



Green technology for the industrial production of biofuels and bioproducts from microalgae: a review

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Abstract

Rise in human population and gradual decline in fossil fuels are increasing the demand for fuels and causing an upsurge in fuel prices and global warming. Concomitantly, consumers have raised their health awareness by taking various supplements, e.g. omega-3 fatty acids, thus calling for advanced nutraceuticals. Both issues are addressed by recent research on microalgae, which can be easily cultivated to produce lipidic biofuels and active drugs. Here, we reviewed the types and biosynthesis of microalgal lipids. We discuss genetic engineering and manipulation of cultivation conditions for enhancing lipid production. We present techniques for lipid extraction, with focus on green techniques. We also discuss the technical and economic challenges in manufacturing microalgae-based biofuels and other bioproducts at the industrial scale. Last, the market potentials and further research directions for future commercialization of microalgae lipids are discussed.

Keywords Microalgae · Lipid · Genetic engineering · Nutrient stress · Extraction

Abbreviations

Acetyl-CoA	Acetyl coenzyme A
ADP	Adenosine diphosphate
AMOP	Aquatic microbial oxygenic photoautotroph
ATP	Adenosine triphosphate
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeat
Fad	Fatty acid desaturase genes
RNAi	Ribonucleic acid interference
SDNs	Site-directed nucleases

TALENs	Transcription activator-like effector nuclease
ZFNs	Zinc-finger nucleases

Introduction

Over the last few decades, microalgae have been studied extensively due to their various benefits. These oil-amassing species have the potential for production of biofuel and other high-value products in industrial scale. The BP statistical review of world energy published in June 2018 highlighted that fossil fuels including crude oil, coal and natural gas are projected to exhaust in the next 50 years (Dudley 2018). This emphasizes the extensive exploitation of fossil fuels and thus elevation of the levels of anthropogenic greenhouse gases in atmosphere. The global greenhouse gas concentration had reached 400 ppm in 2016, which was 146% higher than that of pre-industrial era. To reduce or maintain this concentration, developed nations are expected to phase out all fossil fuel-derived emissions by 2050. The major contributors to the global greenhouse gas emissions are transportation fuels and electricity demands. The demand is expected to rise as the global population booms (Singh et al. 2020). Therefore, the need of the hour is an affordable, renewable and

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sustainable alternative to conventional fossil fuels (Kheshgi et al. 2000).

The catastrophic oil crisis in 1973 forced USA and other European countries to explore for alternate agriculture-based oil crops to meet their energy demands. This sparked the interest in the biofuel research and development. The first-generation biofuels were extracted for almost three decades. They were sourced from oil crops for biodiesel and fermentation of sugar and starch for bioethanol. The first-generation biofuels were inadequate to replace fossil fuels and lower the natural resources as the oil crops competed with food crops for resources (Schenk et al. 2008). The third-generation biofuels were introduced to amend the problems faced by the previous generations. Apart from consuming less space, the raw material used for these biofuels has the capability of reducing CO₂ from atmosphere (Wang et al. 2019), especially the biofuel that is extracted from algae and cyanobacteria. These organisms are also referred to as aquatic microbial oxygenic photoautotroph (AMOP). They are more sustainable and viable options for biofuel production.

Besides, omega-3 oil supplements market has been gaining notable momentum in recent years because the people nowadays are more concerned about their health by taking various supplements to boost their immune system and prolong the life. One of the supplements consumed by most people is omega-3 oil supplements. The main omega-3s are eicosapentaenoic acid, docosahexaenoic acid and alpha-linolenic acid. Omega-3 oil has many health benefits, including lowering the risk of cardiovascular diseases by increasing the levels of “good” high-density lipoprotein cholesterol, lowering triglyceride levels, reducing blood pressure and preventing the formation of plaques in the arteries. Most of the omega-3 fatty acids have been obtained from the fish but there some issues arise: unsuitability of fish oil for

vegetarian consumption; marine pollution; and increased pressure on global fish stock market.

Among them, algae, especially microalgae, promise a greater potential as the only renewable source to meet demands of transportation of fuel and nutraceutical products in the future. Microalgae are diverse, microscopic in size and can be categorized according to habitat and morphology (Aravind et al. 2020). They do not affect the supply of food and nourishment while reducing the anthropogenic carbon residues and tackling global warming concurrently (Faried et al. 2017). The microalgae cell oil content is in the range of 25–77% of dry harvested biomass weight. That is why they are also regarded as best alternative to first-generation biofuels and replace the fish oil to produce omega-3 supplements. Microalgae have a production capacity of 20,000–80,000 L of crude biooil per acre/ year (Table 1). This is 7–31 times higher than a conventional first-generation biofuel source like palm tree (Al Hattab et al. 2015).

The photoautotrophic strains require sunlight or artificial light in the suitable spectrum to produce algal biomass via photosynthesis. For large-scale commercial production of microalgae, utilization of sunlight as a natural and free resource is crucial (Koyande et al. 2019a). This is an important factor in cost reduction of final product (Mata et al. 2010). Apart from sunlight, phototrophic microalgae also require CO₂ as an organic source. Microalgae have ability to fix CO₂ in three different pathways, namely CO₂ from atmosphere or air, CO₂ from flue discharge or chimneys and CO₂ from soluble carbonates (Koyande et al. 2019b). In an open system, CO₂ is absorbed from atmosphere at which air contains about 400 ppm CO₂. The CO₂ tolerance of microalgae is observed as high as 150,000 ppm (Koyande et al. 2020). Therefore, it is safe to direct flue gas or discharge gas from power plants. This

Table 1 Characteristics of different oil-producing crops and microalgae, such as biomass oil content, land required as well as oil yield and biodiesel production per year

Type of source	Biomass oil content (wt %)	Yield (L oil/ha year)	Land required (m ² / kg biodiesel year)	Biodiesel production (kg/ha year)
Corn	44	172	66	152
Hemp	33	363	31	321
Soybean	18	636	18	562
Jatropha	28	741	15	656
Camelina	42	915	12	809
Rapeseed	41	974	12	862
Sunflower	40	1070	11	946
Castor	48	1307	9	1156
Palm oil	36	5366	2	4747
Microalgae (low lipid content)	30	58,700	0.2	51,927
Microalgae (medium lipid content)	50	97,800	0.1	86,515
Microalgae (high lipid content)	70	136,900	0.1	121,104

Adapted from Koyande et al. (2019b)

further reduces the global atmospheric CO₂ concentration. The microalgae can assimilate CO₂ from soluble carbonates like NaHCO₃ and Na₂CO₃ (Pahl et al. 2013). The microalgae require a medium to sustain. This medium should be rich with nutrients like nitrogen and phosphorus. The best source of soluble nitrogen is urea or ammonia; however, some species can absorb nitrogen from air with the help of NO_x gases present in the atmosphere. Ammonia can be sourced from wastewater streams like sewage (Brennan and Owende 2010; Van Der Hulst 2012).

