

1 **Advances in Algal Biotechnology: Sustainable Production of Nutrient-Rich Biomass and**
2 **High-Value Bioproducts**

3 **Abstract**

4 Microalgae (MiA) have been portrayed as a sustainable alternative for conventional sources of
5 biofuels, several food and feed systems, and renewable energy sources. Microalgal biomass has
6 attracted considerable attention for its potential to produce high quantities of value-added
7 compounds, including pigments, vitamins, polyunsaturated fatty acids (PUFAs), and
8 antioxidants. In this context, a plethora of existing and potential studies and reviews elicit the
9 applied technologies for bio valorization of algal biomass and sustainable and integrated
10 biorefinery approaches for production of high-value products. The focus of the present review is
11 to provide insights on current trends in the production of lipids and pigments from the alga
12 biomass, and employing eco-conscious methodologies for the extraction of compounds from the
13 microalgal biosphere holds significant importance.

14

15 **Keywords:**Microalgae, functional lipids, vitamins, bioactive phenolic compounds, extraction,
16 algal biomass, utilization

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19 **1. Introduction**

20 Current trends of data available on the per capita availability of food resources directs towards a
21 scarcity of production both as food stock and feedstock i.e., the current food production capacity
22 and potential is not completely sustainable to contain and fulfill the enormous increment in
23 requirement of food (25% - 70%) (Hunter et al., 2017) relative to the rising population, expected

24 to be at 9.7 billion by 2050, neither quantitatively nor qualitatively. Alternatively, new evidences
25 portray that approximately 690 million people (8.9% globally) were found to be suffering with
26 nutrient deficiency in 2019, with the figure expected to rise to 840 million by 2030. Asia, Latin
27 America, Africa, countries of the Caribbean region are the most affected (WHO, 2020). Thus,
28 there is a requirement of novel sources of food which are adequate in terms of amount,
29 nutritional profile, sustainability, and compatibility with the existing resources. (Jones et al.,
30 2020; Bertsch et al., 2021). There is a tremendous diversity of micro-algae (MA) species, with a
31 high occurrence in the marine systems, fstramenopiles such as *Chaetoceros muelleri*, and
32 *Thalassiosira pseudonana* and dinophyta such as *Crypthec* are dominant in marine systems
33 (Heimann et al., 2015; Labeeuw et al. 2017; Minggat et al. 2021; Kumaran et al. 2021).

34 Most of the organisms belonging to the algal constitution are aquatic and photosynthetic in
35 nature, but a few are terrestrial. (Gerotto et al., 2020). Approximately for producing 1 kg algal
36 biomass rich in high-quality lipids, digestible proteins and bioactive compounds 1.83 kg of CO₂
37 is required (Khan et al., 2018; Katiyar et al. 2021). Utilizing biorefinery approach wherein the
38 waste streams can be utilized as nutrients source to produce microalgal biomass single algal
39 specie can be cultivated for valorization, as 90% of algal biomass can be valorized into food,
40 feed, energy, or compounds with high value as opposed to higher plants. (Premaratne et al., 2022;
41 Sarma et al., 2021). Algae biomass is commercially utilized in human nutrition, nutraceuticals,
42 pigments, biofuels, animal feed, cosmetics, and bio-fertilizers (Suganya et al., 2019; Garcia et
43 al., 2018). Algae and cyanobacteria pose versatile advantages which deem them suitable for
44 aforementioned applications. Firstly, water serves the purpose of electron donor for oxygenic
45 photosynthesis also, the present an inordinate biomass productivity/ acre in comparison to
46 oilseed crops and such yield is not achievable using current agricultural systems, Moreover, they

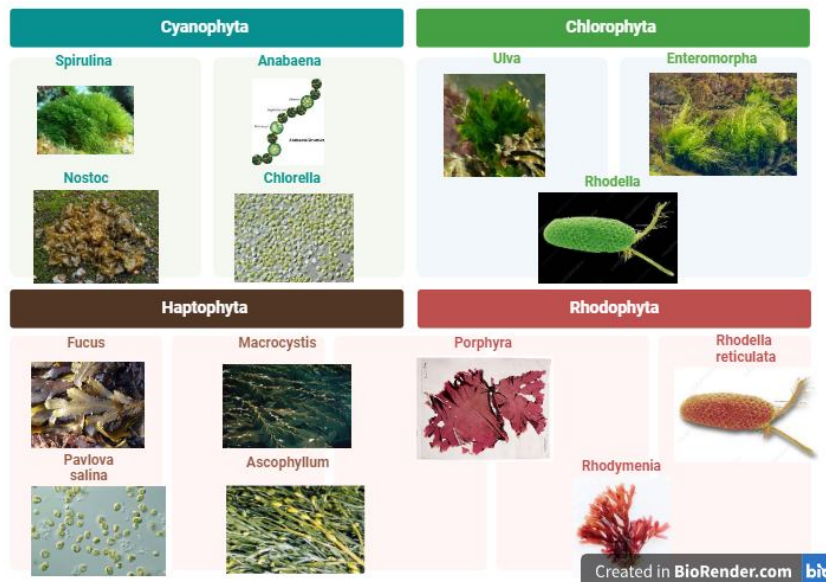
47 are a nonfood feedstock thus are resolution for food vs feed resources. Their culture is rapid and
48 doesn't require agriculture land, they are efficient adaptors of growth conditions such as
49 seawater, brackish water, and wastewater. Finally, they can be utilized to formulate a diverse
50 array of products of sustainable nature (Nguyen and Hoang, 2016; Bhowmick et al., 2019;
51 Haeder et al., 2022; Khan et al., 2018; Shin et al., 2018;; Hosain et al., 2019; Yap et al. 2021).

