

Review

The Potential of Marine Microalgae for the Production of Food, Feed, and Fuel (3F)

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Abstract: Whole-cell microalgae biomass and their specific metabolites are excellent sources of renewable and alternative feedstock for various products. In most cases, the content and quality of whole-cell biomass or specific microalgal metabolites could be produced by both fresh and marine microalgae strains. However, a large water footprint for freshwater microalgae strain is a big concern, especially if the biomass is intended for non-food applications. Therefore, if any marine microalgae could produce biomass of desired quality, it would have a competitive edge over freshwater microalgae. Apart from biofuels, recently, microalgal biomass has gained considerable attention as food ingredients for both humans and animals and feedstock for different bulk chemicals. In this regard, several technologies are being developed to utilize marine microalgae in the production of food, feed, and biofuels. Nevertheless, the production of suitable and cheap biomass feedstock using marine microalgae has faced several challenges associated with cultivation and downstream processing. This review will explore the potential pathways, associated challenges, and future directions of developing marine microalgae biomass-based food, feed, and fuels (3F).

Keywords: marine microalgae; feed and food; biofuels; water footprint; bioactive compounds

Citation: Mahata, C.; Das, P.; Khan, S.; Thaher, M.I.A.; Abdul Quadir, M.; Annamalai, S.N.; Al Jabri, H. The Potential of Marine Microalgae for the Production of Food, Feed, and Fuel (3F). *Fermentation* **2022**, *8*, 316. <https://doi.org/10.3390/fermentation8070316>

Academic Editors: Abdelrahman Zaky and Abd El-Fatah Abomohra

Received: 28 May 2022

Accepted: 3 July 2022

Published: 5 July 2022

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1. Introduction

Climate change, increasing population, and continuous improvement in the standard of living over the past decades have led to greater consumption of fossil fuel, freshwater, and land and water-based products as food and feed ingredients. [1,2]. Due to extensive exploration and utilization of fossil oil reserves, for most countries, the production capacity has either already surpassed or is close to its maximum capacity [3]. Excessive consumption of non-renewable fossil fuels has caused environmental pollution, global warming, and climate change. Therefore, renewable alternative energy resources obtained through biological routes (biofuels) are currently needed to reduce the rate of fossil fuel consumption and sequester CO₂ from the environment [4]. Over the past few decades, biofuels have been researched worldwide as an alternative to fossil fuel-derived petroleum products. Similarly, a wide variety of bio-based products are either currently in use or being developed. However, there have been several challenges faced by their production technologies. Some of these challenges are (i) the selection of appropriate feedstocks that could directly compete with food-bearing crops, (ii) a large requirement of arable land and freshwater for the cultivation of terrestrial plants and crops (e.g., *Jatropha*, *Pongamia pinnata*, sugarcane, etc.), (iii) seasonal and low biomass productivity, and (iv) upstream and downstream processing costs involved in food, feed, and fuel (3F) production.

Terrestrial plants and their products are the major sources of bio-based products. The reported maximum photosynthetic conversion efficiency (ϵ_c) is 4.6% for C3 and 6% for C4 plants. These values are calculated based on total full-spectrum solar radiation [5]. On the contrary, microalgae have photosynthetic conversion efficiency ranging from 8–10% [6]. Lipids, proteins, and carbohydrates are the primary metabolites of microalgae; although the relative concentrations and their compositions may vary among strains, the whole-cell biomass or their specific extracts could be utilized in a variety of applications [7,8]. Furthermore, microalgae are rich in high-value bioactive compounds such as fatty acids, pigments, amino acids, polysaccharides, vitamins, and minerals [9,10]. Already, over 30,000 microalgal strains have been identified [11]; these strains vary by their metabolites and have different growth requirements. Because of their much higher areal biomass productivity, the simultaneous production potential of lipids, carbohydrates, and proteins by specific microalgal strains could still exceed the productivity of any other terrestrial plants, making microalgal biomass an ideal candidate for 3F production [12]. Compared to the limited freshwater reserve, seawater could be a practically non-exhaustible source of water for producing renewable biomass feedstock, as 97% of the world's water is available as seawater. Furthermore, in most cases, the production of microalgae biomass with specific metabolite contents could be achieved by using both marine and freshwater strains. Therefore, it is logical to produce marine microalgae biomass as a feedstock. Although there are several possibilities for utilizing marine algal biomass for food, feed, and fuel production, apart from a limited number of applications, most of these are yet to be commercially exploited, as there remain several limitations. This review will highlight the existing challenges for 3F production from marine microalgae. Furthermore, potential future research directions will also be identified to tackle these challenges.

2. The Potential of Reducing Water Footprint by Marine Microalgae

The primary source of water loss in open pond cultivation is evaporation. Although marine microalgae do not need freshwater to grow initially, they eventually may need it to compensate for evaporation-induced water loss and maintain the salinity. Elevated salinity could inhibit microalgal growth and reduce biomass productivity [13]. Increased culture salinity could also affect the cellular metabolites' composition [14]. Seawater instead of freshwater could be used to compensate for the water loss due to evaporation; however, this would increase the culture salinity over time. Several strains, such as *Dunaliella* sp., *Tetraselmis* sp., etc., displayed high biomass productivity in open raceway ponds over a wide range of salinity levels [15]. Additionally, a few other techniques can be used to reduce pond water evaporation; these include reducing the exposed surface and the wind effect [16]. For example, the wind barriers can be used to reduce the wind effect, while floating covers on the water surface can be used to reduce the exposed surface. In addition, evaporretardant-based chemical treatment can be used to reduce water evaporation from an open pond. In the above cases, the meteorological factors that influence evaporation can be monitored and used to determine which strategies to implement.

Figure 1 compares the water footprint of various crops used for feed and biofuel with that of microalgae. The figure shows that microalgae biomass is clearly competitive in terms of total water footprint when compared to other conventional feedstocks for fuel and feed. Particularly, the water footprint of feed ingredients, such as soybean and wheat, and sources of first-generation bioethanol, including corn and cane molasses ethanol, have a higher water footprint than microalgae biomass. In relation to freshwater microalgae cultivation, the biomass production of marine microalgae has a very low water footprint, as shown in Figure 1. Therefore, marine microalgae are a highly sustainable source of feed and fuel due to their low demand for potable water.

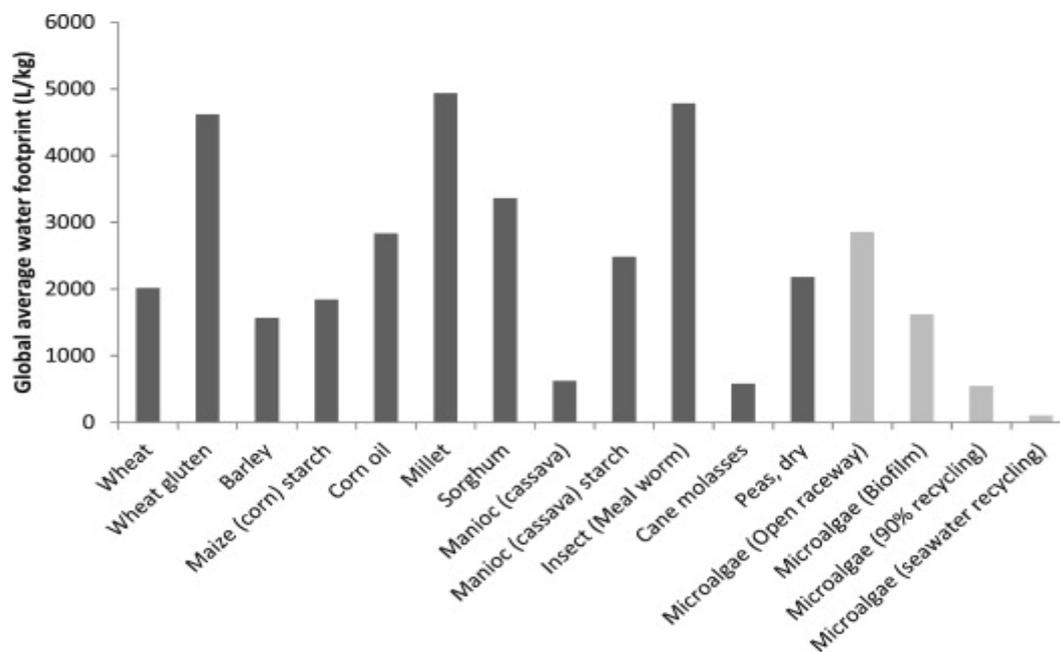


Figure 1. The average global water footprint of different feed ingredients [17].