Apart from uptake of natural resources, microalgae are able to secrete various biomolecules (Halim et al. 2011). Lipids from microalgae are of major interest for biofuel production such as bioethanol and biodiesel (Hajilary et al. 2019; Kanda et al. 2020; Srivastava et al. 2020). In this review paper, the biosynthesis and classification of microalgal lipids are discussed. Additionally, the manipulation of conditions of surrounding environment during microalgae cultivation for enhanced biomolecules production, particularly lipid is explained (Fig. 1). The extraction techniques for microalgal lipids are also reviewed. Finally, current market potential and challenges related to commercialization of microalgal lipids are highlighted.

General views of lipids in microalgae

Microalgae are the unicellular microorganisms which have been shown to produce lipid which can be utilized as biofuel, food ingredients, nutraceutical or pharmaceutical products. The lipid productivity in microalgae is high as compared to the traditional oil-bearing crops, for instance corn, soybean and palm tree, and can be up to 85% for oil-producing microalgae (Goh et al. 2019; Gupta et al. 2019). Due to the high lipid productivity, microalgae are able to synthesize 58,700 L oil per hectare (Chisti 2007), showing the potential as an alternative sources for biofuel or other lipid-based bioproducts. The lipid yield by the microalgae is affected by the strain species, surrounding environment and cultivation conditions (Chen et al. 2018). There are various microalgae species whose biomass contain high lipid contents, ranging from 20 to 80% of dry biomass or dry cell weight, such as *Botryococcus braunii* (25–80%), *Dunaliella tertiolecta* (35.6%), *Chlamydomonas reinhardtii* (21%), *Chlorella vulgaris* (14–22%), *Chlorella protothecoides* (57.9%), *Nannochloropsis* sp. (37–60%), *Isochrysis* sp. (25–33%), *Neochloris oleoabundans* (35–54%), *Nannochloris* sp. (30–50%), *Schizochytrium* sp. (50–77%) and *Cryptothecodium conhi*

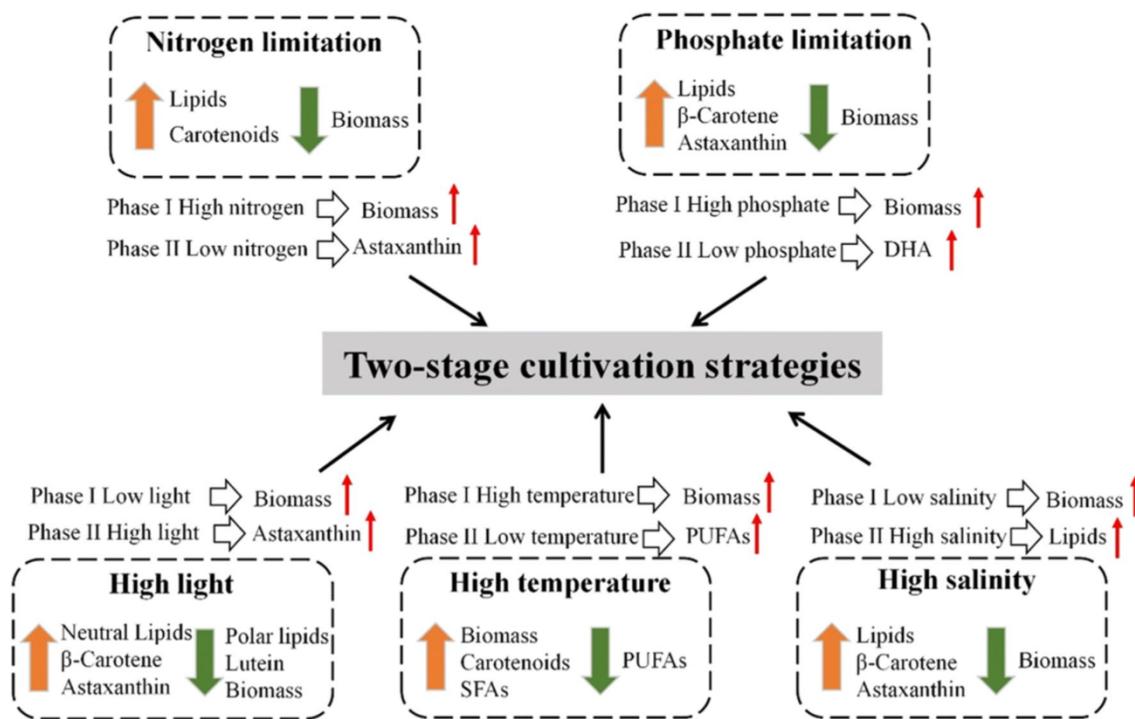


Fig. 1 Manipulation of surrounding cultivation conditions of microalgae culture, for example salinity, temperature, light intensity, nitrogen concentration and phosphate concentration to increase the production of lipids, biomass and other compounds in a two-stage process (reprinted with permission of microalgae for the production of lipid

and carotenoids: a review with focus on stress regulation and adaptation, biotechnology for biofuels from Sun et al. (2018). DHA: omega-3 docosahexaenoic acid, SFA: saturated fatty acids, PUFA: polyunsaturated fatty acids

(20%) (Chisti 2007; Ma et al. 2014; Mata et al. 2010; Wu et al. 2012). The microalgae lipids can be categorized into two major groups which are polar lipids, such as phospholipids or glycolipids, and nonpolar lipids, for example sterols or free fatty acids. Generally, the polar lipids constitute 41 to 92% of total lipids, whereas nonpolar lipids or neutral lipids comprise 5% to 51% of total lipids in the microalgae (Mimouni et al. 2018).

Microalgae lipids consisting of glycolipid and phospholipid as the common polar lipids are functioning as the bridge compounds between the nonpolar carotenoid and polar protein. Polar lipid or structural lipid assists in building up membrane structure commonly found in the cellular wall membrane from the microalgae biomass. Apart from maintaining the cell structure, polar lipids that consist of long-chain fatty acids can undergo a series of metabolic reactions to produce polyunsaturated fatty acids, for instance eicosapentaenoic acid and docosahexaenoic acid. Generally, the majority of polar lipids are found as phospholipids of which a glycerol acts as a backbone and phosphate as the polar molecule that is attached to fatty acids (Liang and Wen 2014). The interaction of the polar lipids with cellular membrane has influenced a series of the signal processes and enzymatic functions of the cells (Küllenberg et al. 2012). Phospholipids have beneficial potential for human body health, especially in the prevention of coronary heart disease and cell mutation. Phospholipids also can deliver fatty acids effectively to the membrane as the immune compounds. On the other hand, glycolipids are mostly found in eukaryotic cell. The molecules of glycolipids can perform cell surface recognition with amphipathic nature to bind physically to antibody, pathogen, toxins and intact cell. This assists the discovery of smaller molecules of the affinities and specification through thin-layer chromatographic and surface plasmon resonance (Lopez and Schnaar 2006). Glycolipids have two classes which are glycolipids and glycosphingolipids, where glycolipid is found in plants while glycosphingolipid is commonly found in animals.

