




Production of microalgae with high lipid content and their potential as sources of nutraceuticals

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Abstract In the current global scenario, the world is under a serious dilemma due to the increasing human population, industrialization, and urbanization. The ever-increasing need for fuels and increasing nutritional problems have made a serious concern on the demand for nutrients and renewable and eco-friendly fuel sources. Currently, the use of fossil fuels is creating ecological and economic problems. Microalgae have been considered as a promising candidate for high-value metabolites and alternative renewable energy sources. Microalgae offer several advantages such as rapid growth rate, efficient land utilization, carbon dioxide sequestration, ability to cultivate in

wastewater, and most importantly, they do not participate in the food crop versus energy crop dilemma or debate. An efficient microalgal biorefinery system for the production of lipids and subsequent byproduct for nutraceutical applications could well satisfy the need. But, the current microalgal cultivation systems for the production of lipids and nutraceuticals do not offer techno-economic feasibility together with energy and environmental sustainability. This review article has its main focus on the production of lipids and nutraceuticals from microalgae, covering the current strategies used for lipid production and the major high-value metabolites from microalgae and their nutraceutical importance. This review also provides insights on the future strategies for enhanced microalgal lipid production and subsequent utilization of microalgal biomass.

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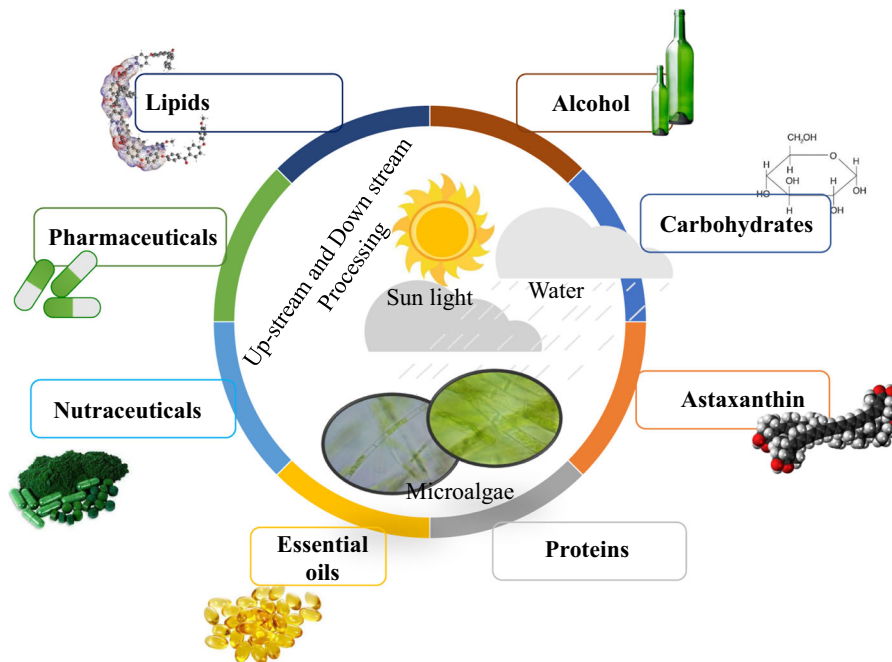
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Graphical abstract



Keywords Microalgae · Lipids · Nutraceuticals · Biorefinery · High-value metabolites · Food and feed

Abbreviations

EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
PUFAs	Polyunsaturated fatty acids
ALA	Alpha linoleic acid
PDT	Photodynamic therapy
DAF	Dissolved air flotation
TAG	Triacylglycerol
FAEE	Fatty acid ethyl esters
FAME	Fatty acids methyl esters
ppb	Parts per billion
ppm	Parts per million
CO ₂	Carbon dioxide
vvm	Volume by volume
ha	Hectare
wt	Weight
FDA	Food and Drug Administration
PE	Phycocerythrin
PEC	Phycocerythrocyanin
PC	Phycocyanin
APC	Allophycocyanin

Introduction

During recent years, microalgal cultivation has gained increasing attention for the production of biofuels, functional food, and nutraceuticals (Udayan et al. 2017) with emphasis on adaptation of biorefinery approach, which could be of different types, e.g., biomass-based biorefinery (Fig. 1). A biomass-based biorefinery is a production process and system in which biomass is fractionated to different chemical moieties which are then converted to different end-products (desired products). This could be achieved via biochemical or thermochemical pathways to produce marketable value-added metabolites, fuels, chemicals, etc. (Hingsamer and Jungmeier 2019; Sirohi et al. 2020; Awasthi et al. 2021). The biorefinery approach can be efficiently utilized for sustainable and efficient product accumulation with increased environmental and economic benefits. The development of a new and efficient biorefinery concept with the already existing industrial approaches can reduce the cost of production as well (Fig. 1).

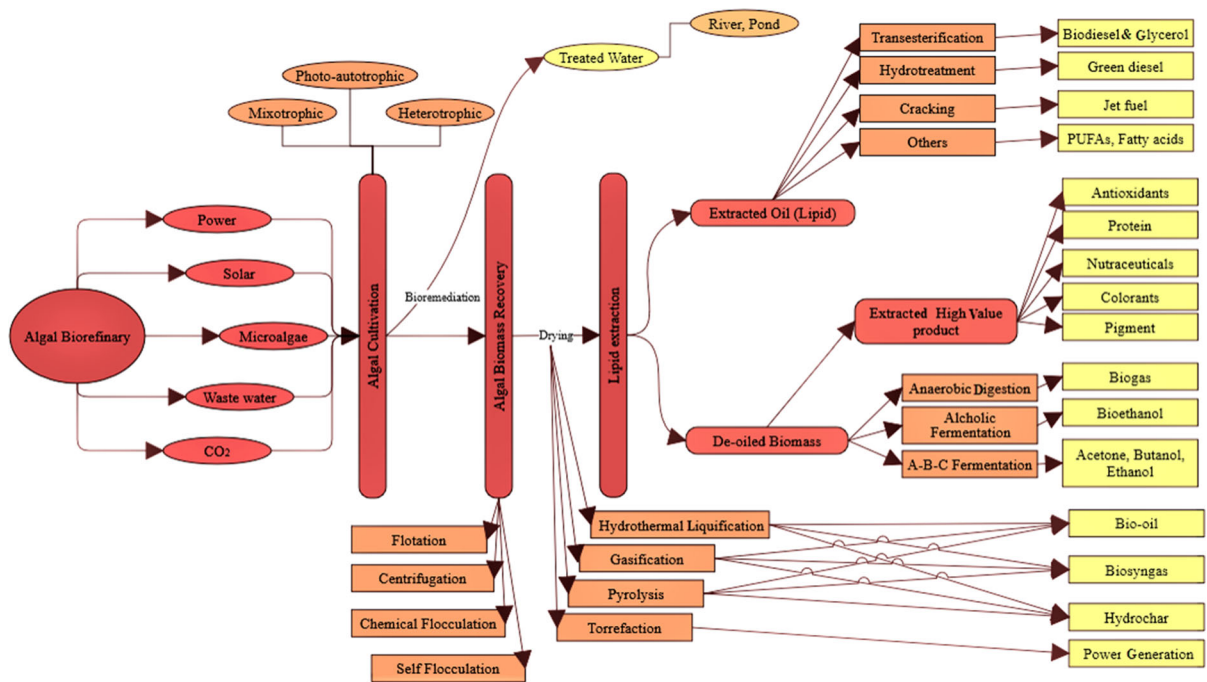


Fig. 1 Process flow diagram for microalgal biorefinery including cultivation, different operation units and downstream processing. The major criteria are the selection of a potential microalgal strain depending on the specific product and supply of low cost nutrients for microalgal growth and metabolite production. Microalgal biorefinery should consider the factors such as (1) better mixing and light penetration, (2) minimum

investment and operational costs, (3) high biomass production and ivEfficient downstream processing. Downstream processing involves the harvesting of biomass, cell disruption and intracellular metabolite extraction and fractionation of the metabolites to increase the number of commercial products. (**PUFAs* polyunsaturated fatty acids)

Microalgae are microscopic photosynthetic organisms and primitive eukaryotic plants on the planet earth. They are unicellular to multi-cellular organisms with different sizes ranging from one to hundred microns. Microalgae have different habitats like freshwater and ocean systems and they play the role of primary producers in the food chain (Udayan et al. 2021). These biological cell factories are the natural nutritional base and primary source for the aquatic food chain. Microalgae are primitive plants belonging to Thallophytes without stems, leaves, and roots and have *chlorophyll a* as primary photosynthetic pigment. Other examples of Thallophytes are fungi, lichens, and some classes of bryophytes, bacteria, and slime molds. Among these fungi and microalgae are widely studied for biofuel applications. Photosynthetic machinery of land plants is evolved from microalgae and are considered as the primary producers of the aquatic ecosystem. From ancient times, microalgal biomass has been utilized for the production of fuels, food, medicine, etc.

In the present global scenario, the major fuel sources used are petrol, diesel, coal, and natural gas (Enamala et al. 2018). At the current rate of consumption and overexploitation, it is estimated that the existing fossil fuels will be depleted in the coming 50 years (Martins et al. 2019). The level of greenhouse gases such as CO_2 , CH_4 , and N_2O have increased tremendously in the atmosphere to 409.8 ppm, 1875 ppb, and 331.1 ppb as of 2017 compared to the 1800s, which was 289.9 ppm, 750.8 ppb, and 273 ppb, respectively (Köhler et al. 2017). This alarmingly high increase in CO_2 and other greenhouse gas emissions could be reduced by using renewable liquid biofuels.