52 Microalgal biomass has been portrayed as a sustainable alternative for conventional sources of
53 biofuels, several food and feed systems. (Lever et al., 2020) One of the secondary advantages of
54 incorporating the usage of microalgal technology in the industry is the potential of algae to trap
55 CO₂ round the application and biomass production processes, hence reducing CO₂ emissions and
56 carbon footprint. (H Onyeaka et al., 2021).

57 The industrial application requires cultivation at a mass level which encounters the cost
58 constraint in comparison to the raw materials of other origin for similar purposes. (Roostaeie et
59 al., 2018). Enhancing cost-effectiveness and economic feasibility of utilizing MiA biomass can
60 be accomplished through various avenues such as optimizing the efficiency of bioreactors,
61 Utilizing cost-effective nutrient sources, namely domestic and industrial wastewater, can
62 significantly contribute to reducing expenses and enhancing the economic viability of MiA
63 biomass utilization. And lastly, enhancing the completeness of extraction of target compounds
64 from the biomass. (Rafa et al., 2021; Gu et al., 2023; Ahmad et al., 2022). The fundamental
65 processes for recovering microalgae from their growth medium encompass bulk harvesting, co-
66 cultures with bacteria, fungi, or multiple strains, gravity sedimentation, filtration, and
67 concentration stages. Advances in coculture algae-flocculation are facilitating the production of
68 natural bioproducts with potential applications in fuel and food additives. Additionally, genetic

69 tools and resources are being developed to enhance harvesting efficiency and the creation of
70 novel bioproducts.

71 In this context, a plethora of existing and potential studies and reviews elicit the applied
72 technologies for bio valorization of algal biomass. The focus of the present review is to provide
73 insights on current trends in the valorization of algal biomass and sustainable and integrated
74 biorefinery approaches for production of high-value products. Also, a framework for the use of
75 algae as a possible origin of bio-products rich in nutrients, natural reservoir for valuable
76 biochemicals, including proteins with high biological value and availability, bioactive peptides,
77 minerals, polysaccharides, vitamins, dietary fibre, functional lipids, fatty acids (FAs), pigments
78 etc.



79

80

Figure 1 Classification of algal species

81

82 2. Nutritional composition of algae

83 The abundantly available nutrients in the conventional crops such as rice, wheat corn, tubers and
84 other grains are predominantly carbohydrates as they are primary constituents which despite of
85 the equal significance of proteins and lipids their occurrence stands comparatively limited, with
86 soybeans serving as the predominant source for these crucial components, particularly sought
87 after for their protein content within the biomass. (Verma et al., 2018). Providently algae
88 presents a viable solution as they contain significantly high levels nutritionally available,
89 digestible, and adequate. (Fernandez et al., 2023). Remarkably, certain algae species are already
90 integrated as food supplements to augment the nutritional profile of various consumables,
91 including cereal-based products, dairy derivatives, and even meat-based items. (C Aware) .
92 Noteworthy is the exceptional protein content of algae, comprising all essential amino acids
93 crucial for human dietary needs, frequently at levels comparable to or exceeding those found in
94 conventional crops (P Geada et al., 2021; Quintieri et al., 2023).

95 **3. Functional lipid production**

96 Functional lipids are an element or ingredient of functional foods. Algae particularly MiA are
97 contain high amount of carotenoids and Omega-3 polyunsaturated fatty acids (PUFAs) which are
98 widely known illustrations of useful lipids. (Remize et al., 2021; Ren et al., 2022). Terpenes ,
99 phospholipids, glycolipids, sulpholipids and sterols constitute further examples of functional
100 lipids. (Domínguez et al., 2022). Marine MiA are the main producers of omega-3
101 polyunsaturated fatty acids (PUFAs), with lower concentrations found in marine macroalgae (2-
102 4.5 % on dry weight basis) (Barta et al., 2021; Remize et al., 2021; Polat et al., 2020). 68 PUFAs
103 have been identified in MiA include docosahexaenoic acid and eicosapentaenoic acid and γ -
104 linolenic acid. (Otero et al. 2018; Li et al., 2019; Kumar et al., 2019) As reported by Barba et al.
105 (2015), certain green MiA (*Chlorella* sp., *Haematococcus pluvialis*) are excellent carotenoid

106 producers. The lipids from MiA have been found to possess anti-cancer and anti-inflammatory
107 and anti-viral activities as well as anti-oxidant potential making them feasible for nutraceutical
108 and pharmaceutical applications. They also have presented anti-hypertensive effect, anti-diabetic
109 effect, cardioprotective effect. (Carrizzo et al., 2020; A Saide et al., 2021; Rehmanji et al., 2022;
110 Silva et al., 2024).

