties that are highly sensitive in metrics of sustainability, including DLUC, protein content, and specific energy consumption in cultivation, cell rupture for bioproduct recovery, and conversion processes. We provided in our research state-of-the-art processes and model input uncertainties that must be considered in the sustainability

assessment of bioproducts from high-protein algal biomass. Probabilistic life cycle energy results demonstrated that environmental benefits are more likely to be achieved in scenarios displacing animal-feed and highly resource-intensive products, including nylon 6 and polystyrene. Probabilistic life cycle $CO_2$eq emissions showed that even net-negative carbon pathways are possible under the best conditions for cultivation, protein content, low-energy intensity processes, and carbon capture and utilization in the form of bioproducts stocks.

#### Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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