Liquid biofuels can be used without any major changes compared to the other renewable energy sources such as solar, tidal, and wind energy generation and release of toxic gases into the environment, which contributes to a major reason for global warming which is the major drawback of fossil fuels. The importance of biofuels has increased recently due

to the above-mentioned reasons. Biofuels are produced from different biomass sources from forestry, agricultural and aquatic sources. The current scenario of producing first-generation biofuels by utilizing food crops such as corn, soybeans, sugar cane, etc., and uncontrolled use of agricultural land for energy production creates an ethical threat of “food crops *versus* energy crops”. Second-generation biofuels mainly utilize nonfood parts of crops including leaves, grass, stem, whole crop maize, lignocellulose biomass, and industrial waste from the food and pulp industry. The use of first and second-generation biofuels is restricted because of their conflict with edible crops, agricultural lands, low market availability, potable water requirement, application of fertilizers, and ecological imbalances (IEA 2017). Several shortcomings of these biofuel feedstocks can be partly solved by switching to third-generation biofuels using algal biomass.

Microalgae have a high rate of lipids production per hectare yield which is 7–31 times higher than other oil crops (Table 1). Microalgal biofuels are non-toxic, highly biodegradable with no sulfur content. The utilization of CO₂ by algae during the growth makes it a feasible CO₂ mitigation agent from power stations and industrial plants, which could lead to environmental pollution (Sreekumar et al. 2018; Joun et al. 2021; Sirohi et al. 2021b). Microalgae represent primitive land plants and depend on photosynthesis for the production of chemical energy from solar energy. The chemical energy synthesized by microalgae is stored in the oils, proteins, and carbohydrates. While considering biofuels, the lipid yield from a particular species highly affects its energy production efficiency (Sreekumar et al. 2016). Microalgae have a higher growth rate, high photosynthetic efficiency, adaptability to harsh environmental stress conditions, and can grow in wastewater with high nutrient uptake ability. Also, the simple cellular structure simplifies the technical limitations of downstream processing and product recovery, which makes it a prominent feedstock for nutraceutical production (Costa et al. 2019).

It has been reported that microalgae can contribute around 40–50% of the atmospheric oxygen and subsequently utilize CO₂, nitrogen, and phosphate to grow autotrophically, which makes microalgae attractive for CO₂ mitigation and reduced environmental pollution (Costa et al. 2019). Microalgal biofuel has an

advantage over the petroleum feedstock because of its high oxygen content (10–12%) over petroleum fuel (4%). Higher oxygen content helps to speed up the combustion process. It has been estimated that around 16% of global transport fuel requirements will be satisfied by biofuels in 2040 (Energy and Change 2017). Currently, many microalgal production systems are focused on the production of lipids and their subsequent use as nutraceuticals (Table 2), but the major problem facing all the industries is low biomass production and high downstream processing costs. Therefore, it is indeed necessary to understand the problems in commercial lipid and nutraceutical production from microalgae. Especially during the Covid-19 pandemic situation the world is in search for efficient immune boosters and health promoting substances, microalgal metabolites have many health benefits including immune system boosting activities (Udayan et al. 2021). Moreover, many studies have focused on the biofuel applications of microalgae rather than its food and nutritional applications (Table 2) Therefore, the main objective of this study is to give insights on the current methods used for the production of lipids from microalgae, their challenges in commercial production and future of microalgae in nutraceutical industries.

Production of lipids by microalgae

Microalgae have active participation in overtaking the transport fuel sector if cost-effective and efficient large-scale production systems could be achieved. The development of a cost-effective and coherent biorefinery system for biofuel production from microalgae along with high-value metabolites for food and feed applications is very necessary. It has been reported that the total cost of production of dry microalgae ranges from 2 to 10 USD per kg based on a different mode of cultivation (Hingsamer and Jungmeier 2019). Taking USD 2 per kg and 25% oil content, the oil cost will be USD 8 per kg. In the last decade, crude oil has traded in the range of USD 60–140 per barrel of 159 L (Hingsamer and Jungmeier 2019). The crude oil price must rise to more than USD 1272 per barrel or algae production cost has to reduce to 0.2 USD per kg if microalgal oil has to become competitive. This difference in cost of production can only be solved by technological break-through. A critical

Table 1 Comparison of oil production between oil crops and microalgae

Species Oil crop	Galon oil/accre	Fat content (%)	SFA (%FA)	MUFA (%FA)	PUFA (%FA)	References
Soybean	46	20–25	15.9	24.8	59.3	Dorni et al. (2018), Tamagno et al. (2020)
Rapeseed	122	45–50	18.2	59.3	21.7	Hossain et al. (2019)
Peanut	109	45–55	19.3	53.8	27.0	Dorni et al. (2018), Wang et al. (2012)
Sunflower	98	35–50	11.4	25.9	62.7	Dorni et al. (2018), Ebrahimian et al. (2019)
Palm	23	46–50	44.8	43.6	11.5	Dorni et al. (2018), Ong et al. (2011)
Mustard	59	31	5.7	67.0	27.3	Dorni et al. (2018)
Cottonseed	33	28–45	28.2	19.7	52.2	Dorni et al. (2018), Liu et al. (2020)
Corn	18	4–5	16.6	33.7	49.7	Dorni et al. (2018)
Coconut	276	65–75	90.8	7.2	1.9	Dorni et al. (2018)
Safflower	80	32–40	9.2	14.0	76.8	Dorni et al. (2018), Ebrahimian et al. (2019)
Microalgae	Oil content (%)	Lipid productivity (mgL ⁻¹ day ⁻¹)				
<i>Phaeodactylum tricornutum</i>	18–57	44.80	20.2	25.3	49.8	Deshmukh et al. (2019)
<i>Schizochytrium sp.</i>	50–77	30–40	19.0	27.0	30.0	Sajjadi et al. (2018), Menegazzo and Fonseca (2019)
<i>Chlorella sp.</i>	28–53	42.1	26.3	70.9	12.4	Ferreira et al. (2019)
<i>Botryococcusbraunii</i>	25–75	5.50	11.7	63.1	25.2	Ferreira et al. (2019)
<i>Nannochloropsis p</i>	31–68	54.80	10.4	39.5	36.9	Deshmukh et al. (2019)
<i>Scenedesmus obliquus</i>	30–50	40–54	18.6	25.9	30.0	Ferreira et al. (2019)
<i>Pavlova salina</i>	12–30	34.8	32.0	5.0	41.0	Deshmukh et al. (2019)
<i>Chlamydomonas sp.</i>	21–27	10–20	63.9	14.1	6.7	Deshmukh et al. (2019)
<i>Phorphyridium cruentum</i>	9–14	34.80	31.1	5.0	40.8	Deshmukh et al. (2019)

understanding of the cultivation parameters will help to improve lipid and biomass production from microalgae and subsequent cost reduction methods are indeed necessary to make microalgae as a renewable and ecofriendly agent for fuels, chemicals and nutraceuticals applications.

The storage of oils and fatty acid depends upon the microalgae species and cultivation conditions including temperature, light availability, and medium constitution (Gifuni et al. 2019). Temperature, light, and nitrogen concentration are the cultivation factors that impart direct influence on the lipid and fatty acids

content in microalgae (Sibi et al. 2016). Among the nutrients, nitrogen concentration has been known to critically impact lipid accumulation such that nitrogen limitation increases the lipid content in microalgae (Viegas et al. 2015; Sulochana and Arumugam 2020). Nutrient stress hinders cell growth but some species still synthesize fatty acids which, under such conditions, are not utilized in membrane lipid formation and instead accumulate as triglycerides (Ratomski and Hawrot-Paw 2021). Oils from microalgae are excellent sources for bioenergy and biomaterials production. Depending upon the species and cultivation

Table 2 Summary of literature about the enhanced lipid production in microalgae (From 2017)

Year	References	Objective of the study	Main focus
2017	Chen et al. (2017)	Environmental stress and stress tolerance of microalgae for enhanced lipid production	Genetic approach and cultivation
2017	Chu (2017)	Strategies for enhanced lipid production in microalgae	Selection of microalgal strain, manipulation of cultivation conditions, wastewater treatment, co-culturing of microalgae
2017	Kim et al. (2018)	Application of microfluidics for the selection of highly efficient microalgal strain	Strain selection, microfluidics, mutation
2017	Zhu et al. (2016)	Molecular approach for lipid production in microalgae for biodiesel applications	Molecular synthesis of lipids and fatty acids, strategies for improving lipid production using molecular approach
2018	Sharma et al. (2018)	Application of microalgae for biofuel production	Genetic engineering for the manipulation of oil synthesis and biomass production, genome editing for strain improvement
2018	Yun et al. (2018)	Wastewater as a nutrient source for biomass production in open raceway ponds	Optimization of cultivation conditions
2018	Shin et al. (2018)	Strategies for enhancing economic viability of microalgae for lipid production	Species dependent factors, optimization of physiochemical properties, culture system improvement
2018	Sajjadi et al. (2018)	Enhancement of lipid and fatty acid production in microalgae	Taxonomic characterization of microalgae, environmental factors affecting lipid and fatty acid production in microalgae
2019	Nagappan et al. (2019)	Two stage microalgal cultivation systems for biofuel applications	Modification of cultivation conditions
2019	Piligaev et al. (2019)	Production and catalytic processing of microalgal lipids for biofuel applications	Strain selection, modification of media composition, use of wastewater for lipid production, biodiesel applications
2019	Menegazzo and Fonseca (2019)	Recovery of microalgal biomass and lipid extraction	Harvesting of microalgal biomass and lipid extraction methods
2020	Nguyen et al. (2020)	Molecular approach for lipid induction in microalgae	CRISPR-Cas9 technology
2020	Poh et al. (2020)	Application of stress conditions for lipid induction in microalgae	Induction of stress conditions
2020	Aziz et al. (2020)	Two stage cultivation for enhanced biomass and lipid production in microalgae	Culturing parameters and harvesting
2020	Bhatia et al. (2021)	Wastewater based microalgal biorefinery	Different types of wastewater, cultivation systems, harvesting techniques
2021	Lee et al. (2020)	Lipid extraction for microalgal biofuel production	Biomass production, harvesting, lipid extraction
2021	Orejuela-Escobar et al. (2021)	Biomaterials from microalgae for biorefineries	Bioactive compounds from microalgae, health benefits, industrial applications
2021	Udayan et al. (2021)	Industrial applications of microalgae	High value metabolites from microalgae, health benefits, industrial applications