3. Bioactive Compounds from Marine Microalgae

Marine microalgae produce several types of bioactive compounds, which have a wide range of applications, ranging from food supplements to bioactive substances for medical therapy. Figure 2 illustrates some of the commercial bioactive compounds derived from marine microalgae. The content and composition of the bioactive compounds in microalgae vary from species to species. The major components and key bioactive compounds synthesized by marine microalgae are discussed in the following sub-sections.

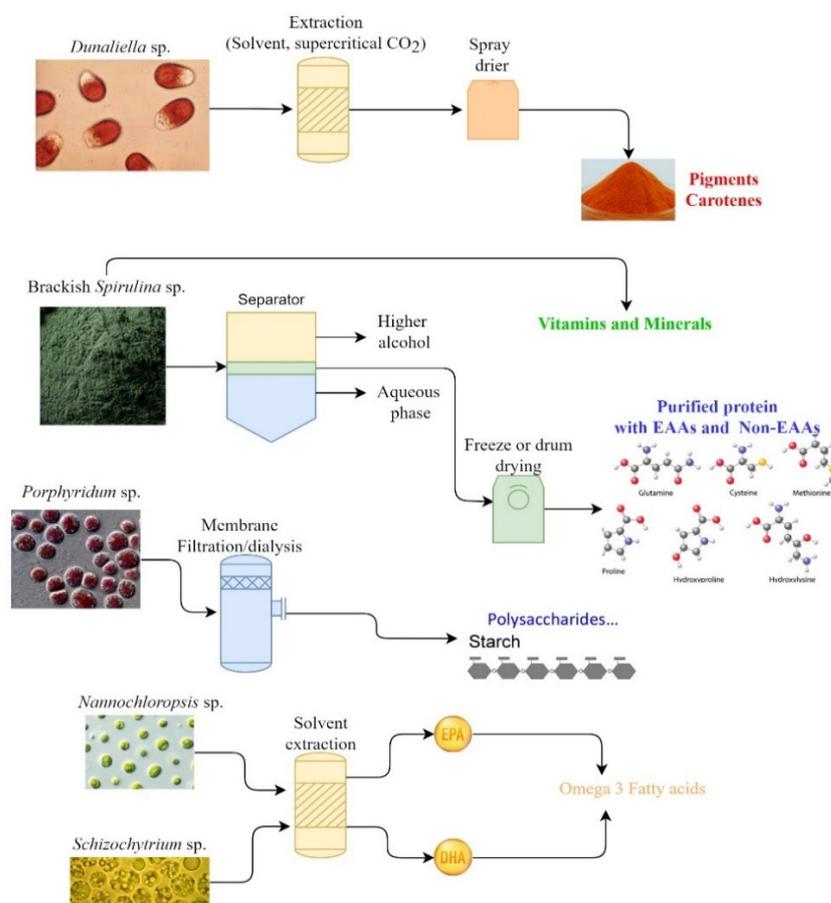


Figure 2. Schematic presentation of various bioactive compounds from different marine strains.

Extraction of carotenoid from *Dunaliella salina* can be performed by supercritical carbon dioxide [18]. Likewise, ethanolic extraction methods could be used for simultaneous solvent extraction and fractionation of EPA from *Nannochloropsis* [19]. Proteins could be extracted from microalgae using a three-phase partitioning system [20]. Extracellular polysaccharides (EPS) are reported to be extracted and fractionated from *Porphyridum* sp. and *Cyanotheca* sp. using the ethanolic precipitation technique [21].

3.1. Amino Acids

Microalgae can synthesize a variety of amino acids, which are the building blocks of molecular proteins. Due to its high protein content of up to 40–60% (*w/w*), microalgae can be utilized as a promising protein source in food industries (Table 1) [22,23]. Tibbetts et al. (2015) reported a crude protein content of 57% *w/w* in *Dunaliella salina* [24], whereas Schwenzfeier et al. (2011) quantified that *Tetraselmis* sp. contains 65% *w/w* protein of total biomass [25]. Unlike other protein-rich diets, algae-derived amino acids are preferable because microalgae can synthesize almost all amino acid molecules [26]. Moreover, genetically modified microalgae can synthesize various proteins efficiently. For example, compared to wild strains, genetically manipulated marine microalgae using techniques such as lithium acetate-polyethylene glycol-based transformation, glass beads, and electroporation have successfully overproduced proteins like human canstatin, soybean kunitz trypsin inhibitor, and the virus VP28 [27–29]. Essential amino acids (EAAs) such as threonine, leucine, valine, isoleucine, lysine, methionine, and histidine are unable to be produced by the human body and must thus be obtained externally [22]. Some protein sources, such as poultry meat, eggs, and fish, have met the need for EAAs [30]. Nevertheless, there is a lack of preference among vegans and vegetarians, as the majority of plant-based proteins do not meet the EAA profile. To resolve this issue, an alternate source that

fits both the criteria of having a balanced protein profile while being affordable is necessary. Marine microalgae can be the ideal vegan protein alternative due to their ability to meet the EAA profile. Microalgae also contain non-essential amino acids, such as proline, arginine, aspartic, serine, glycine, cysteine, tyrosine, and glutamic acid, which have numerous health benefits [7,31]. These substances contribute to the immune system by modulating gene expression, antioxidant responses, and cell signaling [7]. *Selenomethionine*, also known as organic selenium, is a selenium-containing protein that has multiple health benefits [32]. Only a few terrestrial plants (e.g., Brazil nuts, cereal grains, soybeans) can produce this compound, although its concentration in these plants is rather low [33]. Several marine microalgae species, such as *Nannochloropsis oceanica* [34] and *Chlorella* sp. [35], can accumulate a high concentration of selenomethionine. Microalgae (e.g., *Phaeodactylum tricornutum*) could also be used as biofactory to produce therapeutic proteins [36].

Table 1. Protein and essential amino acid contents in different marine microalgae.

Strain	Protein (%)	EAA (%)	References
<i>Nannochloropsis salina</i>	40	48.14	[37]
<i>Navicula incerta</i>	50.38	63.5	[38]
<i>Phaeodactylum tricornutum</i>	28.6	57.7	[39]
<i>Isochrysis galbana</i>	36.4	48.7	[39]
<i>Phaeodactylum tricornutum</i>	70	N.A.	[40]
<i>Tetraselmis</i> sp.	27.86	36.86	[41]
<i>Nannochloropsis granulata</i>	34	17.58	[42]
<i>Pavlova</i> sp.	66	21.25	[43]

3.2. Fatty Acids

Microalgal lipids could replace fish oil to fulfill the demands for aquaculture and human consumption because of their high lipid content [44]. Microalgal lipids are rich in essential long-chain polyunsaturated fatty acids (PUFA), including omega-3 and omega-6 oil, which must be included in the regular dietary plan because humans and many animals cannot synthesize them naturally [45]. PUFAs aid human health, as well as animals, by synthesizing prostaglandins and thromboxane, which are bioactive chemicals necessary for the maintenance of cholesterol and triglycerides in body fluids and for protection against certain diseases, such as dermatitis and osteoarthritis [46]. PUFAs such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have numerous health benefits to humans and animals. DHA improves brain function by assisting neurons, promoting both short- and long-term memory, and facilitating the treatment of brain-related disorders, whereas EPA and DHA together aid in fetal development, inflammation prevention, and the treatment of cardiovascular illnesses [47]. Nowadays, marine fish such as salmon and cod are the foremost source of these bioactive compounds for human utilization. However, due to the limited fish-based oil stock, researchers are encouraged to find an alternative source of these essential PUFAs. *Schizochytrium* sp. is a promising source of DHA that can accumulate up to 30% DHA of the total fatty acids [48]. On the other hand, *Phaeodactylum tricornutum*, a marine diatom, contains 46–52% EPA of total fatty acids [9]. *Nannochloropsis* sp. [49,50], *Isochrysis lutea* [51,52], and *Chaetoceros muelleri* [53], and *Nitzschia* sp. [54] are some other strains with a high concentration of PUFAs. PUFA-producing marine microalgae can be autotrophic or heterotrophic, as mentioned in Table 2.

Table 2. Lipid and essential fatty acids content in different marine microalgae.