111 However, lipids extraction is considered a crucial step as it poses difficulties owing to the
112 thickness of polysaccharide and cellulose comprising cell walls which have excellent
113 photosynthesis performing potential and its major fraction is lipids, proteins, and polysaccharides
114 It makes up about 10% of the dry weight of the algal species and the composition varies
115 depending on species and cultivation conditions (Rösch et al., 2018; Oh et al., 2022; Abolore et
116 al., 2023). Lipid content in algal species is generally in the range of 40 to 80 % which is higher
117 than almost all the plant source indicators. (Morales et al., 2021). Several conventional and novel
118 approaches have been studied and discussed for the extraction of lipids from the algal biomass,
119 and employing eco-conscious methodologies for the extraction of compounds from microalgal
120 biomass holds significant importance. (Singh et al., 2021; Mago et al., 2023). Some
121 electrotechnology based techniques for cell disruption such as high-voltage electric discharge
122 utilized in the studies by Zhang et al. (2020), which portrayed increased efficiency of solvent
123 extraction of carotenoids, chlorophyll and other lipids. Han et al. (2018), pulsed electric field
124 (PEF), High-voltage electrostatic field, and comparatively novel technology such as supercritical
125 fluid extraction, Assisted extractions (microwave, radiofrequency, Ultrasound and, enzymatic)
126 pressurised liquid extraction and ohmic heating have been studied. (Pereira et al., 2023;). PEF is
127 among the most popular techniques due to its energy efficiency (4.8 % the energy consumption
128 as compared to the other methods) wherein several short pulses of high-intensity electric fields

129 (20–50 kV cm⁻¹) between 2 electrodes usually in the range of μ s to ms. Kumar et al. (2017);
130 Zuorro et al. (2016); Qiu et al. (2019); Wang et al. (2015) utilized EAE alone or in combination
131 with the other methods of such as ultrasound or high-pressure homogenization (Liang et al.
132 2018; Wang et al. 2017). The objectives can be attained through the application of cellulases or
133 proteinases, or through the utilization of mixture containing multiple enzymes, such as cellulase
134 combined with proteinase, or cellulase with mannanases, or a composite of other hydrolytic
135 enzymes. (Chukwuma et al., 2020; Jayasekara et al., 2019). EAE is an expensive method, but it
136 is also a quick, highly selective, and nontoxic approach that can be used in algae biorefinery
137 (Kumar et al. 2017). Any method capable of attaining satisfactory levels of selectivity and
138 resolution should be deemed useful, for extraction of functional bioactive oil (Satriana et al.
139 2019). SFE has also demonstrated utility in separation of chlorophyll and carotenoid, exhibiting
140 greater efficiency in carotenoid recovery compared to traditional UAE (Sanchez et al., 2019).
141 According to Cheng et al. (2016), the nonpolar properties of CO₂ render ScCO₂ a suitable
142 extraction solvent for semi-selective TGA extraction, preventing the extraction of phospholipids
143 and other polar lipid classes. (Cecchi et al., 2021; Niemi et al., 2021). Because of this, SCCO₂ is
144 a more appealing technique than traditional solvent approaches for precisely isolating
145 triacylglycerols (TGs) from polar lipids (Bjornsson et al. 2020). Considerable decrease in
146 extraction time during extraction of carotenoids and kavalactones using Fluid extraction was
147 reported under pressure from *H. pluvialis* and *Dunaliella salina* (20 minutes in comparison with
148 90 minutes for UAE methods (Sarkarat et al., 2023), making the procedure appropriate for
149 thermolabile compounds, while consecutively reducing solvents quantities. The primary
150 drawback inherent in employing the SFE lies in its high energy demands, thereby exerting a
151 pronounced impact on the overarching operational efficacy. Advancements in lipid extraction

152 methodologies have yielded innovative approaches circumventing solvent utilization, i.e., by
 153 isotonic extraction, osmotic pressure extraction, and enzyme-mediated extraction techniques.
 154 (Sivaramakrishnan et al., 2022). The PLE procedure's selectivity can also be improved by
 155 utilizing solvent mixes (Attard and Hunt 2018). However, the significant costs of extraction
 156 owing to the drying procedure prevent its commercialization, however, the extraction from wet
 157 biomass has emerged as a potential solution. (Howlader and French, 2020; Amorim et al., 2020;
 158 Zou et al., 2021).

159 *Table 1 Extraction technology of lipids from algae, operations conditions and major findings*

Extraction technique	Algae	Target lipids(s)	Operation conditions	Main findings	References
PEF	Chlorella	Carotenoids and chlorophyll	50 % DMSO; 3 kV/cm; 99 kJ/kg 44 pulses, for 0-180 seconds	Extraction of target lipids was affected by the solvent and increase in content from 0-180 seconds for all the lipids	Wang et al. (2023)
PEF	<i>Chlorella zofingiensis</i>	Carotenoids and	Ethanol; 20 kV; 50 pulses; 30-150	Treatment with fifty kV	Pereira et al. (2024)

chlorophyll μ s utilizing 50
 1 pulses was
 most efficient
 for the
 extraction
 which is eco-
 friendly

HVED Phaeodactylum Lipids CHCl₃/MeOH HPH was Zhang et al.
 tricornutum (2:1 v/v) for more suitable (2020)
 lipids and 95 % as compared
 EtOH for to HVED for
 pigments; 40 kV; extraction of
 200 pulses at 1-3 pigments
 min interval; 0.1- (carotene and
 8 ms chlorophyll)
 and lipids