conditions, microalgae oil production can be as high as 75% of their biomass. This could be further enhanced by inducing modifications in the microalgae life cycle,

cultivation parameters, and extraction and recovery methods (Chen et al. 2011; Abreu et al. 2012). However, the application of such modifications and

selection of appropriate recovery and extraction strategies can be a challenging task to be performed at an industrial scale because of the associated technical and economic obstructions. Microalgae with the highest lipid content, ranging from 10 to 67%, belong to *Chlorella*, *Dunaliella*, and *Scenedesmus* species (Islam et al. 2013; Nascimento et al. 2013).

Strategies for enhanced lipids production in microalgae

The growth and constitution of microalgae are substantially influenced by the environmental factors and composition of the medium in small-scale and large-scale culturing systems. The amount of nutrients added to microalgae culture, besides other parameters, decides the quantity and quality of biomass and metabolites obtained. At commercial scale production where the focus is on the synthesis of biomolecules, the amount of micro and macronutrients are varied to minimize the microalgae growth and increase the accumulation of metabolites of interest. Carbon, nitrogen, phosphorus along some micronutrients are the essential nutrients that assure minimum microalgae growth conditions (Chu 2017). In addition to this wastewater has also been used to increase the lipid productivity in microalgae with a combination of other methods like light, temperature, salinity, nutrient stress, etc. (Table 3).

In the case of lipids production, the production performance is mainly evaluated based on lipid content i.e. % lipid per biomass dry weight and lipid productivity is the amount of lipid produced per liter working volume per day. Accumulation of oils in microalgae cells is favored during conditions of stress which leads slower growth rate of cells and this aspect is particularly important when the focus is on lipid productivity (Aratboni et al. 2019). Production efficiency of most microalgae products including lipids is critically influenced by factors like light intensity and illumination pattern, temperature, and nitrogen source. Besides these factors, pH, salinity, and mineral salts are also major stimuli affecting lipid production (Zhu et al. 2016). In a study, the high lipid content of about 32.5% was obtained from biomass of *Chlorella* sp. cultivated with $MgSO_4$ (150 ppm), salinity (12.5%) and low light intensity, however, the lipid content noticeably decreased to 12.5% on lowering the concentration of salt and increasing salinity and light

intensity (Shekh et al. 2016). Carbon is a macronutrient and is essential for any cell cultivation because it forms the basic constitution of major biomolecules i.e. proteins, carbohydrates, lipids, and nucleic acids as well as other organic substances like vitamins. Nitrogen too has a major role in protein formation and its adequate presence in culture medium ensures sufficient concentrations of proteins, carotenoids, and chlorophyll (Table 3).

Nutrient stress

Nitrogen limitation is the most efficient stimuli for creating stress conditions in cells and therefore enhances lipid accumulation in microalgae (Suloचना and Arumugam 2020). A lipid content study by Hu and colleagues showed that under conditions of nitrogen limitation, the lipid content of microalgae varies from 10 to 20% (Hu et al. 2008). The same study reported that stress conditions in cyanobacteria caused less than 10% lipid content production in cells. A two-stage nitrogen limitation and a one-stage limitation method are the two main approaches utilized to encourage lipid aggregation in microalgae cells. In the two-stage strategy, the cells are initially provided with adequate nitrogen conditions for a definite time to incite cell growth. The second step is to collect the grown cells and expose them to nitrogen limited conditions, encouraging lipid accumulation. On the other hand, in one-stage approach a defined level is set for the initial nitrogen concentration such that the time after which nitrogen starvation occurs could be monitored. The culture cells initially grow and as the nitrogen content in the medium reduces over time, the microalgae culture will switch to the nitrogen limitation stage on its own.

Under normal growth conditions, microalgae produce high biomass but will not accumulate high-value metabolites like lipids (Piligaev et al. 2019). Under nutrient stress conditions, microalgae will change the metabolism of fatty acids towards the synthesis and accumulation of triacylglycerol's which consists of up to 80% of the total lipid content in the cell (Ratomski and Hawrot-Paw 2021). When nitrogen is limited in the cultivation medium, microalgae will accumulate large amounts of lipids but due to the lack of nitrogen, the cells will not produce sufficient amounts of proteins which results in lower biomass production. Chlorophyll content also changes under the nutrient

Table 3 Strategies for enhanced lipid productivity in microalgal biorefinery

Strategy	Microalgae used	Lipid productivity (g L ⁻¹ day ⁻¹)	References
Salinity stress	<i>Scenedesmus</i> sp.	0.607	Xia et al. (2013)
	<i>Nannochloropsis oculata</i>	0.324	Shokravi et al. (2020)
	<i>Dunaliella salina</i>	0.565	Abomohra et al. (2020)
Salinity stress + nitrogen starvation	<i>Tetraselmis</i> sp.	0.285	Park et al. (2018)
	<i>Chlorella vulgaris</i>	0.8	Mirizadeh et al. (2020)
Phytohormones	<i>Chlorella sorokiniana</i>	0.502	Guldhe et al. (2019)
Phytohormones + nitrogen starvation	<i>Chlorella sorokiniana</i>	0.69	Babu et al. (2017)
Combination of NaCl/CaCl ₂	<i>Chamydomonas reinhardtii</i>	0.109	Hang et al. (2020)
Salinity + nitrogenstarvation + wastewater	<i>Chlorella vulgaris</i>	0.080	Mirizadeh et al. (2020)
Municipalwastewater + seawater	<i>Phaeodactylum tricornutum</i>	0.054	Wang et al. (2019)
Two stage photoautotrophic and mixotrophic cultivation	<i>Chlorella vulgaris</i>	0.108	Shokravi et al. (2020)
Farm wastewater	<i>Chlorella sorokiniana</i>	0.083	Shokravi et al. (2020)
Wastewater + glycerol	<i>Chlorella vulgaris</i>	0.163	Ma et al. (2016)
Wastewater	<i>Chlorella vulgaris</i>	0.02	Ge et al. (2018)

stress conditions, which is the indicator of photosynthesis and photochemical processes during which the energy accumulated in ATP is generated (Rai et al. 2015). Therefore, the supply of appropriate doses of nutrients is indeed necessary for achieving high production of lipids as well as high efficiency of biomass production along with optimal culture conditions like temperature, pH, light, and mixing (Ratomski and Hawrot-Paw 2021). The supply of nutrients for the production of microalgal biomass and lipids can be associated with the purification of the aquatic environment and bioremediation.

Light intensity

The types of the light source, intensity, duration of illumination as well as wavelength range are known to have an acute impact on lipid productivity and biomass growth. In laboratory and closed cultivation systems, artificial lights are used to simulate natural conditions with the help of fluorescent lamps. For

increased lipid production, light distribution should be uniform within the photobioreactor. However, to an extent, practically this is unattainable because as more cells grow (or when biofilm forms) in the reactor, the cells on top layers mask those in lower layers thus reducing light penetration to them (Chia et al. 2018). Proper mixing of cells within the reactor is one way to overcome light penetration issues while keeping in mind that the mixing device does not cause cell shear. It has been reported that the content of neutral storage lipids increases while that of total polar lipids decreases when the culture is exposed to high light intensity (Seo et al. 2017). Increase light supplementation favors the formation of short-chain fatty acids, thus, demonstrating its role in deciding the level of fatty acid saturation (Islam et al. 2013). Application of light intensity of 700 $\mu\text{mol photons/m}^2/\text{s}$ to *Nannochloropsis* culture resulted in a lipid accumulation of 47% of dry weight (Pal et al. 2011). Accumulation of lipids in *Scenedesmus* sp. was found to be increased to 11-fold when the light intensity has been increased

to 400 $\mu\text{mol photons/m}^2/\text{s}$ from 250 $\mu\text{mol photons/m}^2/\text{s}$ (Liu et al. 2012). Providing light intensity of 1500 $\mu\text{mol photons/m}^2/\text{s}$ to *Ettlia* sp. has resulted in the highest lipid accumulation of 291.4 mg/L/day (Seo et al. 2017).

Temperature

When considering biomass growth, the temperature of microalgal cultures commonly ranges from 15 to 26 °C. Metabolism is impeded by high temperatures and carbon bioconversion is limited by 20–30% (Cheah et al. 2015), although some strains of *Chlorella* show contradicting behavior by reportedly tolerating temperatures as high as 42 °C. Culture temperatures below 15 °C are also considered unsuitable for photosynthesis and growth. High culture temperature has been reported to increase saturated fatty acids (Nadzir et al. 2018) and lipid content as is observed in cases of *Nannochloropsis salina* and *Ochromonas danica* although, cultures of *Chlorella sorokiniana* showed almost no variation in lipid contents with changing temperatures (Chowdury et al. 2020). Even though high temperatures increase saturated fatty acids, the

unsaturated fatty acids percentage reportedly decreases (Chowdury et al. 2020).