Marine Algae Strain	Cultivation Condition	Lipid (% wt./wt.)	Essential Fatty Acids		References
			EPA (%)	DHA (%)	
<i>Nannochloropsis salina</i>	Autotrophic	35	28	N.A	[55]
<i>Pavlova lutheri</i>	Autotrophic	34–36	12.10	5.69	[56]
<i>Prorocentrum triestinum</i>	Autotrophic	3.69	3.66	20.06	[57]
<i>Isochrysis aff. galbana</i>	Autotrophic	51	0.57%	15.2	[44]
<i>Schizochytrium</i> sp.	Heterotrophic	17.83	<1	58.25	[58]
<i>Schizochytrium</i> sp.	Heterotrophic	50.35	N.A.	48.95	[59]
<i>Nannochloropsis oceanica</i>	Autotrophic	35.3	28.9	-	[60]
<i>Pavlova</i> sp.	Autotrophic	16–17	26.6	8.2	[43]

Microalgae are also rich in phospholipids, which are widely employed in the cosmetic industry as liposomes, emulsifiers, solubilizers, and wetting agents. Additionally, they have pharmaceutical values because of their anti-inflammatory and antithrombotic activities [61]. Phospholipids such as phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE) are commonly available in microalgae. Phospholipids can account for 10% to 50% of total lipids in marine microalgae [62]. Manisali et al. (2019) investigated the effect of macronutrient ratio ($\text{NO}_3^-/\text{PO}_4^{3-}$) on the accumulation of phospholipids in *Nannochloropsis oculata* and observed 26% higher phospholipid content in the nutrient ratio of 5:1 than 15:1 [63]. Nitrogen content in the growth medium is also expected to have an inverse relationship with the total fraction of lipids [64].

3.3. Pigments

Microalgae have distinctive colors, which are determined by the presence of pigments within their cells. Pigments are broadly categorized as fat-soluble and water-soluble. Fat-soluble pigments are carotenoids and chlorophylls, whereas phycobilin is a water-soluble pigment [7]. These microalgae-derived high-value products can be beneficial in health applications, including as anti-oxidizing agents, immune boosters, neuroprotective, and vitamin precursors (Table 3) [65]. Recently, it has been suggested to use algal pigments in skin-caring creams as an anti-aging compound [66]. Marine microalgae and cyanobacteria (e.g., *Porphyridium cruentum*, *Arthrospira platensis*) can synthesize up to 8% *phycobiliproteins*, which are used as fluorescent markers [67]. The *phycobiliproteins* can be classified into four primary classes based on their long-wavelength absorption maxima, such as allophycocyanin (650–660 nm), phycocyanin (610–625 nm), phycoerythrin (490–570 nm), and phycoerythrocyanin (560–600 nm) [68]. Among them, phycocyanin and phycoerythrin are the most well-known *phycobiliproteins* because of their wide application in immunity assays [69,70]. Carotenoids are naturally occurring fat-soluble (lipophilic) pigments produced by microalgae in the starvation phase. Primary carotenoids include α -carotene, β -carotene, and lutein, whereas the secondary carotenoids are astaxanthin and canthaxanthin [46]. Although *Haematococcus* sp., a freshwater microalga, is used commercially to produce astaxanthin, several marine microalgae (e.g., *Coelastrum* sp.) could potentially also be used as a source of astaxanthin [71]. β -carotene is the most common type of carotenoid synthesized by *D. salina*, a halotolerant which contains up to 10% *w/w* β -carotene in biomass [67]. Likewise, marine *Spirulina* sp., *Porphyridium* sp., and *Chlorella protothecoides* are rich in natural pigments that could be utilized as food markers, antioxidants, pharmaceutical ingredients, and food additives [67].

Table 3. Pigments from marine microalgae and their application.

Algal Strain	Pigments Content	Benefits	References
<i>Tetraselmis suecica</i>	Lutein, β -carotene	Antioxidant, prevent eye diseases and cancer, skin conditioning	[10,72]
<i>Chlorella salina</i>			
<i>Dunaliella salina</i>	β -carotene	Antioxidant, UV protection	[73,74]
<i>Navicula incerta</i>	Carotenes	Antioxidant	[74,75]
<i>Tetraselmis</i> sp.			
<i>Picochlorum maculatum</i>	Astaxanthin	Treatment of inflammation, improve blood flow and red blood cells	[76,77]
<i>Rhodomonas salina</i>			
<i>Porphyridium purpureum</i>	Phycoerythrin	Immunodiagnostic, tumor treatment, antioxidant, food colorant	[78–80]
<i>Spirulina platensis</i> ,			
<i>Phormidium</i> sp.	Phycocyanin	Anti-inflammatory, antioxidant, natural dye, anti-abetic	[81,82]
<i>Odontella aurita</i>	Fucoxanthin	Antioxidant, anti-inflammatory, treating chronic diseases	[83]

In certain marine diatoms, fucoxanthin, the most prevalent carotenoid, which accounts for about 10% of all carotenoids on the planet, is used as a light-harvesting pigment [84]. Fucoxanthin-producing diatoms synthesize the fucoxanthin-chlorophyll a/c-protein (FCP) complex that allows microalgae to grow in deep-sea water where red light is absent by absorbing blue and green lights [85]. For instance, *Cylindrotheca closterium* produced 25.50 mg·g⁻¹ fucoxanthin under 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ blue LED lights in a bag photobioreactor [86]. It has also been proven that blue lights induced 26% and 17% higher fucoxanthin in the marine diatom *Thalassiosira weissflogii* than white and red lights, respectively [87]. Furthermore, Gao et al. (2021) also observed similar results in *Tisochrysis lutea* by comparing blue-green light with red lights [88]. Apart from light sources, light intensity also has significant effects on fucoxanthin synthesis [84]. Xia et al. (2013) found that a light intensity of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ showed more pigment productivity in *Odontella aurita* than 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [89]. Similarly, McClure et al. (2018) demonstrated more than three folds of fucoxanthin production in *Phaeodactylum tricornerutum* using a light intensity of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ compared with 210 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [90]. Another study conducted by Sun et al. (2019) revealed that lower light intensity such as 30 and 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had higher fucoxanthin production in *Isochrysis* strains than 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [91]. CO₂ supplementation with atmospheric air could also increase the synthesis of fucoxanthin. Li et al. (2019) achieved a 24.53% improvement in pigment content in *Isochrysis zhangjiangensis* while supplying 5% CO₂ instead of air [92]. However, there was no noticeable difference in pigment content between experiments using 2% and 5% CO₂.

3.4. Vitamins and Minerals

Similar to macronutrients such as protein, fat, and carbohydrates, micronutrients (vitamins and minerals) are essential for human body metabolism. They act as co-factors in biochemical pathways and eventually participate in several cellular functions such as boosting immunity, supporting growth and development, and repairing cell damage. Furthermore, vitamin deficiency can result in a variety of disorders, including scurvy, beriberi, and rickets. On the other hand, marine microalgae are regarded as an excellent source of vitamins. For instance, *Spirulina* sp. is abundant in vitamin A and B complex, which directly impacts brain functions, cell metabolism, and prevents infection [7]. Likewise, *Dunaliella tertiolecta* is an excellent source of vitamin B₂, vitamin B₁₂, and vitamin E, and similarly, *Tetraselmis suecica* can synthesize vitamin B complex and vitamin C [93].

Vitamin E is one of the major bioactive compounds in marine microalgae that has several health benefits for treating eye disease, cancer, skin, and heart disease [7,94]. It can maintain male fertility function and acts as a natural anti-oxidant [95]. In 2007, Durmaz examined the effects of nitrogen source and dosage on the production of vitamin E (α -tocopherol) in *Nannochloropsis oculata* [96]. The results showed the maximum content of

α -tocopherol was 2.3 mg. g⁻¹ of biomass in an F/2 medium with 441 μ mol L⁻¹ of NO₃⁻. On the other hand, Carballo-Cárdenas et al. (2003) achieved 1.08 mg. g⁻¹ α -tocopherol (dry biomass basis) in *Tetraselmis suecica* using a NO₃⁻ concentration of 16 mmol. L⁻¹ [97]. The authors also reported that *Dunaliella tertiolecta* could accumulate more vitamin E during the growth phase. Nevertheless, vitamin content in *Dunaliella tertiolecta* was less as compared to *Tetraselmis suecica* [97].

Minerals such as calcium, phosphate, potassium, magnesium, sodium, iron, zinc, and copper are essential for body growth and maintenance. The deficiency of calcium and phosphate leads to skeleton deformities, whereas an inadequate potassium diet can cause convulsions. Likewise, magnesium, iron, copper, and zinc have significant roles in muscle movement, skeleton formation, eyesight, and preventing fatigue. Favorably, marine microalgae are comprised of these minerals that could be utilized for human or animal diet. For instance, *Isochrysis galbana*, *Chlorella stigmatophora*, *Tetraselmis suecica*, and *Dunaliella tertiolecta* could be promising mineral sources for fish diets [98]. On the other hand, marine *Spirulina platensis* can be utilized as a model source of minerals for feed preparation [99].