PLE *Galdiera phlegrea* Carotenoi Ethanol; 12 % Imbimbo et
 d pressure at 100 enhancement al. (2020)
 bar; at 50 °C in the yield of
 temperature for lipids as
 30 minutes compared to
 conventional

methods						
EAE	<i>Dunaliella salina</i>	Carotenoids	and	Choline chloride with urea/ oxalic acid;	67.41 % ± 6.07	Asevado et al. (2023)
coupled		lipids			achieved; 1	
with deep					pot method	
eutectic					prevented	
solvents					carotenoid loss	
EAE	<i>Euglena gracilis</i>	Paramylon	n-hexane/Ethanol		Increased rate of extraction for lipids from 73% - 96% (paramylon at 58.3%)	Zheng et al. (2023)
EAE	<i>Nannochloropsis</i> sp.	EPA rich oil	Trichoderma sourced Cellulases;	50 °C; 12 hours	Enzyme assisted extraction extract yielded 77%	Bhattacharya et al. (2023)
					TFA with 11% EPA	

					fraction
UAE	<i>Microchloropsis</i> <i>gaditana</i>	glycolipids	Ethanol; 37 kHz; 100W; at 50 °C for 30 min	185% yield was achieved in comparison to traditional extraction	Pühringer et al. (2024)
UAE	<i>Nannochloropsis</i>	lipids	hexane/isopropanol; 0.45 W/mL	Some positive impact on yield recorded, attributed to ultrasonic heating	Meinis et al. (2024)
UAE	<i>Chlorella</i> sp	PUFA	EtOH, CPME, DMC, 2-MeTHF; 20:1 (v/w); 60 °C; 40 minutes	Ethanol-2- MeTHF- extracted lipids showed dominance in linoleic acid, α -linolenic	

				acid, and palmitic acid	
UAE	<i>Ulva lactuca</i>		40 KHz; 60 W; 1 h, 50% ethanol, and temperature of 25 °C was the optimized condition for		
MAE	<i>Nanochloropsis</i> sp	EPA	2:1 (IL:biomass) 90 °C; 25 min, 3.3% w/v solid-loading	37.28 mg g ⁻¹ EPA which was 8.1 times as opposed to Soxhlet extraction	Motlagh et al. (2024)
SFE	<i>Chlorella vulgaris</i>	Carotenoid, chlorophyll 1	CO ₂ /EtOH; 250 bar, 60 °C; 100 kgCO ₂ /kgbiom	β-carotene- 24.88 mg/g; Chlorophyll- 7.06 mg/g; SFE presented	Georgiopoulou et al. (2023)

short duration

operation

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161

162 **4. Vitamin production**

163 A variety of chemical compounds that serve as vital micronutrients for life are included in the
164 class of vitamins. (Godswill et al., 2020). These molecules perform a diverse array of biological
165 functions, serving as coenzymes, antioxidants, hormones, cell signaling mediators, and
166 regulators of the cells and tissues growth or differentiation. (Mitra et al., 2022; Surana et al.,
167 2023). The majority of the vitamins are of photosynthetic origin, whereas vitamins B and vitamin
168 K are acquired through diet and primarily synthesized by bacteria. (Simes et al., 2020; Mondro et
169 al., 2020). Vitamins are essential for life, but neither humans nor animals can synthesise them
170 very well, so they must be continuously ingested through food, such as plants, fruits, or seeds.
171 (Ofoedu et al., 2021). It is highly advised that people consume diets rich in various vitamins to
172 prevent vitamin deficiencies in humans. Nevertheless, not all plants possess every vitamin, and
173 certain vitamins such as D and K, along with several B vitamins, are rarely present in plant
174 sources. (Godswill et al., 2020). Algae (marine and terrestrial) are appreciated for synthesizing a
175 wide array of vitamins, and MiA—photosynthetic, unicellular, rapidly dividing organisms—may
176 prove to be particularly beneficial as vitamin producers. (Edellman et al. 2019; Jung et al. 2019)
177 .One such example of MiA is the vitamin-rich *Spirulina platensis*, which is already well-known
178 as a "super food." Vitamins like B12 (active and bioavailable form contained by *C. vulgaris*), K,
179 or D that are absent from higher plants can be found in MiA. (Islam et al., 2017; Wang et al.,
180 2020; Jung et al., 2019; Edelmann et al., 2019; Kiran et al., 2021). Marine microalgae have the

181 capability to synthesize and accumulate a diverse array of vitamins, including pro-vitamin A,
182 several B-group vitamins (B1, B2, B3, B5, B6, B8, B9, and B12), vitamin C, and vitamin E,
183 among others. (Singh et al., 2022; Kraan et al., 2013). This synthesis and accumulation are
184 correlated with the growth phase (Some vitamins such as C and E were most adequately
185 harvested during exponential phase whereas, extraction of provitamin A and B2 was inefficient
186 during stationary phase) and prevailing growth conditions. (Rehmanji et al., 2022; Richmondi et
187 al., 2017). Vitamin B6 and B9. Variation in different photic conditions induced varying vitamin
188 concentrations for different species. (Aratboni et al., 2019). Pseudo form of vitamin B-12
189 furnishes false positive results which forms the base of vague claims regarding nutritional
190 supplements thus, a credible method for analysis of the same is required. (Oever et al., 2022;
191 Herbert et al., 2001). A decline in vitamin E synthesis was attributed to the intensity of photons
192 received by the reactor whereas the administration of UV-B light $\leq 4.4 \text{ kJ/ m}^2$ in *Chlorella*
193 *vulgaris* improved the production of provitamin-A and Vitamin-E. (Suyono et al., 2015;
194 Mutschlechner et al., 2022). Contrarily, it was found to increase with decreasing nitrogen
195 concentration in the media. All these findings indicate that vitamin expression is a species-
196 dependent process and is modulated by environmental conditions, such as light and nutrient
197 availability, as well as the harvesting stage. Different Microalgal sources have been found
198 suitable for different vitamins such as spirulina for Vitamin B2, *Chlorella* for B3, *N. gaditana* and
199 *Chlorella* sp. for B9 (6 times higher in comparison to spirulina powder i.e., $20.8 \mu\text{g g}^{-1}$ and 25.9
200 $\mu\text{g g}^{-1}$ respectively). Also, Vitamin K1 which is predominantly synthesized via chemical protocols
201 has been procured in significant amounts from cyanobacterial algae *Anabaena cylindrica* ($200 \mu\text{g}$
202 g^{-1}) using photobioreactors.