Carbon dioxide

Apart from light, temperature, and nitrogen concentration, the amount of CO_2 also significantly influences microalgae growth and lipid accumulation (Table 4). CO_2 dissolved in the medium is consumed in the presence of light but if the concentration of CO_2 in the medium gets high, it can inhibit the growth of microalgae (Muylaert et al. 2017). The growth inhibition can be attributed to the formation of carbonic acid in the media because of the increased dissolution of CO_2 , which results in the drop of media pH. As a result of pH reduction, the growth of some species gets slowed down and they require optimal pH between 7.9 and 8.3 for survival (Zhao and Su 2014). Strategic application of various stress stimuli in culture is used for enhancing the production of lipid or other molecules of interest, the strategies varying according to microalgae employed (Wang et al. 2014). When microalgal culture is supplemented with high concentrations of CO_2 a portion of the carbon can be used by the cells for participating in photosynthesis and the

Table 4 Effect of CO_2 on lipid accumulation in microalgae

Microalgae	CO_2 concentration	Lipid accumulation	References
<i>Chlorella</i> sp. BTA 9031	3% (v/v)	Accumulated 25% of lipid as a percentage of dry cell weight	Aratboni et al. (2019)
<i>Chlamydomonas</i> sp. JSC4	4% (v/v)	Generated maximum lipid content (65.3%) and productivity (169.1 mg/L/day)	Aratboni et al. (2019)
<i>Chlorococcum littorale</i>	5% (v/v)	Lipid content increased up to 34% wt	Mondal et al. (2016)
<i>Scenedesmus obliquus</i> CNW-N	The optimal CO_2 consumption rate was 1420.6 mg/L/day	The highest productivity of lipid (140.35 mg/L/day) is achieved	Ho et al. (2017)
<i>Synechocystis</i> sp. PCC6803	3% (v/v)	The total lipid content increased up to 14% of dry weight	Cuellar-Bermudez et al. (2015)
<i>Porosira glacialis</i>	20–25% levels of CO_2	The total lipid content increased from 8.91 to 10.57% in cell dry mass	Artamonova et al. (2017)
<i>Attheyolongicornis</i>	20–25% levels of CO_2	Did not show any significant increase in total lipid content	Artamonova et al. (2017)
<i>Nannochloropsis oculata</i>	3% (v/v)	Demonstrated high lipid content (53.2 wt.%)	Udayan et al. (2017)
<i>Scenedesmus</i> sp.	10% CO_2	Lipid productivity reached up to 20.65 mg/L/day	Yoo et al. 92010)
<i>Chlorella vulgaris</i>	30% CO_2	The highest lipid content 45.68% is obtained	Aratboni et al. (2019)

remaining carbon could be converted to carbonic acid. Production of carbonic acid will result in the acidification of the medium which could affect cell growth and metabolic pathways.

Extraction of lipids

The lipids present in microalgae cells are polar membrane lipids (phospholipids and glycolipids) and non-polar reserve lipids (triacylglycerols, glycerides, carotenoids, sterols) (Ryckebosch et al. 2014). High fatty acid content and presence of only glycerol as other constituent makes triacylglycerols a preferred choice for biodiesel production (Breuer et al. 2013). The amount of lipid obtained from a cell is influenced by the employed cell disruption and extraction method. The yield depends upon fatty acids solubility, solvent characteristics, and solvent potential to permeate the disrupted cell membrane. To minimize lipid loss and degradation and to maintain economic feasibility, it is important to select a method that is fast, sensitive and efficient (Koutra et al. 2020). After cell disruption and lipid release, the cell debris is

separated by techniques like filtration and centrifugation and the lipids are removed from the extraction solvent by techniques such as distillation and evaporation (Halim et al. 2012). Organic solvents or supercritical fluid are mostly used in lipid extraction techniques (Table 5) (Li et al. 2014; Baumgardt et al. 2016; Khoo et al. 2020). Direct transesterification into fatty acids is an emerging technique incorporating lipid extraction and transesterification in a single process (Fig. 2) (Torres et al. 2017). Lipid extraction from microalgae has been reported by the use of different physical and chemical processes like solvent extraction, ball mill, microwave, ultrasound, and results of each method varying with the microalgae species (Table 6) (Hidalgo et al. 2016; Lee et al. 2020).

Sequential extraction of microalgal lipids can also be considered as a new strategy for the efficient extraction of lipids (Lee et al. 2020). Such integration in unit operations can be used to achieve effective extraction of lipids with a minimum number of processing steps (Table 6). In this method, the solvents used for the extraction can penetrate the cell membrane and efficiently interact with the intracellular

Table 5 Solvent assisted lipid extraction: Pros and Cons in biorefinery approach

Type of solvent	Pros	Cons	References
Ionic liquids	High thermal stability, selectivity can be adjusted, nonflammable, can be used for wet biomass directly	High toxicity, high cost of chemicals, requires complex steps for the purification of lipids from ionic lipids, more studies are needed for technical viability in scale up process	Wahidin et al. (2018)
Supercritical fluid extraction	Low toxicity, rapid process, easy end product recovery, safe for thermal labile compounds, because of the low critical temperature, selectivity can be adjusted by varying the temperature and pressure, applicable for wet biomass directly without any drying process	High operating cost, high cost of equipment, polar co-solvent need additional polar solvent extraction	Patel et al. (2018)
Organic solvent	Simple method, rapid extraction, easy recovery of end product by distillation or heating	High flammability, high toxicity, need dried microalgal biomass which will make the process more complex	Callejón et al. (2020), Lakshmikandan et al. (2020)
Deep eutectic solvents (DES)	Biodegradable, low toxicity, low volatility, simple process, low material cost, properties can be changed by adjusting hydrogen bond donors and acceptors	Low decomposition temperature, further studies are needed to explore the potential of DES, technical viability at large scale needed to be explore	Sed et al. (2018)
Switchable solvents	Switchable polarity and hydrophobicity, efficient lipid extraction, easy recovery of end product, can be used for wet biomass directly	High water sensitivity, technical viability need to be explored for large scale applications	Al-Ameri and Al-Zuhair (2019)

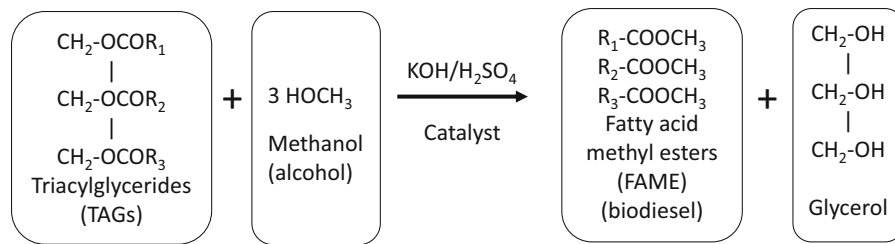


Fig. 2 Transesterification reaction for the conversion of TAG to fatty acid methyl esters

lipids, which helps in the easy extraction. Moreover, the use of a specific solvent, during cell disruption will help to prevent the degradation of targeted lipid and reduce the release of unwanted impurities in the crude extract which thereby simplifies the subsequent downstream process (Lee et al. 2020). This single-step sequential extraction can also be used for the processing of wet biomass, which excludes the incorporation of the drying process and can reduce the cost in microalgal biorefinery. Moreover, the production of lipids for nutraceutical applications from microalgae depends on various factors. Integration of lipid extraction methods will have many benefits in terms of energy and chemical savings. Therefore, more research is needed to obtain high efficiency in the processing of wet microalgal biomass.

Microalgae as a source of high-value metabolites and nutraceuticals

Microalgae have many reported health benefits and have been used as a medicine from 1500 BC (Moheimani and Borowitzka 2011). But more focus has been given recently to the use of microalgae as chemicals and nutraceuticals. High-value metabolites extracted from microalgae can act as nutritional supplements and can be used for food and feed applications. Consumption of such natural nutritional supplements can impart many health benefits to humans and animals. Microalgal biomass can replace fish meal and has more attracted to researchers as a potential renewable and ecofriendly source of nutrients in human diet and animal feed (Adarme-Vega et al. 2014; Udayan et al. 2017). These high-value bioactive metabolites have great potentials for the protection and therapy of many diseases (Udayan et al. 2018). Microalgae and macroalgae-derived

compounds show different health-promoting activities (Lauritano et al. 2016).

Microalgae serve as a potential source for treating different health conditions and deficiency diseases occurred in populations worldwide. Microalgae can be used for the therapy of cancer, diabetes, hypertension, autoimmune diseases, neurodegenerative diseases and they can also be used for boosting immunity and maintenance of the proper brain and heart health by consuming a sufficient quantity of microalgae per day (Barkia et al. 2019; Kiran and Venkata Mohan 2021; Udayan et al. 2021). Microalgae have also been used as a moisturizing agent and sun protectants in cosmetics (Udayan et al. 2021).