3.5. Polysaccharides

Polysaccharides (PS) produced from marine microalgae are promising therapeutics for atherosclerosis because of their advantageous economics, availability, and minimal toxicity. In addition, marine microalgae-based PSs are promising compounds for antioxidant, anticancer, immunomodulatory, antiviral, and anticoagulant purposes [100]. Several red microalgae (e.g., *Porphyridium* sp., *Cochlodinium polykrikoides*, etc.) could also produce sulfated polysaccharides which have anti-inflammatory properties [101–103]. Polysaccharides are mostly composed of fructose, glucose, xylose, and galactose, with minor amounts of sulfate, protein, and uronic acid (Table 4) [67]. It can broadly be categorized as intracellular, extracellular, and structural glycans. Nevertheless, extracellular polysaccharides (ECP) or exopolysaccharides (EPS) are of keen attention because of their rheological and biological activities. Microalgae-derived EPS are predominantly heteropolysaccharides with no repetition in monomers and are frequently connected with non-sugar components such as sulfates. Microalgal EPS synthesis is light-dependent and greatly facilitated by continuous illumination and high light intensities; likewise, the dark/light regime dramatically affects EPS production [104]. However, light intensity and dark/light regime may not have an influence on the composition of algal PS [105]. Marine microalgae, such as *Synechococcus* sp., *Dunaliella* sp., *Porphyridium* sp., *Rhodella* sp., and *Tetraselmis* sp., are reported as EPS producers [106,107]. EPS obtained from *Porphyridium* sp. and *Rhodella* sp. were found to be good sources of high molecular weight (2.3 × 10⁶ g mol⁻¹) EPS, which might be attributed to the formation of ionic bridges formed through divalent cations [108]. On the other hand, Yingying et al. (2014) reported the highest molecular weight of 15.934 kDa of the polysaccharides obtained from *Isochrysis galbana* [109].

Table 4. EPS synthesis from different marine microalgae.

Strain	Type of Polysaccharide	Concentration (mg L ⁻¹)	Monomers	References
<i>Cylindrotheca closterium</i>	sPS	3.23–6.10	Glucose, xylose	[110]
<i>Isochrysis galbana</i>	Sulphated EPS	54.9 *	Glucose, galactose, rhamnose	[109]
<i>Porphyridium cruentum</i>	Transparent EPS	-	-	[111]
<i>Arthrospira platensis</i>	Calcium spirulan PS	-	Rhamnose, xylose, ribose, fructose	[112]
<i>Heterosigma akashiwo</i>	Sulphated EPS	-	Rhamnose, Galactose, fructose	[107]

sPS: soluble PS; sEPS: soluble EPS; * mg/g.

It was observed that nutrients might influence the physicochemical properties of polysaccharides, and monosaccharide patterns vary by species [113]. For example, at low nutrient concentrations, *Nannochloropsis gaditana* displayed more complicated monosaccharide patterns than *Isochrysis* sp. and *Rhodomonas marina*, whereas *Isochrysis* sp. and *R. marina* favored synthesizing glucans to the tune of up to 75% of identified monosaccharides [114]. Compared with lipids, marine microalgae-based PSs are still poorly studied. Further investigations are required to understand the functional mechanism of PS biosynthesis.

4. Utilization of Marine Microalgae as Feed and Food Supplements

Despite their prominence in biofuel production, microalgae have received much interest as a source of feed and food supplements mainly because of their capability of synthesizing several bioactive compounds, as discussed above. Algal bioactive compounds have several benefits over chemically produced ingredients, particularly in improving the immune system and antiviral actions. In addition, these bioactive compounds could be used in pharmaceutical industries.

4.1. Marine Microalgae as Feed for Aquatic and Terrestrial Animals

The whole biomass of microalgae or their specific metabolites could be used as aqua and animal feed [115]. Recently, it has been reported that microalgal nutritional compounds are ideal for the diet of cattle, chicken, goats, and pigs for the improvement in meat grade with higher PUFAs, especially EPA and DHA, and amino acids [116]. He et al. (2002) revealed that the addition of algal biomass in the diet of pigs not only enhances their body weight by 10% but also provides the potential to produce iodine-rich pork [117]. Likewise, the presence of PUFA-rich *Arthrospira maxima* in pigs' diet could produce meat with a balanced lipid profile [118]. Urrutia et al. (2016) studied the effect of *Schizochytrium* sp. as an ingredient in lamb's feed and concluded that adding algae biomass in the feed could improve meat quality and lower feed ingestion [119]. The marine red algae, *Porphyridium* sp., comprises several soluble PS and PUFAs. It is reported that 5% microalgae in chicken feed could reduce cholesterol by 10% and increase arachidonic acid and linoleic acid levels by 29% and 24%, respectively, in chicken eggs [120]. However, algae supplementation had no significant effect on the bodyweight of poultry chickens [121]. Furthermore, marine *Spirulina platensis* can be used in hens' diets to enhance egg yolk color by replacing synthetic pigments [122]. Similarly, because of its high DHA content (37% DHA of total lipids), the biomass of *Schizochytrium* sp. was studied as hen's feed to improve egg quality [123]. Tam et al. (2021) investigated the effect of *Thalassiosira weissflogii* as live feed for white-leg shrimp cultivation at the larvae stage [124]. They observed an improvement in body weight, length, and survival rate by 35.75%, 21.17%, and 33%, respectively, compared to the control diet. Likewise, fishes fed with microalgal consortium manifested well-maintained digestive histomorphology because it can enhance the enzymatic activity of maltase, sucrase-isomaltase, and γ -glutamyl transpeptidase in the distal intestine [125]. A recent study has demonstrated the benefits of using microalgae as a substitutive feed ingredient in dairy cattle diets [126]. Further, microalgal biomass could be utilized as feed for ornamental birds, dogs, cats, and aquarium fishes since it has beneficial effects on the physiology of birds and animals [127].

Live microalgae as feed could be promising for aquaculture because it excludes the cost associated with drying and other processing steps. Rasdi et al. (2015) compared *Nannochloropsis oculata* and *Tisochrysis lutea* as live feed for copepod in terms of fatty acids [128]. The results concluded that *T. lutea*-fed copepod had higher EPA, DHA, and arachidonic acid than the copepod fed with *N. oculata*. Similarly, rotifers, a most common live prey, can grow on live microalgae [129]. Whereas *Nannochloropsis* sp. is often utilized as feed for rotifers to improve its EPA content, *Isochrysis* sp. is used to enhance DHA content [130]. Moreover, Basford et al. (2020) observed an increment in phospholipids of *Amphiprion latezonatus* when live *Proteomonas sulcate* was used as feed [131].

Increasing demand for algal lipid in biofuel production leads to de-fatted biomass being used as an ingredient in animal feed. Valente et al. (2019) explored the de-fatted *Nannochloropsis* sp. obtained from a biodiesel industry as a fish meal substitute for European sea bass fish [132]. In the study, the growth performance of the fish was comparable with the conventional feed; however, fish given 10% algal biomass had a substantially higher nutrient digestibility than the control feed. The algae-fed fish's final whole-body composition and nutritional gain were also similar to the control fish. In addition, there were no notable changes in the gut morphology. A similar study conducted by Sørensen et al. (2017) obtained analogous results for Atlantic salmon fish using the de-fatted biomass of *Nannochloropsis oceanica* [133]. Likewise, Austic et al. (2013) used the defatted diatom *Staurosira* sp. biomass as the source of protein in diets for broiler chickens and concluded that the de-fatted biomass (left-over biomass after lipid extraction) could replace 7.5% of soybean meal or corn [134]. Recently, Pereira et al. (2020) also revealed that the de-oiled biomass of *Tetraselmis* sp. has the potential to be a sustainable replacement for soybean in aquafeeds [135]. The investigation showed the higher digestibility of proteins and reduced cholesterol level in juvenile gilthead seabream fed with de-fatted biomass as a substitute.

4.2. Marine Microalgae as Human Food

In recent years, increasing concerns about the health and food safety of processed food products have led national agencies, such as The American Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), to restrict the use of synthetic colors in diets owing to increased oncogenesis or allergic responses. Therefore, natural ingredients, for example, algae-based compounds, will be more acceptable in food industries [136]. The most common marine microalgae (e.g., *Spirulina* sp. and *Dunaliella* sp., etc.) are being consumed in the form of tablets, capsules, and dried powder as a source of functional foods [7]. Additionally, they may be used as a constituent in various foods, including cookies, sweets, snacks, noodles, and carbonated beverages. The application of microalgae (e.g., *Dunaliella salina*, *Isochrysis galbana*, etc.) in pasta to enhance quality was explored by several researchers [137]. Qazi et al. (2021) investigated the impact of *Tetraselmis chunii* biomass on bread formulation and observed a significant increase in protein content in the bread [138]. Dough rheology and bread color, however, were negatively affected by the green pigments and volatiles present. In this regard, ethanol treatment was employed to negate the negative impacts [138]. On the other hand, Khemiri et al. (2020) found enhancement in the sensory properties (color, odor, taste, texture, and global appreciation) of bread made of 3% *N. gaditana* [139]. Additionally, iron and calcium content also increased by two folds. Furthermore, bioactive-rich gluten-free bread can be produced using *Tetraselmis chunii* biomass [140]. Similarly, the addition of *Spirulina* biomass as an ingredient of extruded snacks resulted in a nutritional gain of 22.6% in proteins, 28.1% in lipids, and 46.4% in minerals without substantially altering the physical properties of the snacks [141]. Likewise, protein and phenolic content in cookies were increased by adding algal biomass as an alternative ingredient without affecting the in vitro digestibility [142].