Triglycerides, steryl esters and wax esters are among the group of neutral lipids which are without charge in the molecular structure. These neutral lipid particles have the hydrophobic cores, which are often surrounded by the phospholipid monolayer and mobilized by lipases with hydrolases product for membrane formation (Athenstaedt and Daum 2006). Neutral lipids, especially triglycerides, are normally used for the energy storage or as the components of lipid droplets (Mimouni et al. 2018) to ensure the adequate supply of metabolite energy and molecules of energy storage for the cell. Sterols or also known as phytosterols are also one of the important components of the microalgae that control the fluidity and permeability of membranes. Phytosterols produced by microalgae are shown to exhibit cholesterol-lowering, anti-inflammatory, anticancer, antioxidant,

antibacterial and antidiabetic activities (Luo et al. 2015). In addition, fatty acids are defined as the straight or branched long aliphatic chains of carboxylic acids, which can be in saturated or unsaturated forms, for example saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. Saturated fatty acids have an essential role in deciding the fuel properties. On the contrary, omega-3 or omega-6 fatty acids are the examples of polyunsaturated fatty acids and have been explored widely as the nutraceuticals due to beneficial health properties. Polyunsaturated fatty acids are essential to human health to maintain brain function and cell growth. Polyunsaturated fatty acids lower the low-density lipoprotein cholesterol, triglycerides and the build-up of plaque in the arteries and thus reduce the risk for cardiovascular disease. Polyunsaturated fatty acids also help to reduce the risk for diabetes by controlling the blood sugar. However, our bodies do not synthesize these essential fatty acids which must be got from the food or supplements. In short, the composition of lipids produced is varied among the microalgae species and different lipid classes have various health and fuel properties.

Lipid biosynthesis in microalgae

The biologically derived fossil fuels are heavily dependent on the lipid compounds for producing biofuels. The techniques via metabolic engineering to increase the lipid yield are the most common effective methods for enhancing lipid production. Before that, the understanding of the lipid metabolic pathway within the cell is crucial. The lipid metabolism is either broken down to generate energy or synthesized for a new lipid compound. Lipid metabolism is linked with carbohydrate metabolism, starting from simulation of glucose by kinases followed by conversion of glucose via enzyme into lipid pathway for most eukaryote cell. The formation of neutral lipids and triglyceride is led by the reaction from Calvin cycle through the conversion of diglyceride and diacylglycerol from the precursor of glycerol-3-phosphate (Tanguy et al. 2019). Diacylglycerol is formed from the diacylglycerol kinases and the acyltransferases, which is a type of transferase enzymes to convert acyl group including acetyl coenzyme A (acetyl-CoA) to lipids or vice versa. The acetyl-CoA converted into acetyl-CoA carboxylase subsequently through conversion of adenosine diphosphate to adenosine triphosphate for malonyl coenzyme A. The coenzyme is a biomolecule with a tiny cellular structure, which is specialized for fatty acids metabolite mechanism a step before triglycerides have been formed within the cell as in Fig. 2. The compound is also an important precursor of fatty acid biosynthesis in plants to produce lipids, triglycerides in the eukaryote cell (Hayashi and Satoh 2006).

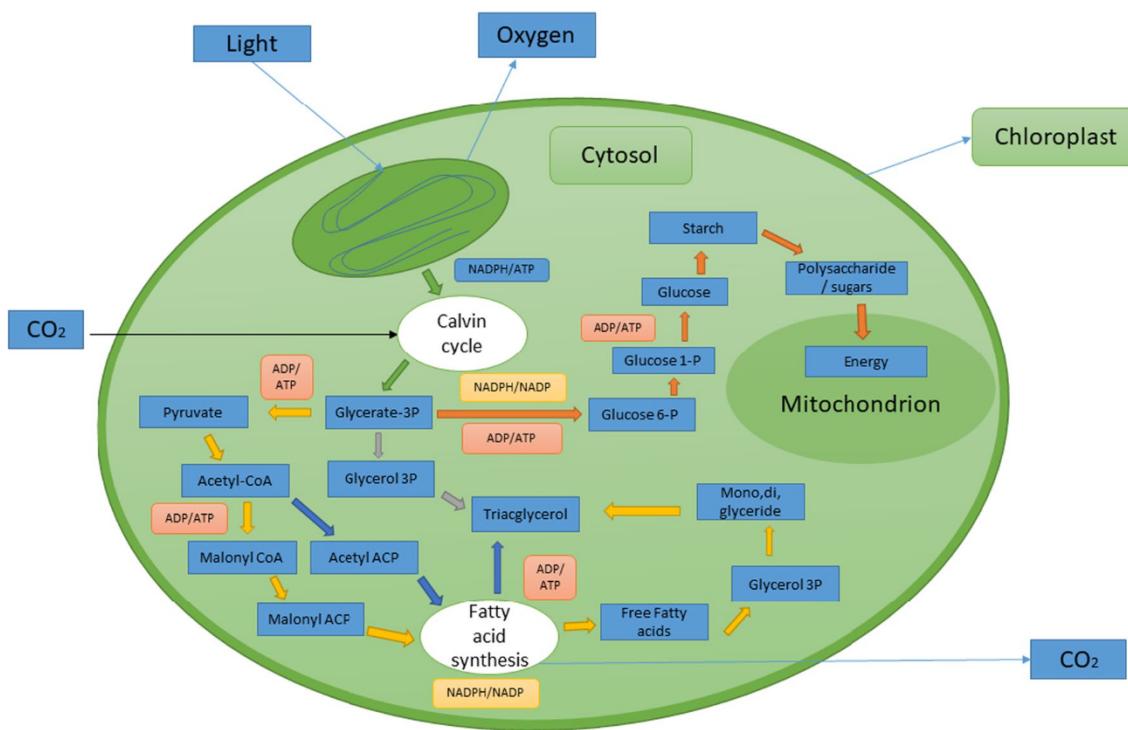


Fig. 2 ¹During photosynthesis, as the first step of the light-independent reactions for glycerate 3-phosphate (glycerate-3P) to produce pyruvate through catalyses by adenosine triphosphate (ATP)/adenosine diphosphate (ADP). ²Glycerol-3-phosphate is a component for glycerophospholipids frequently found in cell wall membrane as intermediate compounds to produce triacylglycerol. ³Glucose 6-phosphate is one of the metabolite pathways for glycolysis to form polysaccharide. Nicotinamide adenine dinucleotide phosphate (NADP⁺) is coenzyme in the anabolic reaction with NADP hydrogenase (NADPH) as reducing agent for fatty acid synthesis pathway

and formation of glucose-6-phosphate compounds. Abbreviation: Acetyl ACP: acetyl acetyltransferase; Acetyl-CoA: acetyl-coenzyme A; ADP: adenosine diphosphate; ATP: adenosine triphosphate; CO₂: carbon dioxide; Glucose 1-P: glucose 1-phosphate; Glucose 6-P: glucose 6-phosphate; Glycerate-3P: glycerate 3-phosphate; Glycerol 3P: glycerol 3-phosphate; Malonyl ACP: malonyl acetyltransferase; Malonyl CoA: malonyl coenzyme A; NADP: nicotinamide adenine dinucleotide phosphate; and NADPH: nicotinamide adenine dinucleotide phosphate [adapted and modified from Zhu et al. (2016)]