Microalgae that are used in nutraceuticals production include *Tetraselmis*, *Chlorella*, *Chaetoceros*, *Spirulina*, *Chlorella*, *Nannochloropsis*, *Cryptothecodinium*, *Dunaliella*, etc. due to their potential of producing bioactive compounds. Additionally, these edible microalgae are a rich source of major micro and macro nutrients (Table 7). Owing to the potential health benefits of microalgae, the global market for microalgal biomass and high-value metabolites are getting more attention recently. In the present scenario, more funding has been attracted to screen high-value metabolites from microalgae. The different bioactive metabolite was identified and extracted from microalgae and macroalgae, such as sulfated polysaccharides, carotenoids, beta carotene, omega 3 fatty acids, polyphenols, vitamins, and proteins.

It is very important to access the purity of nutraceuticals obtained from microalgae for nutritional purposes. Many of the microalgae are not known to produce any toxic products and their safety is well established (Udayan et al. 2021). In the recent years, quality of specific microalgal supplements are put in doubt because of the detection of cyanotoxins, and the coexistence of toxigenic microalgal species in the large scale cultivation systems. There are also

Table 6 Sequential extraction of microalgal lipids

Cell-disruption method	Species	Cell-disruption condition	Extraction method	Production efficiency (mg/g cell)	Reference
<i>Mechanical method</i>					
Shear force					
Bead milling	<i>Nannochloropsis oculata</i>	Bead milling under 1750 bar pressure	Chloroform/ Methanol	2.8 mg lipid/ g cell	Bharte and Desai (2021)
High-pressure		homogenization	<i>Chlorella saccharophila</i>		
Homogenization at 200 to 1000 bar	t-Butanol, Ammonium Sulfate	400 mg lipid/g cell	Mulchandani et al. (2015), Patel et al. (2018)		
	<i>Nannochloropsis oculata</i>	pH 6.0; homogenization at 125 MPa	Petroleum Ether	200 mg lipid/g cell	Shene et al. (2016)
Hydrodynamic cavitation	<i>Nannochloropsis salina</i>	Autoclave (5 kW); hydro cavitation (1.27 kW); ultrasonication (0.75 kW)	Hexane	191.5 mg lipid/g cell	Lee and Han (2015)
Wave energy					
Ultra-sonication	<i>Haematococcus pluvialis</i>	Ultra-sonication in acetone	Hexane, Isopropanol	19.8 mg lipid/g cell	Dong et al. (2016)
Microwave	<i>Chlorella</i> sp.	Time-20 min; MAS-II microwave reaction system for synthesis/ extraction	Chloroform/ Methanol	0.095 mg lipid/g cell	Cheng et al. (2015), Alhattab et al. (2019)
<i>Electric force</i>					
Pulsed electric field	<i>Chlorella vulgaris</i>	25 kV/cm–100 μ s; electrode distance 0.25 cm; electrode area 1.76 cm ² ; applied voltage 6.25 kV; applied current 55 A	Ethanol	0.4 mg pigment/g cell	Luengo et al. (2015), Sati et al. (2019)
<i>Heat</i>					
Steam	<i>Nannochloropsis oceanica</i>	0.1 s pressure release for 5 min; steam at set pressure 1.0 to 2.1 MPa	Hexane, Methanol, Ethanol, Isopropanol	763 mg lipid/g cell	Cheng et al. (2015), Onumaegbu et al. (2018)
Hydrothermal liquefaction	<i>Nannochloropsis oceanica</i>	Time-60 min; 89 bar; Temp-350 °C,	Dichloromethane	406 mg biocrude/g cell	Yoo et al. (2015), Patel et al. (2018)
<i>Chemical method</i>					
Acid	<i>Chlorella vulgaris</i>	1% H ₂ SO ₄ Temp-120 °C; Time-60 min	Hexane/ Methanol, Chloroform/ Methanol	381.6 mg lipid/g cell	Park et al. (2014), Sati et al. (2019)
Osmotic shock	<i>Chlamydomonas reinhardtii</i>	NaCl or sorbitol 60 g/L	Chloroform/ Methanol	33.4 mg lipid/g cell	Yoo et al. (2012)
Nanoparticle	<i>Chlorella vulgaris</i>	Time-96 h; NiO (< 50 nm), stirring at 80 rpm	Chloroform/ Methanol	900 mg lipid/g cell	Huang and Kim (2016)
Surfactant	<i>Chlorella vulgaris</i>	Temp-120 °C, Time-1 h; 0.2% sodium dodecyl benzene sulfonate; 2% H ₂ SO ₄	Hexane/ Methanol	843.9 mg lipid/g cell	Park et al. (2014), Alhattab et al. (2019)
<i>Biological method</i>					

Table 6 continued

Cell-disruption method	Species	Cell-disruption condition	Extraction method	Production efficiency (mg/g cell)	Reference
Enzymatic lysis	<i>Nannochloropsis oceanica</i>	Lipase; cellulose; protease	Hexane	31.92 mg lipid/g cell in g/L algal culture	Chen et al. (2016a, b), Bharte and Desai (2018)
Algicidal treatment	<i>Chlorella vulgaris</i>	Time-3 days; Co-culture with <i>Flammeovirgayaeyamensis</i>	Chloroform	218 mg lipid/g cell	Chen et al. (2013)

Table 7 High value metabolites from microalgae and its health benefits

High value metabolites	Microalgae	Health benefits	References
<i>Carotenoids</i>			
β carotene	<i>Dunaliella salina</i>	Food colorant, pro vitamin A, anti-oxidant, anti-inflammatory	Sathasivam et al. (2019), Novoveská et al. (2019)
Astaxanthin	<i>C. zofingiensis</i> , <i>H. pluvialis</i>	Pigmenter, anti-oxidant, anti-inflammatory	Novoveská et al. (2019), Dufosse (2008)
Zeaxanthin	<i>C. ellipsoidea</i> , <i>D. salina</i>	Food colorant, anti-oxidant	Raposo et al. (2015)
Canthaxanthin	<i>Chlorella</i> sp.	Food colorant, pigmenter in aquaculture and poultry	Udayan et al. (2017)
Lutein	<i>Scenedesmus</i> sp., <i>Muriellopsis</i> sp., <i>Chlorella</i> sp.	Anti-oxidant	Sathasivam et al. (2019)
Phycobilins	<i>Cyanobacteria</i> , <i>Rhodophyta</i>	Colorant in food and cosmetics, anti-oxidant	Kannaujiya et al. (2020)
<i>Fatty acids</i>			
Arachidonic acid (ARA)	<i>P. purpureum</i> , <i>P. cruentum</i> ,	Improves growth and development of neonates	(Udayan et al. 2017)
Eicosapentaenoic acid (EPA)	<i>Nannochloropsis</i> , <i>P. tricornutum</i> , <i>P. cruentum</i>	Cognition, heart health, protection against arthrosclerosis, anti-inflammatory	Kannaujiya et al. (2020), Weill et al. (2020)
Docosahexaenoic acid (DHA)	<i>C. cohnii</i> , <i>Schizochytrium</i> sp., <i>Ulkenia</i> sp.	Brain and eye health, cardiovascular benefits, nervous system development	Udayan et al. (2017), Kannaujiya et al. (2020), Weill et al. (2020)
Peptides	<i>C. pyrenoidosa</i> , <i>N. oculata</i> , <i>T. suecica</i> , <i>B. braunii</i>	Anti-hypertensive, anti-cancer, anti-oxidant, anti-inflammatory	Udayan et al. (2017)
Sulfated polysaccharides	<i>Porphyridium</i> sp., <i>P. tricornutum</i> , <i>C. pyrenoidosa</i> ,	Antiviral, immunomodulatory, antioxidant, anti-inflammatory	Udayan et al. (2017)
Phenolics	<i>B. braunii</i> , <i>C. vulgaris</i> , <i>Isochrysis</i> sp.	Anti-oxidant	Udayan et al. (2017)

reports on the presence of toxic heavy metals such as arsenic, lead, aluminum due to the improper location of the microalgal cultivation ponds which can lead to the toxicity in microalgal supplements (Udayan et al. 2021). These toxic heavy metals can cause nausea, diarrhea, abdominal pain etc. after consumption. Therefore, it is indeed necessary that the nutritional products should be of high purity and the formulations should be investigated, but still the information in this regard is very limited.

Based on the nature of the substrate, microalgae can be autotrophic, mixotrophic, or heterotrophic. In the autotrophic or phototrophic mode of growth, microalgae utilize CO₂, salts, and light energy its metabolism and primary growth. Microalgae can tolerate extreme stress conditions and even can be grown in non-potable and sea water, without using potable water resources and arable land. High-value metabolites extracted by microalgae are easily digestible also (Udayan et al. 2017).

Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) with 18 or more carbon are categorized as long-chain fatty acids categorized as ω-6 and ω-3 depending on the site of last unsaturation from the methyl end. Long-chain PUFAs found in fish and fish-derived oils are obtained

from the microalgae in aquatic regions that are consumed and digested by fishes and therefore microalgae are rich in PUFAs. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are important for brain function, memory, learning, and associated health benefits (Table 8). Several studies reported that omega 3 fatty acid consumption during pregnancy and breastfeeding will protect the infants from allergies (Table 8). Consumption of omega 3 fatty acids is also important for the maintenance of membrane fluidity and the development of the brain and retina. Improved problem-solving skills have been observed in infants whose mothers consumed omega 3 fatty acids in pregnant time (Judge et al. 2007). Children have also shown significantly higher vision and memory whose mothers have supplemented with EPA + DHA during pregnancy (Dunstan et al. 2008). It has been reported that omega 3 fatty acids can prevent the *cytokine storm* during COVID 19 pandemic and also helps to prevent difficulties in cardiovascular patients due to corona virus infection (Weill et al. 2020). EPA and DHA can lower triglycerides which help to lower the risk of developing *cytokine storms* (Mehta et al. 2020).