5. Biofuels

5.1. Biodiesel

Several oleaginous microalgae, under specific growth conditions (e.g., nutrient starvation, salinity stress, etc.), can accumulate high contents of lipids [143]. The lipid fraction of the biomass can be extracted using an appropriate solvent with or without any pretreatment of the biomass. A comparison of energy requirements for different lipid extraction techniques has been given elsewhere [144]. A transesterification process can then convert the lipid into biodiesel (i.e., esters of fatty acids) in the presence of alcohol and a catalyst (Table 5). As a byproduct of the transesterification process, glycerol is generated as almost

10% (*w/w*) of the lipid [145]. The conversion efficiency of lipid to biodiesel is usually very high, and it could reach as high as 99% [146]. Biodiesel could also be made directly from the biomass using a single-step transesterification process [147]; however, this process requires excess solvent and catalyst, making it economically unfavorable [148].

Table 5. Biodiesel production from different marine microalgae biomass feedstock.

Microalgae	Lipid Content (%)	Extraction Method	Transesterification Process			Biodiesel Yield (%)	Reference
			°C	min	Catalyst		
<i>Tetraselmis suecica</i>	23	Chloroform-methanol (2:1)	80	20	H ₂ SO ₄	78	[149]
<i>Nannochloropsis salina</i>	32.1	Modified Bligh and Dyer	40–45	180	NaOH	60.26	[150]
<i>Nitzschia punctata</i>	16	Modified Folch	40	2880	Lipase	87.2	[151]
<i>Dunaliella tertiolecta</i>	69.6	Bligh and Dyer	80	300	NaOH	-	[152]
<i>Phaeodactylum tricornerutum</i>	36	Methanol-Hexane (2:3)	N/A	N/A	N/A	7-11	[40]

5.2. Bioethanol

Bioethanol from microalgae can be produced either by extracting fermentable sugars from microalgal biomass followed by fermenting it using well-known microbes such as yeast or by directly producing ethanol in broth utilizing genetically engineered microalgae [153]. Microalgal carbohydrates are primarily comprised of glycogen, starch, and cellulose with no lignin and low hemicellulose content, making them ideal candidates as bioethanol feedstocks [154]. Fermentable sugars from microalgae biomass could be extracted by a variety of techniques (e.g., thermal, chemical, and enzymatic hydrolysis), which then can be converted into bioethanol by methanolic microbes (e.g., *S. cerevisiae*, *E. coli*, etc.) in the fermentation process [155]. One common cultivation strategy for enriching microalgae biomass with carbohydrates is nitrogen starvation. Carbohydrate content in microalgae can reach as high as 50% [156]. Although most studies of bioethanol production from microalgae biomass were carried out on freshwater microalgae, a few studies explored marine microalgal biomass as feedstock (Table 6).

Table 6. Bioethanol production from different marine microalgae biomass feedstock.

Microalgae Strain	Carbohydrate Content (%)	Carbohydrate Extraction		Fermentation Process		Reference
		Process	Yield (%)	Organism Used	Glucose to Bioethanol Conversion (g/g glucose)	
<i>Tetraselmis seucica</i>	27	NaOH, 120 °C	N/A	<i>S. cerevisiae</i>	0.073	[157]
<i>Synechococcus</i> sp.	60	Lysozyme hydrolysis	80	<i>S. cerevisiae</i>	0.37	[158]
<i>Chlorella vulgaris</i>	N/A	H ₂ SO ₄ , 120 °C	22	<i>E. coli</i> SJL25260.4		[159]
<i>Chlorococcum infusioinum</i>	43.8	NaOH, 120 °C	79.9	<i>S. cerevisiae</i>	0.26	[160]
<i>Dunaliella tertiolecta</i>	37.8	Lipidextraction, Chemo-enzymatic	81.7	<i>S. cerevisiae</i>	0.44	[161]

Apart from the fermentation of extracted sugar, two other alternative routes were also explored for ethanol production: (i) dark fermentation and (ii) photofermentation. Several microalgae (e.g., *Chlorococcum littorale*, *Cyanothece* sp., etc.) are capable of dark fermentation; the strains convert cellular starch and lipid into ethanol and other byproducts as the dark conditions are applied [162]. The ethanol released in the culture could reach as high as 20.7 mg/g of biomass [163]. Genetically modified strains of freshwater cyanobacteria can directly produce ethanol [164]; however, more studies would be required to screen and identify marine strains and optimize the downstream processes.

5.3. Biomethane

In the anaerobic digestion (i.e., AD) process, organic matter gets converted to biogas (methane, hydrogen, and CO₂) in the presence of specific microbes. Methane yield from the microalgal biomass was reported to be as high as 0.56 L CH₄/g of vs. [165]. The methane yield mainly depends on biomass quality (e.g., C/N ratio, hydrolysable organic matter) [166]. The suitable C/N ratio in the feedstock was found to be 25–30:1 due to the high requirement of carbon for bacterial metabolism [153]. A pretreatment (physical, chemical, thermal, enzymatic, etc.) of the biomass to break the cell walls is often used to improve the methane yield [167]. Unlike green microalgae, cyanobacteria do not have rigid cell walls, which could eliminate the need for biomass pretreatment [168]. While the whole microalgae could be used to generate methane, in several studies, lipid-extracted biomass was also used to generate methane [169]. However, the method of lipid extraction could influence methane production [170]. Biomass with low lipid content, typically obtained from wastewater treatment, could be used as a whole for AD without any pretreatment due to its low quality and low calorific value. However, when the intention of cultivating microalgae is to produce pigments, lipids, or any other high-value metabolites, the residual biomass could also be used to generate methane. Table 7 shows the methane yield from different algal species having different compositions.

Table 7. Methane production from different marine microalgae biomass feedstock.

Microalgae	Feedstock Composition			Pretreatment of Biomass	Methane Yield (L CH ₄ /g VS)	Reference
	Carbohydrate	Protein	Lipid			
<i>Isochrysis galbana</i>	6.5	15.3	22.8	Acid hydrolysis	0.016	[171]
<i>Nannochloropsis salina</i>	11.5	17.2	37.2	No treatment	0.56	[165]
<i>Phaeodactylum tricornutum</i>	19	26.5	7.2	No treatment	0.34	[165]
<i>Nanofrustulum</i> sp.	9.0	12.5	13	No treatment	0.51	[165]
<i>Tetraselmis</i> sp.	NA	11.3	NA	Supercritical fluid	0.24	[172]

5.4. Biocrude Oil

Hydrothermal liquefaction (HTL) is a promising technology that can convert a variety of biomass, including microalgae, into biocrude oil in the presence of heat and pressure where the moisture in the biomass act as a solvent [173,174]. Under optimized conditions, the HTL process could recover more than 80% of the calorific value of the microalgal biomass feedstock [175]. The HTL process could convert all the metabolites into biocrude, although the conversion efficiency would vary as lipid > protein > carbohydrate [176]. The biocrude yield from microalgae biomass would vary based on the metabolite composition [177], HTL operating conditions, and catalyst type (Table 8). Apart from biocrude, three other byproducts are formed during the HTL process: biochar, aqueous and gaseous compounds [178]. The gaseous fraction is mainly comprised of carbon dioxide [179], which could be injected into the cultivation system. Although the HTL results in a higher conversion of biomass to biofuel (as biocrude), the biocrude contains unwanted heteroatoms (e.g., nitrogen and oxygen), which need to be removed by catalytic upgrading, and the upgraded biocrude oil still needs to be processed in a petroleum refinery [180]. While most HTL studies on marine microalgae were conducted in batch mode, there are several studies on continuous HTL operation [181].

Table 8. Biocrude oil production from different marine microalgae biomass feedstock.