Besides, the discussion regarding the nitrogen deprivation which will increase during the accumulation of neutral lipids in microalgae can be learned from the model of diatom *Phaeodactylum tricornutum* (Yang et al. 2013). The nutrient deprivation is to create a stress condition for the microalgae cell to enhance the signal for Calvin cycle for the lipid production in the cytoplasm. Conditions such as light intensities, temperature and nutrient deficiency would have an important role in enhancing an optimum lipid production (Yew et al. 2019b). Most microalgae species' growth requires adequate light for the photosynthesis reaction in the cytoplasm. Therefore, the performances of the microalgae lipid production can be enhanced by adequate light intensity and duration known as photo-assimilates (Zhu 2015). The microalgae *Nannochloropsis sp.*, *Chlorella sorokiniana*, *C. Viscosa* and *C. Emersonii* had found high accumulation of lipids under 600 μmol photons s⁻¹ m⁻¹ to 700 μmol photons s⁻¹ m⁻¹ (Pal et al. 2011; Takeshita et al. 2014). Effect of temperature on growth and lipid production is closely related to high lipid of yield. The accumulation of high-lipid

compounds in the microalgae cell varies on different species, depending on the surrounding temperature. Microalgae *C. vulgaris* had found an optimum accumulation of lipid at 25 °C, while the lipid content decreases as the temperature reduced (Xin et al. 2011). Nutrient deficiency is one of the control methods for enhancing the lipid accumulation of the microalgae cell. The macronutrient such as nitrogen and phosphorous could lead to the altered macromolecules composition in the cell (Chew et al. 2018; Zhu et al. 2016). The reported microalgae cell under nutrient stress would enhance the lipid content, especially for triglyceride (Sibi et al. 2016).

The objective of recent genetic alteration at targeted gene selectively for bioproduct and biofuels is to achieve favourable yield. Genetic techniques included knockdown and knockout methods such as clustered regularly interspaced short palindromic repeat (CRISPR-Cas9), ribonucleic acid interference (RNAi), zinc-finger nucleases (ZFNs) and transcription activator-like effector nuclease (TALENs). The DNA sequencer data from microorganism may adapt

genome editing tools with site-directed nucleases (SDNs) for multilayer and complex genome to adjust the metabolite physiology, especially for increasing the lipid yield (Shan et al. 2020; Shockley 2020), particularly for CRISPR-Cas9 activity at a recent technique which is directed to the targeted sites specifically by protospacer region referred to short DNA fragment. CRISPR technique has relatively short duration, low cost, high accuracy and enhances the efficiency compared to other existing DNA editing tools (Doudna and Charpentier 2014). The application of these genome editing technologies can enhance the quality and quantity of the desired biocompounds from the microalgae biomass and have been practical tested. The targeted DNA microalgae gene undergoes the double-stranded breaks and nuclease-induced to lead for stimulation for a higher recombination efficiency. Gene editing for plants cell has a great improvement and revolutionized in CRISPR/Cas9 editing technique, which has improved plant's nutritional value, disease resistance and biomolecules production from the cell (Arora and Narula 2017). In summary, the understanding of the lipid biosynthesis pathway of the microalgae provides a pathway to induce or accumulate the lipid in the microalgae for the production of biofuel and other bioproducts through the employment of genetic modifications, addition of stimulators or repressors as well as change in the cultivation conditions.

Strategies to improve lipid production in microalgae

The quantity of lipid produced by the microalgae is varied among the species, but the yield can be modified or enhanced by modifying the lipid metabolism in different approaches. The cultivation elements such as supplied nutrients, growth phases and surrounding environment conditions, e.g. humidity, pH and temperature, can influence the microalgae biomass composition and lipid yield. The two main approaches that will be discussed in this review are the biochemical engineering and genetic engineering approaches to increase the storage and synthesis of the lipids inside the microalgae.

The biochemical engineering approach or the modification of cultivation condition involves the concept of nutrient stresses. The essential nutrients required during the microalgae growth, such as salinity, nitrogen, pH, temperature and light, are modified to promote the accumulation of lipid in microalgae as shown in Table 2. In microalgae, nitrogen is required to synthesize nucleic acids, amino acids and chlorophyll for the cell growth, photosynthesis and energy metabolism. Phosphorus regulates the protein function and cell energy metabolism. Sulphur is also important in the synthesis of amino acids, vitamins, cofactors and other cell wall constituents. The carbon supply also affects microalgae

biomass production as the microalgae utilize carbon dioxide for photosynthesis and produce storage metabolites. Lack of nitrogen, sulphur, phosphorus and carbon will exert stress on the microalgae and result in the accumulation or enhanced production of energy storage metabolites, such as carbohydrates and lipids (Ran et al. 2019). Besides, light intensity and wavelength are also essential in the microalgae growth, the production of biomass and the lipid. Hence, the change in these cultivation parameters can help to induce the lipid production of the microalgae.

Among the nutrient stress, nitrogen and phosphorous stresses are the most popular techniques to induce microalgae lipid production but they will result in low biomass production which adversely affects the lipid productivity (Singh et al. 2016). Besides, stress manipulation approach is time-consuming as the researchers need to figure out the optimized stress conditions to induce the lipid production and the mechanisms involved in lipid production when the microalgae respond to stress are unclear (Shahid et al. 2020).

Therefore, another easy and eco-friendly approach has been suggested to solve the conflicts between the microalgae's low biomass production and its associated lipid content, which is the use of exogenous phytohormones, hormones produced by the plants to elevate algal biomass accumulation, promote metabolite accumulation, which in turn increases the lipid biosynthesis in microalgae. Auxin, abscisic acid, brassinosteroids, cytokinin, ethylene, salicylic acid, gibberellic acid and jasmonic acid are the examples of the phytohormones. Song et al. (2020) combined the nutrient stress approach with phytohormones to induce the microalgae lipid production without affecting the biomass production. Parsaeimehr et al. (2017) used two classes of low-cost exogenous bioactive molecules, which were phytohormones and antioxidants classes to enhance the growth and lipid content, especially alpha-linolenic acid fraction of *Chlorella protothecoides*. Bhuyar et al. (2020) employed different types of phytohormones such as 2,4-dichloropheenoxyacetic acid, 6-benzylaminopurine, indole-3-butyric acid and gamborg's B-5 vitamins with various concentrations to enhance the growth and lipid production of *Chlorella* sp. Among these, indole-3-butyric acid showed a satisfactory result for the biomass and lipid oil production. Besides, to obtain high biomass and lipid yield in *Graesiella emersonii* NC-M1, Mandal et al. (2020) used a combination of phytohormones (indole acetic acid and kinetin) in nitrogen-limited medium. The lipid yield rose by 2.5- and 1.08-fold as compared to the nitrogen-limited condition and standard condition. Chen et al. (2020) study showed that the highest content of the lipids coproduced with astaxanthin was 64.5% with the productivity of 445.7 mg/L/d using indoleacetic acid as the stimulators in *Chromochloris zofingiensis*.