Many bacteria, fungi, microalgae, and plants are presently being explored as sources of EPA and DHA. In the aquatic ecosystem, microalgae are the initial EPA and DHA producers. They can grow in

Table 8 Potential beneficial effects of PUFAs and its physiological functions

Type of PUFA	Deficiency associated diseases	Physiological functions	
ALA	Cardiovascular diseases, cancer, coronary heart disease, cardiac arrhythmias, myocardial infarction	Maintenance of serum cholesterol level, blood pressure, decreased platelet aggregation, adhesion of monocytes to blood vessels, vascular dilation, inflammatory processes and immune functions, neural integrity, learning and visual abilities, development of retina and retinal functions	Udayan et al. (2017)
EPA and DHA	Coronary heart diseases, fatal myocardial infarction, inflammatory diseases, bipolar disorder, cognitive decline, aggression, age related maculopathy	Maintaining the production of Prostaglandin metabolites and Thromboxane A ₂ , inflammatory processes and immunity, increased activity of Rod photoreceptor, visual acuity and neural function, maintenance of serum cholesterol, development of brain and retina (infants)	Kannaujiya et al. (2020), Weill et al. (2020)
LA	Cardiovascular diseases	Maintenance of cholesterol, lipid levels, platelet aggregation, maintenance of blood pressure, inflammatory processes and immune responses	Udayan et al. (2017)

autotrophic, mixotrophic, and heterotrophic culture conditions naturally at a fast rate with the production of high long-chain ω -3 fatty acids. EPA and DHA are among the major commercially produced PUFAs and microalgae are known to accumulate these in large quantities. Considering the commercial interest regarding EPA and DHA production, strategies for the screening of high omega-3 fatty acid yielding microalgal strains, genetic manipulation, process optimization, and innovation of efficient cultivation systems have been explored. Ren and colleagues studied the effects of air sparging rates on omega 3 fatty acid production by *Schizochytrium* sp. Fed-batch fermentation in a bioreactor with 1500 L capacity was carried out with varying aeration rates. This approach resulted in high biomass, lipid production, and DHA content at 71 g/L, 35.75 g/L, and 48.95%, respectively with high DHA productivity (Ren et al. 2010). In another strategy, the production of DHA was investigated through a double stage culturing process which resulted in 154 mg DHA/L/ha by the use of *Aurantiochytrium limacinum* SR21 (Rosa et al. 2010). This study was based on the understanding that microalgae growth and accumulation of different value-added metabolites in cells require different nutritional conditions. Udayan et al. 2020 reported that the addition of Salicylic acid, a major stress phytohormone increased the EPA production in *N.oceanica* up to 1.5 fold compared to control (Udayan et al. 2020). The addition of Kinetin and IAA to *N.oceanica* CASA CC201 increased the percentage of omega 3 fatty acid fourfold and twofold in comparison with the control (Udayan et al. 2018).

Factors affecting the synthesis of omega 3 fatty acids

Microalgal metabolite induction can be achieved by changing the growth conditions or through the modification of nutrient composition. Increased accumulation of starch or lipids can be correlated with microalgal survivor mechanisms in response to different stress conditions such as temperature, pH, UV radiation, or nutrient limitation (Udayan et al. 2017). During stress conditions or nutrient limitation, microalgal growth will shift towards the accumulation of high-energy-rich compounds like lipids and unsaturated fatty acids (Udayan et al. 2020). Omega 3 fatty acid production can be increased by modification of

nutrient conditions and environmental stresses like light intensity, temperature, pH, and UV radiation.

Light intensity

Light intensity is considered as one of the most important parameters for microalgal growth and biomass production. Apart from growth aspects, sufficient light intensity is required for photosynthesis to generate ATP and NADPH for the production of metabolites necessary for growth (Niccolai et al. 2019). Light intensity increases the growth and biomass production of microalgae up to a specific point after that it leads to photo inhibition. Insufficient light intensity decreases the growth of microalgae. Stress conditions with low or high light intensity lead to a decrease in biomass production (Sun et al. 2018). Lipid and fatty acid production also depend on the level of light intensity (Sun et al. 2018). But the production of polyunsaturated fatty acids and lipids on different light intensities are species-specific. In some species, the low light intensity increased the production of EPA while high light intensities induced the production of DHA. PUFA levels were found to be increased at low light intensities to adapt to the low light stress by increasing PUFA synthesis (Sun et al. 2018).

Temperature

The temperature has a major role in growth, lipid accumulation, and fatty acid production in microalgae. During low temperatures, PUFA content was found to be increased to overcome the low-temperature stress and maintain the cell membrane fluidity. Low-temperature treatments resulted in higher EPA and PUFA production in microalgae, with a significant reduction in growth rate and biomass production (Aussant et al. 2018). However, there is a variation in temperature required for growth from species to species with no overall consistent correlation between temperature and the number of double bonds in fatty acids. Therefore, the highest overall production yields of PUFAs and omega 3 fatty acids cannot be achieved at lower temperatures.

Nutrient stress

Nitrogen, phosphorous, and sulfur are very essential nutrients for the growth of microalgal cells. Yang et al. (2018) reported that nitrogen deficiency and phosphorous deficiency will inhibit microalgal growth and cell division (Yang et al. 2018). Micronutrients like Cu and Zn which are required in small amounts have a strong impact on microalgal growth because they mediate and control many enzymatic activities in the cell (Yang et al. 2018). Lack of nitrogen source in the culture media affects microalgal growth and other biosynthetic pathways and the microalgal metabolism will shift towards lipid accumulation (Conde et al. 2021). Nitrogen depletion has resulted in the highest EPA productivity in *N. oceanica* IMET 1. Nitrogen starvation increased EPA and Triacyl glycerol (TAG) production in *N. gaditana* cultivated for 14 days (Janssen et al. 2019).

UV radiation

UV radiation causes damaging effects on different enzymatic and biochemical pathways including the fatty acid synthesis of microalgae (Udayan et al. 2017). Numerous studies have been conducted to analyze the effect of UV radiation in microalgae; however, the results were often contradictory. Exposure of microalgae to UV-B radiation increases the levels of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) with a significant decrease in polyunsaturated fatty acids (PUFAs) (Oliver et al. 2020). EPA content in *P. tricornutum* increased up to 19.84% when exposed to UV light (Liang et al. 2006). Stress induction due to exposure to UV radiation causes the formation of antioxidants in microalgae which could be the reason for enhanced PUFA synthesis.

Reactive oxygen species (ROS) and antioxidants

Alleviation of Reactive oxygen species (ROS) generation and lipid peroxidation can also cause an increase in omega 3 fatty acid production in microalgae. Zhang et al. (2018) reported that over expression of the superoxide dismutase (SOD) gene in *Schizochytrium sp.* has significantly increased the PUFA content by 32.9% (Zhang et al. 2018). The addition of antioxidants has also been used to increase omega 3 fatty acid

and PUFA synthesis in microalgae. For example, DHA productivity in *Schizochytrium sp.* and *Cryptocodinium cohnii* has increased after treatment with antioxidants ascorbic acid and sesamol (Liu et al. 2015).

Genetic engineering

Currently, the biosynthesis of fatty acids in microalgae is not extensively studied and most of the information has been acquired from studies on plant metabolism. Researchers have been made efforts to produce recombinant sources of omega 3 fatty acids in different systems, but the success rate was very less (Table 9). Recombinant Canola seeds were produced by over-expressing the $\Delta 15$ desaturase from *Brassica napus* to synthesize omega 3 fatty acids (Oliver et al. 2020; Sirohi et al. 2021a). In the future, it could be possible to increase the production of omega 3 fatty acids in microalgae by regulating the expression of enzymes involved in the fatty acid synthesis. Another possible mechanism to increase productivity can be the inhibition of PUFA degradation in peroxisomes during β -oxidation. However, the exact mechanism of PUFA synthesis in microalgae is still at the early stages of research.

Carotenoids

Most photosynthetic organisms contain carotenoids which are red, yellow, or orange pigments, insoluble in water. Microalgal carotenoids have gained increasing global attention due to their unique properties, especially health-associated benefits and new avenues for their production (Novoveská et al. 2019). They seem penetrating well in the global carotenoids market which was US\$ 1.24 billion in 2016 and was estimated to reach 1.53 billion in 2021 (Ambati et al. 2019). Astaxanthin, lutein, lycopene, and canthaxanthin are the major carotenoids present in the chloroplast of most algae and prevent photo-oxidative damage caused due to high-intensity light exposure. There are many methods like continuous, batch, fed-batch using different reactors such as closed systems (photobioreactors), open pond systems. Among all the technologies photobioreactors are the most economic method of carotenoid production from microalgae perhaps due to the cost of microalgae production. Table 10 represents the commercial methods and

Table 9 Genetic engineering strategies for enhanced lipid and fatty acid accumulation

Microalgae	Targeted genes	Strategy	Effect on lipid accumulation	References
<i>Chlamydomonas reinhardtii</i>	<i>ACS2</i>	Overexpression	2.4 Fold increase in Triacyl glycerol content under nitrogen starvation conditions	Rengel et al. (2018)
<i>Nannochloropsis oceanica</i>	<i>NoMCAT</i>	Overexpression	Neutral lipid content was increased to 31%	Chen et al. (2017)
<i>Schizochytrium</i> sp	<i>MAT</i>	Overexpression	PUFA content was increased to 24.5%	Li et al. (2018)
<i>Chlamydomonas reinhardtii</i>	<i>DtTE</i>	Heterologous expression	69% Increase in neutral lipids	Tan and Lee (2017)
<i>Scenedesmus quadricauda</i>	<i>ACC1</i>	Heterologous expression	1.6 Fold increase in lipid content	Gomma et al. (2015)
<i>Cyanidioschyzon merolae</i>	<i>Acyl-ACP-reductase</i>	Heterologous expression	Threefold increase in Triacyl glycerol content	Sumiya et al. (2015)

factors affecting the carotenoid production from microalgae.