Microalgae	Feedstock Composition (%)			HTL Condition				Biocrude Yield (%)	Reference
	Protein	Lipid	Carbohydrate	Temp (° C)	Time (min)	Solid Content (%)	Catalyst		
<i>Tetraselmis</i> sp.	37.5	14.0	18.5	350	10	20	N/A	45.4	[182]
<i>Nannochloropsis oculata</i> .	57.0	32.0	8.0	350	60	10	N/A	38	[183]
<i>Picochlorum</i> sp.	31.0	26.0	24.0	325	30	15	N/A	39.6	[174]
<i>Dunaliella salina</i>	N/A	N/A	N/A	300–450	60	10	Ni/REHY	72	[184]
<i>Nannochloropsis salina</i>	60.0	6.0	19.0	310	120	25	N/A	46	[185]
<i>Pavlova</i> sp.	46.9	13.9	28.0	350	>60	14	Na ₂ CO ₃	47.1	[186]

5.5. Biohydrogen

In comparison to traditional energy sources such as fossil fuels, hydrogen (H₂) is a promising future clean energy carrier because of its calorific value (142 kJ g⁻¹) and the combustion product being water rather than greenhouse gases [187]. Microalgal hydrogen production can be categorized into bio-photolysis and fermentation processes [188]. Dark fermentation (DF) is well known for the fermentative conversion of algal biomass to molecular H₂ by a diverse group of anaerobic bacteria. In DF, biomass undergoes a sequential process. Firstly, various pretreatment techniques break down complex algae molecules (carbohydrates, protein, and lipid) into fermentable sugars by various pretreatment techniques [189]. Secondly, the simple substrate is fermented to H₂, CO₂, and volatile fatty acids by acidogenic bacteria [190]. Two distinct biochemical pathways, the acetate and butyrate pathways, can accomplish the generation of molecular hydrogen with the help of specific enzymes. The stoichiometry of the process shows that 4 moles of hydrogen are generated from 1 mol of glucose when pyruvate is oxidized to acetate as the only metabolic product, whereas it produces 2 mol of hydrogen when pyruvate is converted to butyrate [191]. Generally, the yield of H₂ from algal carbohydrates varies in the range of 160.1–448.0 mL H₂/g VS, which is 32.2–90.0% of the theoretical yield [192]. Typical bacteria involved in DF are *Bacillus* sp., *Clostridium* sp., *Klebsiella* sp., and *Enterobacter* sp. [193]. In photolysis, algae split water molecules into H₂ and O₂ with the help of sunlight and release them into the surrounding environment. Moreover, photolysis can be divided into direct and indirect photolysis. In direct photolysis, H₂O is split directly into H₂ and O₂ in a single stage (H₂O is split into H⁺ and electrons (and O₂), and electrons are transferred to hydrogenase, which catalyzes the H₂ formation), whereas in indirect photolysis, CO₂ is fixed by algae for carbohydrate biosynthesis (first stage) and further fermenting of the carbohydrate to produce hydrogen with the help of hydrogen-producing enzymes (second stage) [188]. Microalgae involved in photolysis are *Platymonas subcordiformis*, *Chlorococcum littorale*, marine *Anabaena* sp., and *Synechococcus* sp. [194,195].

6. Challenges and Future Prospective of Producing 3F from Marine Microalgae

6.1. Selection of a Suitable Strain

The selection of a strain and its cultivation conditions are intended to generate biomass of desired quality. Much of the earlier focus on strain selection was dedicated to oil-rich microalgae or the cultivation process that assisted the strains in accumulating a high amount of lipid content. Therefore, a vast number of fast-growing, halo-tolerant, easily harvestable microalgae/cyanobacteria were not considered in earlier investigations. Extensive strain screening is needed to identify suitable strains and their specific growth

conditions that prevent or minimize contamination from other microalgal strains or predators. The cellular concentration of N and P in microalgae would vary from strain to strain and the growth conditions. Typically, commercial fertilizers are used as sources of N (urea, ammonium nitrate, urea-ammonium nitrate, etc.) and P (monoammonium phosphate, diammonium phosphate, NPK, etc.) [196]. Therefore, biofuels made from N and P-rich microalgal biomass could negate the photosynthetic energy conversion by the microalgal cells.

Furthermore, a suitable microalgal strain should have high biomass productivity and be capable of accumulating energy-dense biomolecules and/or high-value products. Eventually, the bioactive compounds would be utilized as supplements in food and feed. Recycling the growth media is crucial as it would allow efficient nutrient utilization. In this regard, several halo-tolerant microalgae, diatoms, and cyanobacteria such as *Dunaliella salina*, *Tetraselmis* sp., *Amphora* sp., and *Cyanothece* sp. have been found to be promising. Since harvesting accounts for 20–30% of overall cost [197], self-settling halo-tolerant strains would be the ideal choice as potential strains for biofuel, feed, and food.

The selection of strains for feed and food is more sensitive than biofuels, mainly depending on the content of proteins, fatty acids, polysaccharides, vitamins, and minerals. Not only the content of proteins and lipids but the presence of different EAAs and PUFAs are also desirable in microalgae for dietary applications [7]. Several recent studies have focused on the genetic modification of wild strains to improve productivity and the compositional values of microalgae [198].

6.2. Cultivation

Although higher biomass productivity could be achieved in closed cultivation systems, construction and operating energy cost in the closed systems could be prohibitively high for microalgal biofuel production [199]. For example, the average biodiesel production cost in a commercial photobioreactor can be up to 2.5 times higher compared to an open pond [200]. On the contrary, an open cultivation system (e.g., raceway pond) could offer cost and energy-effective microalgal cultivation by compromising biomass productivity, increasing evaporation water loss, and contamination potential by unwanted microalgae and predators. Nevertheless, a controlled cultivation system is recommended for feed and food applications. Water loss due to evaporation in an open pond is one of the major problems in cultivating marine microalgae. Depending on the location of the cultivation site and weather conditions, the rate of evaporation loss could vary from 0.1–2.0 cm/d [201]. Supplying fresh water to compensate for the evaporation loss would undermine the advantages of selecting marine strains to produce different feedstock. Instead of adding fresh water, seawater could be used, and an appropriate halotolerant strain could be used. Carbon content in microalgal cells could be as high as 50% [202]. The atmospheric diffusion of CO₂ into the culture is limited, and hence to enhance the biomass productivity and yield, flue gas could be injected as a CO₂ source [203]. The requirement of carbon dioxide for producing microalgal biomass was estimated as 1.83 kg CO₂/kg biomass [204]. However, the collocation of a flue gas source and cultivation site would be essential. Apart from carbon, two other macronutrients for microalgae are nitrogen and phosphorus. In addition, because of the lower culture depth in an open raceway pond, the CO₂ loss to the atmosphere could be substantial [205]. On the other hand, a deeper culture may resist the light penetration in the broth. Microalgae could also utilize organic carbon in either a mixotrophic or heterotrophic growth mode [206]. Although two-phase cultivation could be adopted to enhance the lipid content [207], with a mixotrophic phase being the second phase, recycling the growth medium after biomass harvesting could be challenging because of the presence of residual organics in the growth medium. Further, mixotrophic cultivation of microalgae may face the challenges of (i) the supply of a cost-effective and suitable organic carbon source and (ii) competition from faster-growing bacteria and yeast. Hence, different waste sources need to be explored as alternatives to commercial fertilizers (especially for N and P). The authors' group has successfully cultivated marine

microalgae, including *Tetraselmis* sp. and *Picochlorum* sp. (not published yet), using waste nitrogen fertilizers from a local fertilizer company [208]. Similarly, as a source of nitrogen and phosphorus, other waste sources, food waste, and digestates of the AD process and the aqueous phase of the HTL process could be used [209]. Consequently, overall cost and energy requirements could be reduced. To avoid contamination, repetitive cultivation of the same microalgae in the same place could help dominate the area with the desired microalgae. Furthermore, appropriate commissioning, scheduling, and maintenance are required for targeted products.