On the other hand, recently, there is a novel approach to induce the lipid production in microalgae, which is a genetic

Table 2 Biochemical engineering approaches used to induce production of microalgae lipids

Microalgae	Parameter change	Lipid content or lipid productivity	References
<i>Coccomyxa subellipsoidea</i>	Nitrogen	One-stage continuous nitrogen sufficiency (control group): 16.51% or 128.43 mg/L/day One-stage nitrogen deprivation (control group): 22.34% or 45.85 mg/L/day One-stage nitrogen limitation: 40.21% or 232.37 mg/L/day (highest lipid productivity with increased saturated and monounsaturated fatty acids) Two-stage batch nitrogen starvation: 50.5% or 186.05 mg/L/day (highest lipid content)	(Wang et al. 2017)
<i>Monoraphidium</i> sp. QLY-1	Nitrogen deficiency and phytohormones, strigolactone	The lipid content in optimized nitrogen deficiency condition treatment was 46.18% and 53.71% when combined with strigolactone, experienced 1.11- and 1.29-fold increases as compared to the control condition (41.57%)	Song et al. (2020)
<i>Chlorella sorokiniana</i>	Nitrogen, phosphorus and metal stress	The biomass productivity and lipid productivity were improved by 1.6 and 2.3 times, respectively. The highest lipid yield $77.03 \text{ mg L}^{-1} \text{ d}^{-1}$ was achieved with the nitrogen and phosphorus stress together with metal stress of high iron, magnesium and EDTA as well as low calcium. The expression of the <i>rbcL</i> gene and <i>accD</i> gene, which were the genes involved in photosynthesis and lipid biosynthesis respectively, also increased by 5.15- and 9.79-fold, respectively	Singh et al. (2017)
<i>Diacronema lutheri</i>	Phosphorus and nitrogen starvation	Under phosphorus and nitrogen starved conditions, the eicosapentaenoic acid proportion in neutral lipids increased by 2.1- and 2.9-fold on day 7, respectively	Huang et al. (2020)
<i>Micractinium reisseri</i>	Nitrogen starvation, salt (sodium chloride) stress	High concentration of sodium chloride enhanced the lipid yield by 206% and lipid content by 152% as compared to control. 75% nitrogen starvation increased the lipid content to 133% as compared to control	El-Sheekh et al. (2020)
<i>Monoraphidium</i> sp. QLY-1	Cadmium stress and γ -aminobutyric acid	2.5 mM γ -aminobutyric acid with cadmium stress achieved the highest lipid content (55.37%), 1.55-fold higher than control group and 1.12-fold greater than cadmium stress group	Zhao et al. (2020)
<i>Desmodesmus</i> sp.	Temperature, ultraviolet treatment, nitrogen	An elevation in lipid content (59 to 62% w/w) was observed in microalgae cultivated via ultraviolet and at low temperature (5°C). The alpha-linolenic acid fraction was increased around 39 to 42%. However, the nitrogen stress reduced alpha-linolenic acid fraction (18%) with the increase in lipid content of 39%	Sijil et al. (2019)
<i>Chlamydomonas</i> sp. JSC4	Temperature	The highest lipid productivity achieved was 177.31 mg/L/d at 35°C . However, the highest total fatty acid content was achieved at 20°C with 202.56 mg/g and increased amount of unsaturated fatty acid	Ma et al. (2020)

Table 2 (continued)

Microalgae	Parameter change	Lipid content or lipid productivity	References
<i>Cylindrotheca closterium</i>	Temperature	The high lipid content was achieved during the low-temperature stress at stationary phase. Eicosapentaenoic acid content increased significantly as compared to control, which was 1252 mg (low-temperature stationary phase) and 1339 mg (low-temperature exponential phase)	Almeyda et al. (2020)
<i>Chlorella vulgaris</i>	pH, concentration of carbon dioxide (CO_2) and light intensity	The lipid content and lipid productivity obtained in this study were 45.68% and 86.03 mg day $^{-1}$ L $^{-1}$, respectively, when conditions were pH 7.0, 2930 lx and 30% CO_2	Huang and Su (2014)
<i>Chlorella</i> sp.	Light	The combination of blue-red light had the maximum biomass production. In 20 cultivation days, LED of red light doubled the lipid dry weight from 30 to 60% in white light illumination	Severes et al. (2017)
<i>Chlorella</i> sp.	Light	The highest lipid content obtained was 0.0921 g L $^{-1}$ when microalgae were grown under red light as compared to 0.0761 g L $^{-1}$ (yellow light) and 0.0813 g L $^{-1}$ (white light or control light)	Rai et al. (2015)
<i>Chlorella vulgaris</i>	Light	The cool white light showed the highest lipid productivity (31.86 mg/L d $^{-1}$), as compared to blue light (25.56 mg/L d $^{-1}$) and red light (12.90 mg/L d $^{-1}$)	Wong et al. (2016)
<i>Chlorella</i> strain KS-MA2	Light	Blue light produced highest biomass, whereas total oil was better obtained under white- or red-light conditions. The production of specific fatty acids could be increased using different light colour during the cultivation. For example, the production of palmitic acid was highest in white light ($38.62 \pm 3.29\%$), blue light for stearic acid ($11.11 \pm 0.14\%$), red light for oleic acid ($30.50 \pm 0.14\%$ of biomass dry weight), whereas green and blue light for linoleic acid ($28.63 \pm 1.36\%$ and $26.00 \pm 0.81\%$, respectively)	Osman et al. (2018)

engineering or metabolic engineering approach. The simpler and unicellular structures of microalgae facilitate and simplify the genetic manipulation process. The recent discovery in microalgae omics together with the signalling and biosynthesis pathway has strengthened the understanding of genes regulation, metabolites change, proteins activity and interaction (Guarnieri and Pienkos 2015). Genetic manipulation has been adopted to develop a new genetically modified microalgae strain to induce lipid production in microalgae (Rastogi et al. 2018). Generally, the genetic engineering works by overexpressing the key enzymes or genes involved in fatty acid biosynthesis or inhibiting the lipid catabolism pathway such as starch synthesis and beta-oxidation.