Astaxanthin

Astaxanthin can be commercially produced using microalgae as a feedstock and its production from microalgae is economic and well established on a large scale (Novoveská et al. 2019). Astaxanthin is known for its strong antioxidant activity. Astaxanthin shows antioxidant property that is 10 times of β -carotene and above 500 times that of α -tocopherol (Dufosse 2008). Astaxanthin is used for pigmentation in the aquaculture industry which is approved by US FDA in 1987 and further in 1999 (Table 2). Being a strong antioxidant, astaxanthin is commercially used in food, nutraceuticals, and cosmetics and has also displayed positive effects on the therapy of inflammatory diseases, diseases of heart, liver, nervous system, cancers, metabolic syndrome, diabetes, and gastrointestinal diseases. Various microalgae such as *Botryococcus braunii*, *Chlamydocapsa* sp., *Chlamydocapsa nivalis*, *Chlorella zofingiensis*, *Chlorococcum* sp., *Haematococcus pluvialis* are capable to produce astaxanthin. Among these species, *H. pluvialis* is accepted as one of the best producers of astaxanthin because of its nature to accumulate enough amounts in harsh conditions (Oslan et al. 2021). Li et al (2020) produced astaxanthin using *H. pluvialis* by a cell transformation strategy and found that 38.02 mg/g of astaxanthin can accumulate which is 2.1 times higher

as compared to the control (Li et al. 2020). Molino and his group were able to accumulate 18.5 mg/g dry weight astaxanthin in *H. pluvialis* at a bench-scale reactor (Molino et al. 2018).

Lycopene

Lycopene is used as a natural colorant and food additive. Its industrial production is usually done through microbial sources, typically by *Escherichia coli*. However, there are other microorganisms such as yeast (e.g., *Candida utilis*) also which have been reported for their production. Genetic and metabolic engineering tools have widely been used to modulate microbial strains for improved production of desired flavonoids (Lee and Schmidt-Dannert 2002; Rathod et al. 2020).

Lycopene production has been evaluated by several microalgal cultures such as *Chlorella vulgaris*, *Nephroselmis* sp, *Dunaliella salina*, etc. (Coulombier et al. 2020; Mazzucchi et al. 2020; Mtaki et al. 2020). Coulombier et al. (2020) conducted the experiments under nitrogen starvation conditions in 10L photobioreactors for 28 days and observed increased lutein content (5.22–7.97 mg g⁻¹ DW) under nitrogen replete conditions. Similarly Mazzucchi et al. (2020), cultivated *D. salina* in Algem photobioreactors under red and blue light for 48 h for beta carotene production. Mtaki et al. (2020) used a low-cost media in which a strain of *Chlorella vulgaris* was cultivated and assessed for its antioxidant and free radical

Table 10 Factors affecting microalgal carotenoid production

Carotenoids	Microalgae source	Method of production	Factors	Processing conditions	Productivity	References
β -carotene	<i>Dunaliella salina</i>	Semi-continuous, outdoor, closed tubular photobioreactor (55 L)	Temperature; pH; Light intensity; Stirred rate	Temperature: 25 °C; pH: 7.5 \pm 0.5; Light intensity: 281 \pm 89 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Stirred rate: 38 $\text{cm s}^{-1} \text{s}^{-1}$	Total carotenoids: 102.5 \pm 33.1 $\text{mg m}^{-2} \text{d}^{-1}$ (β -carotene: 10% of biomass)	García-González et al. (2005)
β -carotene	<i>Dunaliella salina</i>	Continuous turbidostat, flat-panel (2.5 L)	Temperature; pH; Light intensity; Stirred rate	Temperature: 30 °C; pH: 7.5; Light intensity: 200–1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Stirred rate: 0.6 L min^{-1} (N_2)	β -Carotene: 13.5 $\text{mg L}^{-1} \text{d}^{-1}$	Kleinegris et al. (2011)
Lutein	<i>Muriellopsis</i> sp.	Continuous outdoor tubular photobioreactor	Temperature, pH, Light intensity, air flow	Temperature: 28 °C; pH: 7; Light intensity: continuous 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Air flow: 50–100 L h^{-1} (1%, v/v CO_2)	Lutein: 5.5 $\text{mg g}^{-1} \text{L}^{-1} \text{d}^{-1}$	Kleinegris et al. (2011)
Lutein	<i>Scenedesmus almeriensis</i>	Continuous	Temperature, pH, Light intensity, air flow	Temperature: 30 °C; pH: 8.0; Light Intensity: 1700 $\mu\text{E m}^{-2} \text{s}^{-1}$; Air flow: 0.5 (v/v) $\text{min}^{-2} \text{s}^{-1}$; Light/Dark cycle: solar cycle	Lutein: 4.9 $\text{mg L}^{-1} \text{d}^{-1}$	Sánchez et al. (2008)
Lutein	<i>Scenedesmus almeriensis</i>	Continuous outdoor, tubular	Temperature, Light intensity	Temperature: 35 °C; Light Intensity: 1900 $\mu\text{E m}^{-2} \text{s}^{-1}$	Lutein: 5.3 $\text{mg m}^{-2} \text{d}^{-1}$	Sánchez et al. (2008)
Lutein	<i>Chlorella protothecoides</i>	Batch	Temperature; pH	Temperature: 28 °C; pH: 6.5; Light Intensity: absence of light; Metabolic mode: heterotrophic	Lutein: 10 $\text{mg L}^{-1} \text{d}^{-1}$	Wei et al. (2008)
Astaxanthin	<i>C. zofingiensis</i>	Batch	Temperature, pH	Temperature: 30 °C; pH: 6.5; Light Intensity: darkness; Stirred rate: 130 rpm; Metabolic mode: heterotrophic	Astaxanthin: 10.3 mg L^{-1}	Ip and Chen (2005)
Astaxanthin	<i>Haematococcus pluvialis</i>	Continuous chemostat, tubular (50 L)	Light intensity	Light intensity: Day light cycle	Astaxanthin: 8.0 $\text{mg L}^{-1} \text{d}^{-1}$	García-Malea et al. (2009)

scavenging capacity. It was interesting to note that the spectrum of different product formations was quite related to the nature of the substrate. For example, when microalgae were cultivated in a synthetic medium, namely, Bold basal medium (BBM), the *Chlorella vulgaris* cells produced lycopene in the highest quantity, compared to a compost medium or aquaculture wastewater supplemented with NPK. Compost medium resulted in the highest production of phenolics while aquaculture medium produced higher flavanoid and β -carotene compared to other media. Therefore, wastewater could be employed for the cultivation of microalgae to produce carotenoids such as lycopene.

Considering the significance of microalgae in aquatic food chains and their capacity to fix carbon and to produce together another ecological role, microalgae-based biorefinery for the production of carotenoids and flavonoids seems very attractive with potential for commercial-scale exploitation. Also, considering that currently only about 3000 microalgal species have been studied and available in culture collections (from the about 44,000 known species) (Guiry 2012), there is huge potential and perspectives for their exploitation for the production of value-added products. What's more- out of all the known species, apparently only 40 are being commercially used for different applications (Day et al. 2012), showing huge opportunities.

Lutein

Lutein is a primary xanthophyll, a potent antioxidant and among the two carotenoids present in the retina of the human eye and lens (Sun et al. 2015). Lutein protects the DNA, proteins, and unsaturated lipids from oxidation and also provides a protective effect against cataracts. Numerous microalgal species, particularly *Chlorella*, can produce lutein and have been considered as an excellent alternative to plant-based lutein, which is season-dependent. Various strategies have been devised to make the process of lutein recovery from microalgae efficient. For instance, Chen et al. (2016a, b) reported that the use of high pressure followed by extraction with tetrahydrofuran could result in 99.5% lutein recovery from *C. sorokiniana* (Chen et al. 2016a, b). Molino et al. (2020) explored the effect of CO₂ concentration on the yield of lutein from *S. almeriensis* and established that

lutein production can be enhanced at high CO₂ concentrations (~ 3% v/v) due to higher chlorophyll accumulation (Molino et al. 2020). Ma et al. (2020) reported a two-stage bioprocess for enhanced lutein production from *Chlorella sorokiniana* (Ma et al. 2020). They investigated the effect of different temperatures, light intensities, and operating conditions on lutein production. They observed higher lutein production at 33 °C, low light intensity (150 $\mu\text{mol}/\text{m}^2/\text{s}$), and with gradient fed-batch conditions. Barathan et al. (2021) investigated the effect of beijeinck solution (BS), phosphate solution (PS), and hunter trace (HT) on lutein production using *Chlorella pyrenoidosa* (Barathan et al. 2021). It was found that an increase in HT concentration always increased lutein recovery.