6.3. Harvesting

Separation of the biomass is usually achieved in two steps: (i) the preliminary step concentrates the microalgae from the bulk of the culture to a biomass slurry having a solid content as high as 4%, and (ii) in the second step, either a centrifuge or a clarifier can be used to obtain a biomass paste as high as 25% solid content [210]. Some algae cells are large and heavy, and these cells precipitate when the culture is kept undisturbed for some time [211]. Several filamentous cyanobacteria form a tangled mesh that can settle to the bottom or float to the top, making biomass separation relatively easier [212]. Some other microalgae and cyanobacteria produce exopolysaccharides, which assist in forming flocs of the cells; these flocs can settle spontaneously to the bottom [213]. Non-settling microalgal cells can also be harvested by taking advantage of sedimentation exhibiting microalgal or yeast strains—a process known as bio-flocculation [214]. Microalgal cells usually carry negative surface charges in the algal culture, and often, chemicals are added in the form of a coagulant to assist in cell coagulation-flocculation followed by precipitation [215]. Usually, multivalent metal salts (i.e., ferric chloride, alum, etc.) and cationic polymers are used as coagulants; however, the efficacy of harvesting would depend on the cell characteristics (size, zeta potential) [216]. The ionic strength of seawater is much higher than freshwater; hence, the dosage requirement of coagulants for marine microalgae would be higher than freshwater microalgae [217]. The change in culture pH (mostly at elevated pH) could assist in the precipitation of some of the microalgae [218]. Several studies have explored using the electrocoagulation method where a coagulant is generated in situ, as DC power is connected to the electrodes (usually aluminum), to induce microalgal coagulation [219]. The higher the culture salinity, the lower the energy consumption by the electrocoagulation process [220]. Several microalgal strains were successfully harvested using the filtration methods of cross-flow filtration and submerged filtration [221]. While coagulation-flocculation and electrocoagulation introduce contaminants in the culture media, the filtration method eliminates the need for any chemicals, allowing the recycling of the culture media. However, during the filtration process, membrane fouling is initiated, and with time, the fouling becomes severe, which requires the backwashing of the membrane [222]. Furthermore, as the salinity of the culture increases, the frequency of membrane fouling also increases, ultimately requiring more energy per unit of biomass harvesting [223]. Microalgal biomass density in large-scale open cultivation systems often does not exceed 0.5 g/L; the selection of harvesting technique will, therefore, depend on its energy requirement per unit of biomass harvested. Typical energy requirements for harvesting marine microalgae by different harvesting techniques are listed in Table 9. The harvesting method influences the biomass quality [198] and interferes with the final product quality [178]. Drying of harvested biomass is one of the major steps in processing microalgae because it is a time-consuming process [224]. Additionally, solar or any other open system drying has a high risk of contamination [225]. However, it can be negated by employing several advanced technologies such as rotary drying, spray drying, crossflow air drying, vacuum self-drying, and flash drying [226]. The cost involved can be varied in the range of 0.32–0.69 €·kg⁻¹ dried biomass [225]. Furthermore, drum drying was found to be less energy-consuming as compared to spray drying [225].

Table 9. Typical energy requirements for harvesting marine microalgae by different techniques.

Microalgae	Harvesting Technique	Salinity (%NaCl)	Biomass Density (kg/m ³)	Volume Used (L)	Energy Requirement (kWh/kg)	Reference
<i>Picochlorum</i> sp.	Centrifugation	4.0	0.58	2500	2.49	[174]
<i>Tetraselmis</i> sp.	Crossflow filtration	4.64	0.69	200	4.65	[178]
<i>Phaeodactylum tricornerutum</i>	Electrocoagulation	3.0	0.3–0.6	1	1.08	[220]
<i>Tetraselmis</i> sp.	Coagulation-flocculation	4.64	0.69	50	0.49	[178]
<i>Nannochloropsis oceanica</i>	Pulse electrolysis	N.A.	1.0	0.4	1.8	[227]

6.4. Downstream Conversion Process

6.4.1. Biofuel Production

For biofuel production, the extraction of lipids and carbohydrates from the whole biomass is challenging, mainly due to energy-intensive processes like cell rupture of the thick wall. Microwave, supercritical CO₂, and ultrasound have been used as pretreatment to rupture the cell walls of the wet microalgae biomass and assist in lipid extraction; however, the energy requirement in these processes can be very high [228]. In recent times, ionic liquids (i.e., molten salts) have been explored as alternatives to organic solvents for lipid extraction from microalgae biomass [229]. Solvent-extracted lipids typically contain other minor contaminants such as photosynthetic pigments of the cell; additional treatment would be required to remove these contaminants [230]. In this concern, high-value metabolites (e.g., pigments) are extracted and separated before or during the lipid extraction; eventually, the energy demand for the overall extraction process could be offset by the cost of the high-value metabolites. For ethanol and biomethane production from marine microalgae, salinity in the biomass can inhibit the enzymes which are responsible for ethanol and biomethane production. Freshwater could be used to wash the biomass before ethanol fermentation. However, utilization of freshwater in an industry-scale setup should be avoided. The C/N ratio also plays a crucial role in fermentation. Therefore, to maintain the desirable C/N ratio, different waste-based nitrogen sources could be used [187]. Another issue is the drying of the wet biomass. In this case, the production of biocrude using HTL technology is preferable to processing wet biomass. The biocrude conversion yield from the carbohydrate fraction is typically low, and the other byproducts generated from carbohydrates would end up in the aqueous phase liquid. Biocrude from microalgae usually contains 6–7% nitrogen, 10–11% oxygen, and 0–1% sulfur; the presence of these heteroatoms in the biocrude would reduce its calorific value [153]. Additional processes (e.g., catalytic upgrading) would be required to remove these heteroatoms from the biocrude [180]. Although the principle of H₂ production through photolysis is promising, the hydrogenase enzyme, which is mainly responsible for H₂ production, is susceptible to O₂, which strongly inhibits hydrogen production during photolysis [231]. As a result, improving hydrogen generation using microalgal cells is a challenging task. Furthermore, since the maturation of NiFe-hydrogenases needs a large number of specialized maturation enzymes, the heterologous production of O₂-tolerant hydrogenases in cyanobacteria is difficult [188]. Moreover, the development of an appropriate photobioreactor for large-scale H₂ production through photolysis is challenging [188]. DF H₂ production faces an issue associated with the pretreatment of biomass. Besides, it has two main drawbacks such as (i) low energy recovery, as a theoretical maximum of 34% energy can be recovered from the substrate used [190] and (ii) direct disposal of the fermentation effluent [232]. For DF, developing a biorefinery framework could be a promising approach to improve overall energy recovery and mitigate the harmful effects of the volatile fatty acids produced in DF [233]. DF effluent can be used as a feedstock in several second-stage processes such as biomethanation, microbial fuel cell, algal cultivation, biobutanol production, and polyhydroxyalkanoate (PHA) production [187,232]. Furthermore, anaerobic

sludge produced in the H₂-producing reactor can be used as a source of EPS, contributing value to the overall process [197].

6.4.2. Algae-Based Feed and Food

The price of microalgal feed remains high compared to conventional feed; therefore, its price must be lowered to compete with regular feed. The fast expansion of the algal bioeconomy in the food and feed sector is also linked to the development of algae biotechnology. Researchers are working to improve algal productivity because greater biomass productivity implies reduced production costs. Significant advances have been made in bioreactor design, strain selection, rapid sampling methods, genetic and metabolic engineering, and other biotechnology approaches, resulting in increased biomass production and the accumulation of essential metabolites [234]. Another concern of producing feed/food using algal biomass is health safety. As a consequence of the microalgae assimilation of heavy metals, herbicides, and other hazardous substances from seawater, intake of this microalgae biomass is harmful to the health of humans and animals [8]. To overcome this issue, proper safety precaution steps need to be taken care of and follow the permissible limit established by food security agencies such as EFSA and FDA [22]. In this regard, Rzymiski et al. proposed several interdisciplinary methods to evaluate the safety and toxicity of microalgae-based dietary supplements and bioproducts [8]. Additionally, it is necessary to create essential safety laws to measure the environmental risk assessment (ERA) system and ensure that it can keep up with the constantly changing nature of research and development.

Another challenge for microalgae-based feed is its variability in composition. Olsson et al. (2012) observed a seasonal variation in total lipid content and fatty acids profile in *Nannochloropsis oculata* that underlined the importance of light and temperature in algal cultivation and the accumulation of bioactive compounds [235]. Apart from this, the taste and color of food items impact acceptability. The inclusion of microalgal biomass into conventional products has been shown to be difficult in certain circumstances due to its intense green color, fishy taste and odor, and powdery consistency. For instance, Becker observed that the strong fishy flavor is a detriment to the acceptance of these food items among many consumers [23]. All these factors are major areas that require improvements. The color effect may be concealed in products like pasta, but it has not been feasible to hide the taste and odor of microalgae, limiting the quantity that can be utilized. It should be emphasized that the harvesting process does not require chemicals as flocculants because these chemicals might affect the quality of the final products. In this regard, physical techniques such as cross-flow filtration could be employed [17].

In many circumstances, most microalgal biomass is not appropriate for application in animal feed because of its high salt content, which must be eliminated prior to feeding. The microalgae may have collected trace elements, which must be removed in many circumstances. Animals, fish, and humans have been shown to be at risk from certain microalgae toxins, which have been identified in some species of algae [17].