For example, the microalga *P. tricornutum* was genetically modified to accelerate the production of docosahexaenoic acid via heterologous co-expression of an

acyl-CoA-dependent $\Delta 6$ -desaturase with the $\Delta 5$ -elongase gene from the picoalga *O. tauri* (Hamilton et al. 2014). Besides, the genetically modified *Nannochloropsis oceanica* overexpressed different fatty acid desaturase genes (fad), for example $\Delta 5$ and $\Delta 12$ fad encoding sequences, to increase the eicosapentaenoic acid production in microalgae (Poliner et al. 2018). Li et al. (2019) overexpressed a novel bZIP1 transcription factor NobZIP1N that is involved in lipid metabolism in *N. oceanica* to overproduce the lipid without affecting the microalgae growth. Shin et al. (2019) demonstrated the use of genome editing tool CRISPR-Cas9 to knock out phospholipase A₂ gene in *Chlamydomonas reinhardtii* to increase the overall lipid productivities by up to 64.25%. Chang et al. (2020c) generated the mutations of a target gene ADP-glucose pyrophosphorylase (AGP) in the *Tetraselmis* sp. using CRISPR-Cas9 RNP method to inhibit

carbohydrate synthesis and thus to increase the lipid production by 274% as compared to the wild type. Ryu et al. (2020) had produced a mutant Mut68 in *Nannochloropsis salina* through insertion mutation to increase the lipid production. After Mut68 was incubated for 8 days, the fatty acid methyl ester contents and productivity increased by 34% and 75%, respectively, when comparing to the wild type.

The open pond cultivation will expose the microalgae in outermost layers to excessive light energy and result in photoinhibition as well as causing the microalgae in inner layers to not receive sufficient sunlight, thus affecting the growth rate. Therefore, Perrine et al. (2012) modified the light harvesting antennae in *Chlamydomonas reinhardtii* to increase the energy capture and efficient transfer of photosynthetic electrons. Shin et al. (2016) synthesized *Chlorella vulgaris* mutants with reduced chlorophyll antenna size and resulted in the decreases in chlorophyll a and b contents. Next, Fu et al. (2017) introduced intracellular spectral recompositioning of light by modifying *P. tricornutum* to produce green fluorescent protein to convert the absorbed blue light into green light. As a result, photosynthetic efficiency increased by 50%. On the other hand, a nonvector approach has been applied to induce lipid production without the modification as shown in study by Szpyrka et al. (2020). The DNA levels of microalgae *Planktochlorella nurekis* were manipulated by co-treatment with cytochalasin B from plant fungal pathogen *Drechslera dematioidea* and colchicine from the plant *Colchicum arenarium*. The findings showed that the co-treatment improved total lipid content of about 10 to 60% as compared to the wild-type strains.

As compared to the modification of microalgae cultivation conditions, genetic modification is seeming as a popular, faster and more efficient way to induce the lipid accumulation. The approach of modification of cultivation conditions is time-consuming to find the optimum and ideal cultivation conditions, whereas genetic engineering involves the modification or alteration of genes that were involved in lipid production and the effects are overwhelming and satisfactory. Genetic engineering also can produce the microalgae species with faster maturation time and greater yield and thus reduce the harvesting time. Genetic engineering offers the advantages by “tailor-made” microalgae species to show desirable characteristics. Despite this, genetic engineering should be monitored and examined closely, so it does not become a possible ecological hazard. Therefore, the genetically modified microalgae may be prohibited for open cultivation system. The genetic engineering approach will create or introduce a new microalgae strain with the mutant genes and desired traits or known as genetically modified organism with the improved lipid productivity and biomass growth. The new microalgae mutant was able to reduce the production cost in the long term as it can be cultivated in the conditions same as the wild type without any adjustments

or modifications but with the high lipid productivity, it can provide sustainable ways for biofuel and bioproducts production. However, this raises the concern of the scientist about the unknown side effects or outcomes by these microalgae mutants. The release of new genetically engineered microalgae may cause an imbalance in the ecology and limit the genetic diversity by disrupting the natural process of gene flow (Patra and Andrew 2015). Besides, the compounds produced from the genetically modified microalgae may pose a human health risk and additional regulations are required for the introduction of genetically modified microalgae for human consumption. In brief, the lipid accumulation machinery in the microalgae can be triggered by different approaches, such as stress conditions, genetic modification or the addition of exogenous stimulators, which in turn produce lipid in a large-scale and sustainable way.

Techniques to extract, separate and purify lipids

The hard and rigid cell walls of microalgae cause the difficulties in extracting the lipids from the microalgae, which in turns restricts the use of microalgae biomass in commercial scale (Patel et al. 2020). Therefore, before the lipid extraction, the microalgae biomass needs to be pretreated to break down the cell wall and facilitate the release of lipids from the cell wall. There are a few microalgae cell disruption methods, including mechanical methods such as bead beating, grinding, pressing, osmotic shock, electroporation and homogenization; chemical methods using acid or base hydrolysis; physical methods using microwave, ultrasonication and thermal; and biological approaches via enzyme hydrolysis. Grinding is the simplest method that utilizes the mortar and pestle to break down the cell wall mechanically. Bead beating disrupts the cell walls of wet biomass using fine beads in high-speed spinning. Microwave and ultrasonication are the popularly used pretreatment steps prior lipid extraction. Microwaves utilize the electromagnetic wave with the frequency ranging from 300 MHz to 300 GHz, whereas ultrasonication applies ultrasonic waves to disrupt the cell walls in a short time. The chemical treatment method, for example, acid and base hydrolysis, degraded and lysed the cell wall, whereas the enzymatic method employed the enzyme application to degrade the cell walls, aiding the release of targeted compounds (Gonçalves et al. 2013; Sathish and Sims 2012). Among the pretreatment methods, ultrasonication and microwaves are well-known prior extraction steps due to high efficiency and short processing time.

The efficient and effective lipids extracted from microalgae will ensure the sustainable production of microalgae oil. The lipid extraction methods can be mechanical and solvent-based, thermal, membrane, electromagnetic or biological

methods (Chang et al. 2020b; Hassan et al. 2020; Khoo et al. 2020). The ideal characteristics of lipid extraction methods should be easiness to perform, environmental friendliness, short reaction time, cost-effectiveness and ability to be performed in industrial scale (Dvoretsky et al. 2016; Ghasemi Naghdi et al. 2016).

Mechanical approach, for example expeller press and bead beating, can be applied for microalgae lipid extraction. The advantages of mechanical approach are reduced contamination risk and less dependence on the microalgae strain. However, this approach consumes a large amount of energy as the machines require electricity to operate and the heat energy generated during the process may damage the end products, such as proteins and enzymes. Therefore, the installation of cooling system is needed to extract heat-sensitive or heat-intolerance products (Ranjith Kumar et al. 2015). Nevertheless, the thick microalgae cell wall will impede the release of lipids, adding the difficulties or affecting the effectiveness to extract the lipids using mechanical approach.