203 However industrial microbial fermentation has only been established for the production of a few
 204 vitamins such as vitamin K2 using engineered strains of *Bacillus subtilis*, vitamin B12 using
 205 *Sinorhizobium meliloti*, *Propionibacterium shermanii*, and *Pseudomonas denitrificans*, vitamin B2
 206 using the filamentous fungus *Ashbya gossypii* and *Bacillus subtilis*, and vitamin C using
 207 *Ketogulonicigenium vulgare*, *Bacillus megaterium* and *Gluconobacter oxydans*.

208 SCCO₂ based SFE was utilised for extraction of fat-soluble vitamins from *Tetrademus Obliquus*
 209 by Chronopoulou et al. (2019) and they found the treatment with 30 MPa pressure at temperature
 210 of 40 °C to be the best condition for extraction. *Nanochlorosis oceanica* was cultivated for the
 211 production potential for production of vitamin D3

212

213 Table 2 Vitamins found in various algae species

Vitamin	Algae (Genus)		
Vitamin-A	Arthrospira,	Porphyridium,	Chaetoceros, Euglena
Vitamin- D₂ and D₃	Arthrospira,	Tetraselmis,	Skeletonema, Pavlova
Vitamin E	Anabaena,	Coccomyxa,	Chlorococcum, Synechococcus
Vitamin K1	Arthrospira, Anabaena, Tetraselmis		
Vitamin C	Anabaena,	Chlamydomonas,	Nannochloris, Stichococcus, Nannochloropsis
Vitamin B complex	Aphanizomenon,	Dunaliella,	Pavlova,

214
215 Vitamin-A concentration was found to be high in diatoms specially genus *Chaetoceros* (0.52 -
216 0.97 mg/g dw basis) and, in *Porphyridium* a red microalga (upto 0.75 mg/g dw basis).
217 (Bhattacharya et al., 2022). Vitamin A content strongly varied among and inside algal classes
218 hypothesizing that no link between vitamin A concentration and microalgal divisions do exist.
219 (Mondro et al., 2020). A conversion factor between dry and fresh weight for MiA of around
220 10%, furnishes 0.42 and 0.1 mg of retinol equivalents on fresh weight basis per gram (mg RE/g
221 FW) in *Chaetoceros* and *Tetraselmis*. (Mondro et al., 2020). The recorded values significantly
222 surpass those documented in edible carrots, approximately 0.011 mg RE/g FW, and oranges,
223 0.0003 mg RE/g FW. Certain macroalgae species like *P. vulgaris* and *P. palmata* demonstrate
224 noteworthy levels of vitamin B12 content. Furthermore, analyses have identified the presence of
225 retinol, α -tocopherol, and ergocalciferol in *C. barbata*. Notably, algae boast significant
226 concentrations of antioxidant vitamins C and E. Vitamin C plays a pivotal role in warding off
227 scurvy, while vitamin E aids in the management of neurological disorders stemming from
228 impaired nerve conduction, alongside anemia resulting from oxidative harm to red blood cells.
229 (Godswill et al., 2020). Luhila et al. (2022) studied the vitamin B12 composition of Baltic algae
230 for the omega-3-fatty acid content and reported *F. vesiculosus* as the best source among the
231 target species with 5.19 ± 0.32 mg/g on dry weight basis. Eliason studied *Emiliania huxleyi* for
232 vitamin-D content and found that D1 and D2 were produced by the algae to the UV response and
233 is a source for extraction of vitamin-D in its various forms. *Anabeana cylindrica* was cultivated
234 by Tarento et al. (2018) using modified ASM1 media under photoautotrophic conditions and
235 monochromatic white light at $320 \mu\text{molphoton/m}^2/\text{s}$ with dark:light cycle of 12/12 h at 23°C, and

236 were able to achieve the content of $200 \mu\text{g g}^{-1}$. Similarly, *Nanochlorosis oceanica* was studied as a
237 natural source for vitamin D3 production. $1 \mu\text{g g}^{-1}$ of vitamin was produced by the biomass which
238 suggest the suitability for production. (Ljubic et al., 2020).