6.5. Biorefinery Concept

6.5.1. Producing Microalgal Biomass with High-Value Metabolites

At times, the desired product from a microalgae biomass is only a small fraction of the whole biomass. The extraction of these selected products should use greener technologies (e.g., supercritical CO₂ extraction of pigments and lipids) such that the left-over biomass could have wider applications [236]. Although the potential environmental benefits of microalgal biofuels are overwhelming, these biofuels will need to compete with the price of existing petroleum and other sources of fuels. One way to offset the cost of microalgal biofuel is to extract high-value metabolites from the biomass prior to converting the residual biomass into biofuels [237]. A schematic of a cost- and energy-effective microalgal

biofuel and high-value metabolite production pathway is shown in Figure 3. Phycobiliproteins (e.g., phycocyanin, phycoerythrin), pigments (e.g., β -carotene, astaxanthin, lutein, etc.), and lipids (e.g., polyunsaturated fatty acids such as DHA and EPA) are some examples of microalgal high-value products that could be extracted before biofuel production. Screening new strains with a high concentration of these metabolites, optimizing the cellular concentrations of these metabolites, and developing and optimizing the extraction processes of these metabolites could be useful in reducing the cost of microalgal biofuel production.

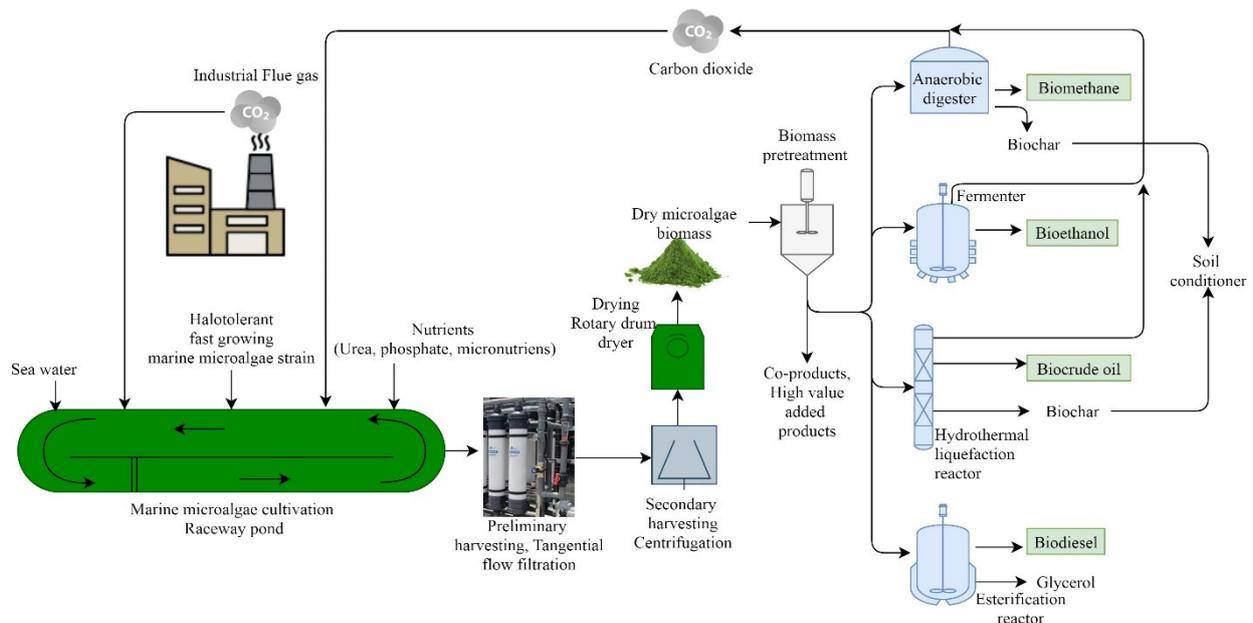


Figure 3. A schematic of marine microalgal biofuel production pathway.

6.5.2. Exploring the Applications of the Left-Over Biomass

Nitrogen, phosphorus, and some other elements are added to the culture to support the cell requirements. The bulk of the consumed nitrogen is converted into protein by microalgal cells. The common source of various forms of nitrogen fertilizers is ammonia, which is produced from atmospheric nitrogen by an energy-intensive Haber–Bosch process [238]. Phosphorus is another microelement required by plants and microalgae. Unlike nitrogen, the stock of phosphate rocks is limited, and the demand for phosphorus fertilizer is expected to increase to cope with future food demand [239]. When specific microalgal metabolites are required, or the desired products do not comprise these elements, appropriate techniques should be developed to optimize the recovery of these elements. Furthermore, to enhance the viability and environmental sustainability of microalgal biofuel, the recycling or re-utilization of these elements, especially nitrogen and phosphorus, should be developed. The applications of lipid-extracted microalgal biomass from a limited number of strains as feed ingredients for cows, fish, and poultry were investigated [24,240]. Therefore, more studies would be required to explore the potential of the left-over biomass of other microalgal strains. The liquid obtained from the AD or HTL of microalgal biomass could be used as a source of nitrogen, phosphorus, and other trace elements for cultivating microalgae [241]. The solid fraction obtained after the AD or HTL process could be used as a soil additive [242].

7. Cost Analysis of Marine Microalgal Biomass Production

The cost analysis is a crucial step for the commercialization of a product because it indicates its economic feasibility. The results assist researchers and investigators in making the right decision concerning scale-up and commercialization. The cost of microalgal biomass depends on several factors such as the mode of cultivation, type of reactors, the composition of the medium used for growth, and the type of harvesting and drying process. The total production cost is estimated by adding depreciation to direct production costs. Over the past few decades, microalgae have been projected as a promising feedstock for producing biofuels, food, and feed. In an earlier study, microalgae cultivation was focused on mainly three systems: open ponds, horizontal tubular PBRs, and flat-panel PBRs [243]. The combined cost of cultivation and biomass dewatering was estimated to be in the range of 4.1–5.96 € per kg. The lowest price was calculated for microalgae produced in open raceway ponds. Furthermore, when all the unit operations were optimized, the cost per kg of microalgal biomass was reduced to as low as 0.7 € per kg [243]. Thus, making microalgal biomass an attractive feedstock for biofuel production. Another study explored EPA and DHA-rich marine microalgal biomass production using three cultivation systems (e.g., flat panel, open pond, and tubular systems) over a 100 ha scale in Spain and the Netherlands [198]. The cost of producing biomass in Spain for flat, tubular, and open ponds was 2.3, 3.3, and 4.2 USD/kg, respectively. In the Netherlands, the cost per dry weight USD/kg for flat, tubular, and open ponds were 4.4, 6.2, and 8.1, respectively. The cost of EPA and DHA oil was reported to range from 39 to 70 USD/kg for Spain and 73 to 135 USD/kg for the Netherlands. In concluding remarks, it was mentioned that the essential fatty acids EPA and DHA in microalgal biomass had a similar price range to a fish oil containing 20% DHA/EPA for 2400 USD/tonne, thereby making marine microalgae a promising feedstock for formulating aquafeeds. Apart from these, a few more studies have been listed in Table 10.

Table 10. Cost analysis for marine microalgal biomass production.

Product	Microalgae Strain	Mode of Cultivation	Cultivation Area (ha) or Volume (m ³) *	Cost (USD Kg ⁻¹ dry wt)	Reference
Biomass	<i>Nannochloropsis oceanica</i>	Open pond	1	56.31	[244]
Biomass	-	Open raceway pond	10	3.06–3.70	[245]
Biomass	-	Tubular photobioreactor	10	4.5–5.2	[245]
Biomass	<i>Phaeodactylum tricornutum</i>	Bubble column photobioreactor	80,000 *	2.12	[246]
Biodiesel	<i>Phaeodactylum tricornutum</i>	Bubble column photobioreactor	80,000 *	0.35 **	[246]
Biomass	<i>Dunaliella salina</i>	Indoor photobioreactor	10 *	4.64–301.61	[247]

* Indicates volume in terms of m³. ** USD L⁻¹.

8. Conclusions

The potential for bioproducts derived from marine microalgae has risen in recent years due to increasing concerns about the world’s expanding population, freshwater scarcity, the future supply of food and fossil fuels, the overuse of these resources, and ecological degradation and pollution. However, wider commercial applications of microalgal food, feed, and fuels (3F) cannot start yet due to the challenges summarized earlier. Production of any microalgal biofuel would require the efficient management of several unit operations. Similarly, safety measurement is a big concern for food and feed production. The selection of a strain is very crucial as it will influence most of the unit operations. Further studies would be required to screen more strains or enhance the performance of a strain. Developing a biorefinery framework could be a promising pathway for sustainable 3F production from marine microalgae. Additionally, the efficiency of downstream processing of microalgal biomass, including harvesting, conversion to biofuels, extraction

of bioactive compounds, separation of co-products, etc., needs to be improved. Genetic engineering could be another helpful tool to make algal products more economically viable.

Funding: This research was funded by the Qatar National Research Fund (QNRF, a member of the Qatar Foundation) grant number MME01-0910-190028.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors would like to acknowledge the support of the Qatar National Research Fund (QNRF, a member of the Qatar Foundation) for providing the funding (under grant MME01-0910-190028) for this study.

Conflicts of Interest: The authors declare no conflict of interest.

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