Hence, here come the solvent-based lipid extraction methods as the organic solvents shown to have the selectivity towards the selected lipid classes. The Folch method (Folch et al. 1957) and the Bligh and Dyer (1959) method are the oldest and most widely practised techniques for lipid extraction. These two methods used chloroform–methanol mixtures to separate the microalgae biomass into two layers. These methods are simple but time-consuming. Besides, chloroform and hexane are toxic chemicals and not suitable to extract lipids for human consumption. Matyash et al. (2008) had modified the Folch/Bligh and Dyer method by replacing the chemicals with methyl-tert-butyl (MTBE) ether, and the findings revealed good lipids recovery. Yew et al. (2019a) had created the hybrid liquid biphasic system by using hydrogen peroxide as a chemical pretreatment method to extract lipids from the *Chlorella sorokiniana* CY-1 strain at which the highest lipid recovery obtained was 50.9% at 30% H₂O₂. Kanda et al. (2020) employed the use of liquefied dimethyl ether to extract lipids from *Pleurochrysis carterae* and *Chaetoceros gracilis* without the pretreatment step because DME has high tendency towards oily substances and can be used to extract neutral and complex lipids efficiently (Bauer and Kruse 2019). The findings showed that the lipid yield extracted using liquefied DME was identical to the lipid yield extracted using Bligh–Dyer method but higher than that extracted using hexane Soxhlet extraction.

Another emerging solvent used to extract microalgae lipids is using switchable solvents, also known as switchable polarity solvents, which can change physical properties reversibly and abruptly. The switchable solvents can be interchanged between nonionic and ionic forms by bubbling CO₂ or N₂ (Jessop et al. 2005), facilitating the recovery of

the extracted material from the extracting solvent. One of the special characteristics about the switchable solvents is that it can be applied in a specific reaction phase in the multistep chemical cycle and can be removed completely before the next reaction phase (Khoo et al. 2020; Phan et al. 2008). CO₂ switchable solvents for extracting lipids from microalgae have been published in various literature studies (Boyd et al. 2012; Du et al. 2013, 2015, 2020; Samori et al. 2010, 2013). Du et al. (2020) showed the use of N-ethylbutyl amine as switchable solvent to extract lipids, and lipid recovery yields obtained can be up to 70%.

The use of solvent in the conventional method, such as hexane and chloroform, will raise the safety and toxicity concerns as well as the pollution issues to the environment. The lipids extracted using hexane, chloroform or other hazardous solvents are not suitable for human consumption. Besides, there is also difficulty faced during the withdrawal of the organic solvents from the extracted lipids. The process of removal of the organic solvents from the microalgae lipids may degrade or reduce the amount of lipids; this will affect the quality and quantity of lipids. Therefore, a solvent-free extraction method has been proposed for microalgae lipid extraction. This extraction method is simple, easy, nontoxic and reduces contamination. One of the examples of solvent-free extraction is using osmotic pressure and isotonic solution. Osmotic pressure can cause the microalgae cell damage through the change in the concentration of salt in aqueous media to produce two osmotic pressures, which are hyper-osmotic and hypo-osmotic pressures (Adam et al. 2012) as seen in *Botryococcus* sp., *Chlorella vulgaris*, *Chlamydomonas reinhardtii* and *Scenedesmus* sp. (Lee et al. 2010; Yoo et al. 2012).

Recently, there is an increased concern of using “green” solution or reducing the application of harmful and toxic solvents to extract lipids from microalgae and ensure the sustainability of the extraction process, for example supercritical fluid extraction or the use of ionic liquids (Akalin et al. 2017; Gonçalves et al. 2013; Gude and Martinez-Guerra 2018; Peng et al. 2020). Nowadays, the use of ionic liquid for microalgae lipid extraction is becoming popular because ionic liquid is known as “green” designer solvent. Ionic liquids are defined as the organic salts, composed of organic cations coupled with inorganic anions and can be held at moderate temperatures, for example at 0 to 140 °C, in liquid state (Wahidin et al. 2016). The benefits of using ionic liquids are that ionic liquids are nonvolatility, good thermal stability, flexibility and recyclability (Chisti 2007). To et al. (2017) demonstrated the use of low-cost choline amino acid-based ionic liquids to extract lipids from *Chlorella vulgaris* and *Spirulina platensis* at which the lipid yields can reach 51%. Krishnan et al. (2020) had demonstrated the feasibility of ionic liquids which were 1-Octyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide, [Omim][NTf] and

1-Octyl-3-methylimidazolium acetate, [Omim] [OAc] to extract lipid from *Chlorella vulgaris* at which the most of the extracted lipids were polar lipids. Tang et al. (2020b) synthesized three switchable and recyclable ionic liquids (C6DIPA-Im, C6DIPA-Pyr and C6DIPA-Triz) to extract and separate lipid from *Chlorella vulgaris*. The lipid contents extracted using C6DIPA-Im, C6DIPA-Pyr and C6DIPA-Triz were 123.8 mg/g, 115.4 mg/g and 109.1 mg/g, respectively, higher than the Bligh and Dyer method (104.8 mg/g). C6DIPA-Im showed the highest lipid extraction efficiencies, and the initial lipid extraction efficiency still maintained more than 83.6% after recycled for five times.

Supercritical fluid extraction is another green technique that can replace the conventional lipid extraction methods. Supercritical fluid extraction has high selectivity towards the target compounds, fast reaction time and the absence of toxic organic solvents (Aresta et al. 2005; Lardon et al. 2009). Supercritical fluid extraction mostly uses carbon dioxide at high pressure and optimized temperature to extract the high-value and specific compounds of interest, especially lipids from the natural materials, including microalgae. This method is known as supercritical carbon dioxide extraction. Vardar et al. (2016) used supercritical fluid extraction to extract lipids from *Schizochytrium* sp. S31, a known docosahexaenoic acid producing marine algae. The results showed that at optimized conditions, the lipid yield (30.2%) and concentration of docosahexaenoic acid (520.1 mg/L) increased. Zinnai et al. (2016) demonstrated that the oil extracted from *Schizochytrium* sp. through supercritical fluid extraction had high ratios of omega-3/omega-6 and docosahexaenoic acid/eicosapentaenoic acid and the reaction time of extraction process was shorter as compared to that of n-hexane. Sanzo et al. 2018 extracted fatty acids from *H. pluvialis* using supercritical carbon dioxide extraction, and the results showed the highest lipid recovery achieved was 93.2% at which polyunsaturated fatty acids had the highest recovery rate. Molino et al. (2019b) extracted eicosapentaenoic acid from *Nannochloropsis gaditana* by using supercritical carbon dioxide extraction with the mechanical approach as the pretreatment step. The maximum eicosapentaenoic acid yield achieved was 11.50 mg/g with the recovery rate of 27.4%. Another study by Molino et al. (2019a) demonstrated that supercritical carbon dioxide extraction resulted in the highest extraction of saturated fatty acids (1.81 to 4.93 mg/g) from *Dunaliella salina*, at which palmitic acid was the most abundant saturated fatty acids extracted and more than 80%. Among the polyunsaturated fatty acids, cis-9-octadecenoic acid (32% of FAs) and linoleic acid- ω -6 (9.17–24.22%) were the highest obtained fatty acids. Leone et al. (2019) used supercritical carbon dioxide extraction for lipid extraction from *Nannochloropsis* sp. The maximum amount of eicosapentaenoic acid and docosahexaenoic acid extracted was 5.69 mg/g and 0.12 mg/g, respectively. The study by

de Melo et al. (2020) showed that the obtained docosahexaenoic acid content from microalgae *Aurantiochytrium* sp. was 3.5- and 7.9-fold higher as compared to the fish oil and other microalgae using supercritical fluid-carbon dioxide extraction. Besides carbon dioxide, other solvents could be used for supercritical fluid extraction. Han et al. (2020) used supercritical methanol as the solvent together with the oxide catalysts for liquefaction of wet *Chlorella vulgaris* for biodiesel production. By comparing the biooil yield, the methanothermal liquefaction was higher (54.5%) than hydrothermal for noncatalytic reaction (30.5%). Despite the high lipid recovery rate, supercritical fluid extraction techniques had some disadvantages such as high equipment cost, not suitable for samples of high moisture and low extraction yield of carotenoids. In addition, during the extraction process, the high temperatures could degrade the molecules, influencing the recovery of selected molecules, especially fatty acids (Molino et al. 2020).