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240

241 **5. Bioactive phenolic compound extraction**

242 Secondary metabolites such as phenolic compounds (PCs) in algae are indirectly involved in
243 primary life processes of metabolism such as cell division, photosynthesis, and reproduction.
244 (Pereira et al., 2021). PCs are found in many algal families and consist of one or more than one
245 phenolic rings, may or may not be halogenated to bestow distinct and frequently stronger
246 biological activity. (Pereira et al., 2021). Both red and brown algae contain phenolic terpenoids.
247 For instance, Sargassaceae contain only meroladiterpenoids (chromanols, plastoquinones and,
248 chromenes), whereas primarily red algae contain diterpenes. (Junopia et al., 2020).
249 Rhodomelaceae (*Laurencia* sp.) contains sesquiterpenes, and *Callophycus serratus*
250 (bromophycolides) forms a macrolide as a result of secondary cyclization. phloroglucinol
251 polymers Phlorotannins including fuhalols, fucols, eckols, phlorethols and, carmalols are major
252 phenolic chemicals identified till now in the Phaeophyceae. (Cotas et al., 2020). They exhibit a
253 wide range of applications; for example, purified PCs portray antiradical, antioxidant, metal-
254 chelation, UV-protective, and antifouling activity, and their activity varies according to
255 molecular-size profile and concentration. (Kumar et al., 2021). Microalgal and cyanobacterial
256 biomasses are processed, primarily by cell disruption, to optimise the yield of phenolic extraction
257 (Kapoor et al., 2022).

258 Various techniques of disruption have been devised for each microalgal group, owing to
259 variations in the composition of their cell walls. The physical approaches include mechanical
260 disruption and/or thermal therapy (high, mild, or freezing temperatures, occasionally through a
261 thermal cycle). (Saravana et al., 2023; Bharte et al., 2021). One type of liquid nitrogen heat
262 treatment ($-196\text{ }^{\circ}\text{C}$) for macroalgae and MiA is called cryogrinding (Keramane et al., 2023;
263 Razz et al., 2024). Using high pressure (as in the French press) or bead milling, mechanical
264 pretreatment breaks the cells. Microwaves are being used more and more, and their value has
265 been shown in the extraction of pigments from diatoms, particularly fucoxanthin, β -carotene, and
266 chlorophyll a. (Gallego et al., 2021; Mehariya et al., 2021). Ultrasonication is actually the most
267 widely used disruption technique for phenolic research. As chemical cell disruption can be more
268 selective than physical approaches, it has been extensively investigated; nonetheless, as this
269 pretreatment is part of the extraction protocol. (Nemre et al. 2021; Khadhraoui et al., 2021;
270 Saravana et al. 2023; Rahman et al., 2022 and Liu 2022).

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274 **5.1 Antioxidants**

275 Antioxidant chemicals can be found in seaweeds. Glutathione (GSH) and ascorbate (vitamin C)
276 are found in fresh seaweed biomass. (Begum et al., 2021). In addition, a variety of secondary
277 metabolites with antioxidant properties can be produced by algae, such as carotenoids,
278 tocopherol, polyphenols including flavonoids, catechins, lignans, tannins, and chlorotannins, and
279 mycosporine-like amino acids. (Kalasariya et al., 2023). Phlorotannins, or brown algal
280 polyphenols, are one particular category among them. It has been noted that brown algae have

281 the highest chlorotandin content of all marine algae. (Ummat et al., 2020). These are 1,3,5-
282 trihydroxybenzene oligomers, with a few exceptions. Eckol, phlorogluconol, dieckol, 6,6-
283 bieckol, and chlorofucofuroeckol from *Ecklonia* sp.; dioxinodehydroeckol, 7-phloroeckol from
284 *Eisenia bicyclis*, and diphlorethohydroxycarmalol from *Ishige okamurae* are the well-researched
285 phlorotannins obtained from macroalgae. (Shrestha et al., 2021; Fernando et al., 2022)

286

287 **5.2 Diterpenes**

288 Dictyotaceae is a family of algae that can create diterpenes and other secondary metabolites.
289 Hydroazulenoids, xenicanes, dolabellanes, and so-called extended sesquiterpenoids are among
290 the several forms that can be identified. Dictyota ciliolate, a marine brown algae, was used to
291 extract diterpenes such as Dictyodial, Dictyol C, and Dicytol H. It has been found that they have
292 algicidal, cytotoxic, and antiviral properties. (Gamal et al., 2023). For instance, herpes simplex
293 virus type 1 infection in Vero cells was suppressed by diterpenes isolated from *Dictyota pfaffii*
294 and *Dictyota menstrualis*. Diterpenes from *D. menstrualis* were tested for HIV-1 resistance.
295 (Rushdi et al., 2022; Cotas et al., 2022; Pagarete et al., 2021)

296

297 **5.3 Plant growth-promoting substances/hormones**

298 All bioactive substances that were separated from the aforementioned algae are mostly
299 advantageous to both people and animals. (Babicha et al., 2022). It's important to consider that
300 algae are also a significant source of chemicals and hormones that promote plant growth.
301 (Kapooore et al., 2021; Wang et al., 2021; Ammar et al., 2022). These have been found in certain
302 seaweed extracts derived from different kelp species, as well as in green, brown, and red
303 seaweeds. (Ali et al., 2021; Hurtado et al., 2021). Commercial applications for these algae