Canthaxanthin

Canthaxanthin, also known as β , β -carotene-4,4'-dione, is a red/orange colored di-ketocarotenoid. Canthaxanthin shows numerous health-promoting attributes including anti-cancer, antioxidant, anti-inflammatory, and immunomodulatory activities (Rebello et al. 2020; Lafarga et al. 2021). It has been widely reported that Canthaxanthin could be better accumulated in microalgae under a stressful environment that could include osmotic stress, thermal stress, oxidative stress, nitrate starvation, and intense solar radiation among others (Lafarga et al. 2021). Canthaxanthin can be extracted from microalgae using different liquid chromatographic techniques specifically, high speed counter current chromatography. *Chromochloris zofingiensis* has been reported to be a valuable source of canthaxanthin along with astaxanthin and adonixanthin (Minyuk et al. 2020).

Phycobilins/phycobiliproteins

Phycobilins are linear tetrapyrroles similar in structure to chlorophylls. Phycobiliproteins are water-soluble substances formed as a result of covalent bonding between phycobilins and polypeptides. Depending upon their absorption spectra, phycobiliproteins are classified as high-energy phycoerythrins (PEs) or phycoerythrocyanins (PECs) (480–580 nm), intermediate-energy phycocyanins (PCs) (600–640 nm), and low-energy allophycocyanins (APCs) (620–660 nm). Phycobiliproteins are used as a potential fluorescent

labeling agent. This fluorescent behavior of phycobiliproteins can be utilized in different research activities (Udayan et al. 2017). Colored variants of phycobiliproteins are produced by many species of microalgae in large amounts. Differently colored phycobiliproteins are present in cyanobacteria which is beneficial to the health of humans (Kannaujiya et al. 2020). But the biosynthetic mechanism of phycobiliproteins in cyanobacteria is still unclear. Phycobiliproteins have a significant role in the food, nutraceutical, and pharmaceutical industries. It can be used as a coloring agent, fluorescence dye, anticancer, anti-inflammatory, antiviral, antibacterial, and antioxidative medicines (Wu et al. 2016). Currently, researchers are focused on developing technologies such as photodynamic therapy, disease diagnosis, solar cell, and disease treatment using phycobiliproteins (Wan et al. 2017). The potential of phycobiliproteins for commercial uses can create more economic growth for the microalgal industry as well as for human welfare.

Carbohydrates

Carbohydrates are mainly present in cell membranes and vacuoles of microalgae and they can also be secreted to the exterior of the cell as exopolysaccharides (Wells et al. 2017). Microalgae can accumulate higher content of carbohydrates under different cultivation conditions (Mayers et al. 2018). Microalgae such as *Chlorella*, *Chlamydomonas*, or *Scenedesmus* can accumulate high carbohydrate content of about

12–32% on a dry biomass basis (Uyaguari-Diaz et al. 2016; Mayers et al. 2018). Carbohydrates possess great significance as additives in the food industry and production of biofuels like biogas or ethanol (Markou et al. 2012; Lam et al. 2014). Nitrogen-depleted condition promotes carbohydrate accumulation (57%) in *Desmodesmus* sp. (Rizza et al. 2017). Microalgal ethanol yield is influenced by the type of species as well as process conditions and production higher than that by conventional feedstocks like corn or sugar beet has been reported. *Chlorococcum* sp. provided the highest reported ethanol yield with 0.52 g/g using acid hydrolysis which is much higher as compared to bioethanol fermentation from wheat yielding 0.23 g/g (Lee et al. 2011). The comparative analysis demonstrated the ethanol production potential of microalgae. For bioethanol production, carbohydrates are converted into fermentable sugars, and hydrolysis of carbohydrates of microalgal biomass through acid or alkaline pretreatment can be a decently effective method for doing so. There are many challenges associated with the development of different products using microalgal-derived carbohydrates as the main source (Fig. 3). The major difficulty occurs during the extraction and downstream processing but is easier in comparison with the plant-derived product because of the absence of lignification of the cell wall (Wells et al. 2017).

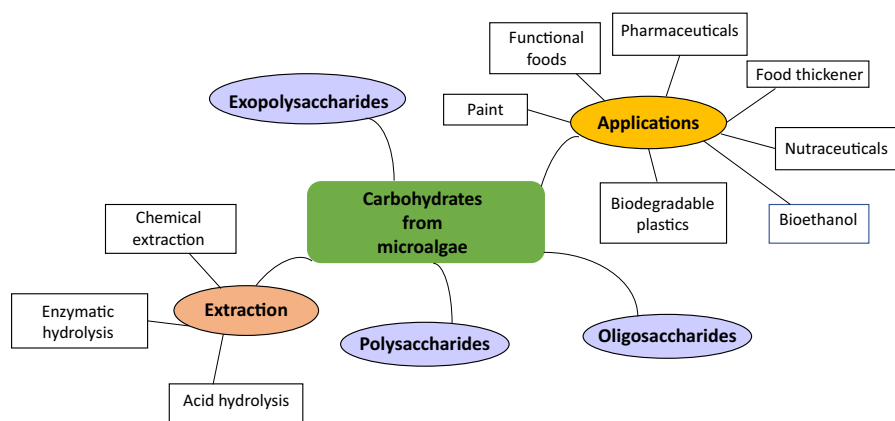


Fig. 3 Applications of microalgal carbohydrates and extraction methods

Proteins

A substantial market for proteins is present around the world due to their increasing demand in industries especially related to food and pharmaceuticals. More than 50% of microalgae biomass is constituted of proteins, creating an opportunity for biorefineries to use this high protein content into value-added products. The high protein content of microalgae such as *Spirulina* (60% protein on a dry basis) and *Chlorella vulgaris* (51–58% dry basis) are being commercialized presently as food supplements (Hariskos and Posten 2014; Trivedi et al. 2015). However, commercial expansion of the use of microalgae protein is often obstructed by the fact that proteins of many microalgae species are biologically indigestible, a drawback attributed to their hard cell walls (Ursu et al. 2014). Chemical hydrolysis methods are mainly used to recover proteins; digestion of biomass with sodium hydroxide at higher temperatures usually provides a recovery efficiency of around 81% (Asiedu et al. 2018). The recovery efficiency varies with microalgae species and also depends on degradation resistance. The selection of extraction and purification technique depends upon the desired form in which protein product is required i.e. concentrate, isolate, or hydrolysate, and accordingly, the type of hydrolyzing agent, pH, and time are also varied (Soto-Sierra et al. 2018). Sonication and beat milling are among other common disruption methods used for protein recovery with beat milling being preferred at commercial scale due to the lower energy input required.

Challenges and future perspectives

Given the current corona virus pandemic situation, more nutrient and health-promoting food are required to meet the dietary requirements of the global population. In this regard, microalgal biorefinery has emerged as a sustainable solution for the production of high-value metabolites and nutraceuticals. The major technological challenges associated with microalgal high-value metabolite production are low biomass and product yield and the high cost involved in the cultivation and downstream processing of biomass. Hence it is indeed necessary to develop strategies for an efficient biorefinery by improving the cultivation process and energy-efficient downstream processing

of metabolites. Economically feasible lipid and nutraceutical production can be achieved by integration of upstream and downstream processing to reduce the energy and cost associated with the process. Consequently, cost and energy analysis should also be performed to understand the economic feasibility of the developed microalgal biorefinery. The prospects should also involve the development of metabolically engineered strains that are capable of high biomass and secondary metabolite production. Metabolic engineering together with bioprocess strategies will be effective for developing genetically modified microalgal strains with high lipid and biomass production for food and nutraceutical applications.

Another major problem associated is downstream processing. Regardless of how much amount of biomass is produced it is very important to develop an integrated biorefinery that permits the extraction of a maximum number of products and by-products, together with the minimum amount of residual or waste generation and maximum return on the investment for downstream processing (Fabris et al. 2020). There are several approaches to achieve this goal. Industry 4.0 is a new manufacturing approach based on the principle of the machine to machine communication technology which is also referred to as “the Internet of Things” (IoT) which involves sensors, automation, and machine learning to develop a self-adapting manufacturing process for understanding the realtime changes (Kumar et al. 2019). This approach can be integrated into microalgal biorefinery for automation of cultivation and harvesting systems to decrease the cost of operation and also to monitor the microalgal growth and productivity in real-time (Whitmore et al. 2014). The basic idea of Industry 4.0 helps to build a simulation, which could be used to predict the future microalgal lipid and metabolites yield and to adjust parameters to reduce the waste generation. Phenomics can be considered as another approach that can make algal biorefinery efficient. But microalgal Phenomics is still in the early development stages, but it has an important role in the use of microalgae in agriculture for food security, nutraceuticals, pharmaceuticals, bioremediation, and carbon sequestration (Fabris et al. 2020). Synthetic biology approaches can be also used for the development of an efficient biorefinery system which includes the application of engineering principles for the rational design of living organisms (Fabris et al. 2020). Application of

synthetic biology to microalgae together with new genetic models with the advantages of a photosynthetic host to generate novel production strains can be used for future nutraceutical and pharmaceutical applications.

Conclusions

Overexploitation of fossil fuels is creating serious environmental problems and ecological imbalances. During the present pandemic conditions, finding an effective solution for the production of edible oils, other immune boosters, health-promoting substances is indeed necessary. To solve the drawbacks to develop an efficient microalgal biorefinery system, it is important to develop efficient process intensification strategies and downstream processing technologies. The present study addresses the strategies for enhanced production of lipids and their nutraceutical applications from microalgae. We also addressed the current challenges in largescale microalgal biorefinery and its solutions in future perspectives. Techno-economically feasible nutraceutical and pharmaceutical production from microalgae can be achieved by integrating the upstream and downstream processes which will help to balance the energy and production cost.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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