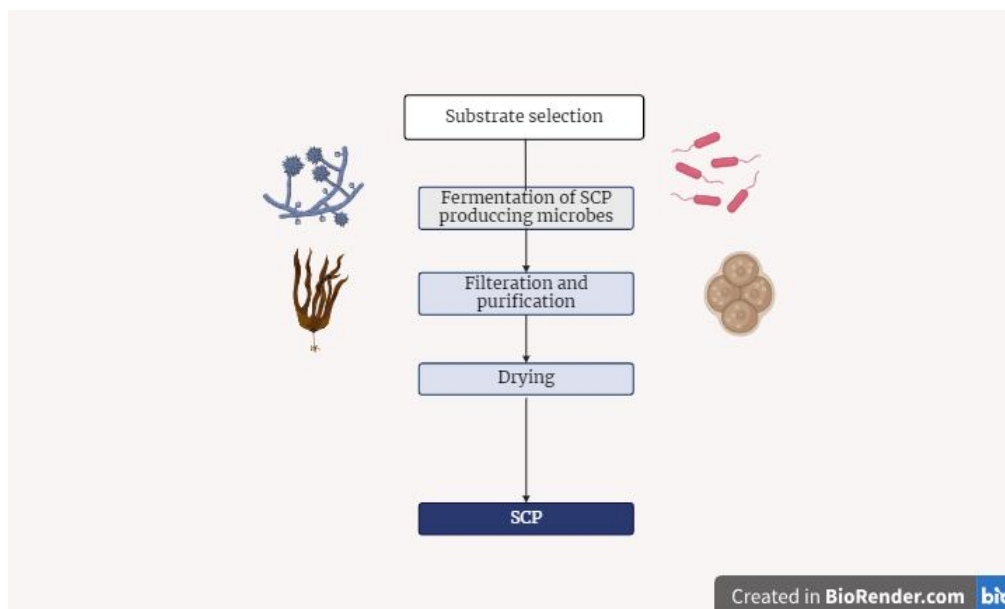
304 extracts include crop production system amendments and growth stimulants. Numerous review
305 publications have provided extensive descriptions of these plant growth-promoting
306 chemicals/hormones, which include auxins, betaine, cytokinins, abscisic acid, gibberellins,
307 ethylene, and polyamines. (Boukhari et al., 2020; Kholssi et al., 2022; Ammar et al., 2022). The
308 amount and kind of growth-regulating chemicals, particularly PGRs, that are present in seaweed
309 biomass are currently the subject of extensive research. (Ma et al., 2022; Parmar et al., 2023;
310 Madruga et al., 2020)

311

312 **6. Single cell protein Production**

313 Owing to their excellent production without arable land achieving a high protein content of up to
314 70%, unicellular microorganisms (cyanobacteria and MiA) have emerged as alternative protein
315 sources (Bratosin et al., 2021; Grossmann et al., 2019; Nyssölä et al., 2022; Onyeaka et al.,
316 2022). In the same light the proteins from MiA are being proposed as a better protein source
317 based on numerous analyses. (Buono et al., 2014; Wells et al., 2017). Single-cell proteins, often
318 known as pure proteins or dried cells, are extracted from high-protein bacteria.
319 *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Spirulina*, *Nostoc* etc., are MiA which constitute all
320 essential amino acids— isoleucine, valine, threonine, leucine, lysine, methionine phenylalanine.
321 (Nass and Nascimento, 2022; Quieroz et al., 2023). Compared to forages, high protein containing
322 SCPs with adequate amino acid profile have present higher protein: carbohydrate ratio, a low
323 lipid content, making them particularly appealing as dietary supplements for humans. (Jach et
324 al., 2022; Bratosin et al., 2021). Fermentation serves as primary operation for production of SCP
325 (Kadim et al., 2017). The selection of substrate is based on several factors, including cost,
326 availability, the amount of oxygen needed for fermentation, the amount of heat generated, the

327 fermenter's cooling capacity, and the cost of post-treatment processing. (Siddiki et al., 2022;
328 Varghese et al., 2022). When the fermentation process is finished, the available biomass is
329 harvested and used as a source of protein. This microalgal biomass at adequately high
330 concentrations has shown the yield of 22 to 44 tonnes hectare⁻¹. Purification, cell disruption,
331 washing, and protein extraction are further processes used to further prepare the biomass to
332 achieve high production rates and high yields (Dhaver et al., 2010; John et al., 2019; Rajput et
333 al., 2024). Micro-algae produce protein rich cellular biomass containing a significant amount of
334 SCPup to 70% by utilizing solar energy. Yields from micro-algae mass culture are 20–50 times
335 higher than those from soybeans. (Hosseinkhani et al., 2022; Chronakis et al., 2018). Various
336 crude substances serve as growth mediums for cultivating micro-algae for production of SCP. In
337 the case of Chlorella, scholarly sources mention tempeh waste, containing 52% protein content;
338 tofu waste, with 52.32% protein content; and cheese waste, with a total protein content of
339 15.43%. (Baltork et al., 2022; Bratosin et al., 2021). Additionally, wastewater has been identified



340 as a viable medium for cultivating *Scenedesmus obliquus* green algae, resulting in a protein
341 content yield of 52%.

Figure 2 General production process for SCP from different microorganisms

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For *Arthrospira (Spirulina) platensis* cyanobacteria, SCP yields range from 48.59% to 56.17% by dry weight, contingent upon the specific culture medium employed. (Lopez et al., 2020; Singh et al., 2022; wang et al., 2022). Although various species of MiA, including *Chlorella*, *Arthrospira*, *Haematococcus*, *Dunaliella*, and *Schizochytrium*, are designated as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration (FDA), their utilization in commercial food and feed formulations requires thorough scrutiny due to potential toxicity concerns. Furthermore, the high nucleic acid content in the algal cells which could lead to kidney related disorders and gout, and high content of chlorophyll responsible for bitterness also, serve as barriers to their application in formulation of food/ feed supplements. (Janssen et al., 2022; Wychen et al., 2021). Moreover, mild pretreatment strategies have been devised to reduce to minimum or nullify the nucleic acid content from the final product. Closed photobioreactors (flat plates, air-lift reactors, tubular systems, bubble columns, large plastic bags) can be engineered for industrial-scale operations. Scalability can be achieved by replicating multiple units.

Table 3 Algal species being used for Single cell protein production

Algae species	Media	Protein content	Important findings	Reference
MiA and methanotroph system	Activated biogas sludge	0.51g/g biomass	Increased iron availability stimulated increased growth;	Zhang et al. (2023)

				Limited nitrogen reduced the growth and protein content upto 0.31g/g	
Chlorella vulgaris and Yarrowia lipolytica	Liquid digestate of dairy wastewater	0.51g/g biomass		Enhanced biomass and protein yields were achieved by mixed culture as compared to C.vulgaris culture	Qin et al. (2018)
Chlorella vulgaris and Arthrospira sp.	Struvite (Phosphorus obtained from wastewater treatment)	-		The protein content of cultivated on struvite was consistently comparable to that obtained when grown on standard media.	Muys et al. (2022)
Chlorella vulgaris	Electrochemically treated potato juice wastewater	0.52g/g biomass		The bioelectrochemical assistance resulted	Pan et al. (2021)

in enhancing the
suitability of
wastewater by
achieving high
rates of removal
for COD, TKN and
Phosphorus, for
pure protein
production

Chlorella	Strong	swine	0.59	pH	had	a	Dinnebier et al.
sorokiniana	wastewater				significant	impact	(2021)
					on	productivity	

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360 **Challenges**

361 The microalgal cultivation and the development of biorefinery based systems have attracted
362 significant attention for their potential to produce high quantities of value-added compounds,
363 including pigments, vitamins, polyunsaturated fatty acids (PUFAs), and antioxidants. (Chew et
364 al., 2017). However, several challenges remain partially unaddressed in this field. The scaling
365 up of algal cultivation is a costly and complex endeavor which requires the optimization of
366 various factors such as the methods, approach and system, the strategies for cultivation and
367 harvesting (mechanical methods like filtration, centrifugation etc.,) and chemical (flocculation,
368 coagulation, etc.) methods for biomass harvesting. These processes are estimated to contribute
369 20–30% to the overall production costs), biomass cultivation, conditions of cultivation

370 (temperature, time light and agitation method and intensity and nutrients) and monitoring the
371 potential environmental risks to be successful and thus is economically not feasible.
372 Efficiency of extraction, which is dependent on the technology of extraction, The hydrocolloidal
373 nature of algal biomolecules, size and compatibility, down streaming processing and recovery of
374 compounds and energy consumption remain as viable concerns as they impact the consumption
375 patterns and selection of the algal compounds for selection. In the food industry, algae
376 cultivation must adhere to the regulations set by the Food and Drug Administration (FDA) to
377 ensure the safety of algae extract for human consumption. Identification of an efficient technique
378 for separation of target compound from algal suspension is crucial in microalgal biomass
379 production, given the substantial environmental and economic costs involved.

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381 **7. Conclusion**

382 Current trends of data available on the per capita availability of food resources directs towards a
383 scarcity of production both as food stock and feedstock i.e., the current food production capacity
384 and potential is not completely sustainable to contain and fulfill the enormous increment in
385 requirement of food (25% - 70%) relative to the rising population, expected to be at 9.7 billion
386 by 2050, neither quantitatively nor qualitatively. Most of the organisms belonging to the algal
387 constitution are aquatic and photosynthetic in nature, but a few are terrestrial. They possess the
388 potential of furnishing a biomass rich in carbohydrates, lipids and proteins utilizing sunlight and
389 CO₂. Commercial applications for algae biomass include human nutrition, animal feed,
390 cosmetics, bio-fertilizers, pigments, biofuels, and nutraceuticals. Functional lipids being
391 extracted from the algal sources are PUFAs (EPA, DHA), MUFAs, Carotenoids, Chlorophyll
392 etc. Electrostatic, enzyme assisted, solvent based and modified solvent based treatments have

393 been utilised for for extraction, which have significant advantage over the existing conventional
394 techniques. Vitamins are essential for life, but neither humans nor animals can synthesise them
395 very well, so they must be continuously ingested through food, such as plants, fruits, or seeds.
396 Marine algae are known to produce a wide variety of vitamins, and MiA—photosynthetic,
397 unicellular, rapidly dividing organisms—may prove to be particularly beneficial as vitamin
398 producers. Phenolic compounds are found in many algal families and consist of one or more
399 phenolic rings. These rings can be halogenated to bestow distinct and frequently stronger
400 biological activity. Cryogrinding, bead milling, and other novel methods have been and are being
401 utilised for the extraction. Because of their efficient development independent of arable land and
402 high protein content of up to 70%, unicellular organisms such as MiA and cyanobacteria have
403 arisen as alternative protein sources. In the same light the proteins from MiA are being proposed
404 as a better protein source based on numerous analyses. Micro-algae have the ability to produce
405 cellular biomass containing a significant amount of SCP (up to 70%) by utilizing solar energy.
406 Yields from micro-algae mass culture are 20–50 times higher than those from soybeans. Thus,
407 the utilization of algae for production of a plethora of nutrient rich biomasses is crucial for
408 making the food systems more efficient and the food nutrient pool more versatile and easily
409 available.

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