The conventional lipid extraction method is solvent-based extraction using hexane or chloroform and involves the intensive use of hazardous solvents that may harm the consumers and environments. Recently, lipid extraction using supercritical fluid extraction or ionic liquid is gaining attention and popularity to manufacture food, nutraceutical and pharmaceutical products. This is because the solvents used are considered as “green” solvents, offer greater yield and are safe for consumption and also reduce the risk of contamination of the end products. Next, carbon dioxide is the most used supercritical fluid. Carbon dioxide exhibits the advantageous properties, such as nonpoisonous, nonflammable, noncorrosive, chemically inert and environmentally friendly. Besides, it is easier to remove carbon dioxide from the extracts after the extraction process (Couto et al. 2010). Therefore, supercritical fluid extraction has now gradually replacing the convention methods to extract value-added products from the microalgae biomass, especially docosahexaenoic acid and eicosapentaenoic acid from microalgal lipids.

In addition, pressurized liquid extraction is another method to extract lipid from microalgae. This method can decrease the amount of solvent needed and give high extraction rate under optimized conditions (Wang et al. 2016). Chang et al. (2020a) utilized the pressurized CO₂ combined with solvents, for instance methanol and castor oil, to extract lipids from *Chlorella vulgaris*, followed by in situ transesterification to produce biodiesel. This method will result in rapid cell wall disruption and achieve high lipid extraction rate in a relatively short time, which was less than 30 min. The maximum fatty acid methyl ester yields from microalgae were achieved at 61.4%. This proposed method showed to have high water tolerance, fast reaction time and requirement of low operating pressure, so the energy used and chemical waste can be reduced. Next, Kamaruddin et al. (2020) had

developed a new ozone-rich microbubble technique for lipid extraction from *Dunaliella salina*. The lipid extraction with methanol was performed at room temperature in the bioreactor with direct ozonation (8 mg L^{-1}). During the extraction with increase in temperature to 60°C and introduction of smaller bubbles, the concentration of palmitic acid and stearic acid was increased significantly to around 88.9% and 150%, respectively. The energy required to extract lipid with ozone was less as compared to the conventional methods, such as mechanical and solvent-based extraction methods, which often used more than 90% of the energy.

To conclude, the pretreatment steps and lipid extraction method should be simple, time-efficient, cost-efficient and environmentally friendly. The extracted lipids need undergoing further purification steps before the lipids are being processed as automotive fuels or for human consumption.

Market potential of algal-based lipids

The main interests of microalgae lipids are the production of biofuel or biodiesel in transportation sector or the essential fatty acids that exhibit beneficial effects to human health. First thing to be discussed is the use of microalgae oil as nutraceuticals, human supplements or as food ingredients. Among these, omega-3 and omega-6 fatty acids, for instance, alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid are consumed by the humans as the health supplements as they are essential to our health and our body cannot synthesize them. Subsequently, there is an increasing market demand for omega-3 and omega-6 oils. We can obtain these omega oils from the marine fish or the vegetable crops. For example, docosahexaenoic acid and eicosapentaenoic acid are present mainly in the fish, whereas alpha-linolenic acid is mainly found in plant oils, such as soybean, flaxseed and canola oils. However, the main issues raised from the consumption of the marine omega-3 oil are not suitable for vegetarian consumption and give unpleasant odour to the consumers. Besides, it will reduce the global fish stock market and the presence of toxins in fish will harm the consumers. The literature studies showed that certain oil-producing microalgae are capable of synthesizing polyunsaturated fatty acids at which polyunsaturated fatty acids content of the microalgae is almost the same as the marine fish, vegetables, fungi and bacteria. Therefore, microalgae are suggested to become a promising alternative source for these valuable resources and have been studied broadly as the alternative to eicosapentaenoic acid- and docosahexaenoic acid-rich fish oil in the future human consumption.

The market prices of the microalgae-based docosahexaenoic acid and eicosapentaenoic acid are approximately USD 50/kg and USD 650/kg, respectively (Koller et al. 2014), showing that microalgae oil has the business potential to

satisfy the customers' needs. *Spirulina* sp. and *Chlorella* sp. are the examples of commercialized oil-producing microalgae. There are a few companies that perform the nutraceuticals research using microalgae oils, especially omega-3 fatty acids, such as Algae Tec, Parry nutraceuticals, Fermentalg, Algaetech International, AlgaeBiotech, Algae to Omega Holdings, Alltech Algae, etc. (Katiyar and Arora 2020). Although the research on the lipids on microalgae in different aspects is still ongoing, there are microalgae oil-based products existing in the market. Pure One™ is one of the supplements sold in the market as the capsules form of algal oil that is rich in eicosapentaenoic acid and docosahexaenoic acid. Source oil Algae Omega 3, extracted from *Schizochytrium* sp., is another approved supplement by Food and Drug Administration. Next, Nordic Naturals Algae Omega is rich in Omega 3, also made from marine microalgae and suitable for vegetarian. The microalgae such as *Cryptecodinium* sp. and *Schizochytrium* sp. are used as poultry feed, particularly chicken to produce omega eggs. In Europe, the purified polyunsaturated fatty acids from the microalgae are added as one of the ingredients of infant grade milk (Pulz and Gross 2004). Besides used as supplements, microalgae oil can also be used as cooking oil. One of the examples is Thrive® Algae Oil cooking oil made from algae that contains low level of saturated fatty acids, as compared to olive oil and avocado oil and high level of monounsaturated fatty acids.

Currently, the fuel consumption increases due to the increasing number of vehicles and the energy use, causing the fuel requirement to raise by 1.4% till 2040. Therefore, there is a need to find the alternative and sustainable fuel sources (Rajesh Banu et al. 2020) due to depletion of natural fuel sources. Therefore, microalgae have been promoted as the renewable third-generation biofuel feedstock, replacing the first-generation biofuels from food crops and second-generation lignocellulosic biomass. Through a series of reactions, microalgae oil can be processed into biofuel, bioethanol, biogas, biomethane and biohydrogen (Chen et al. 2015). This can be seen through the emerging of the company to do the microalgae-based biofuel research with the hope to solve the energy crisis and replace the fossil fuels in the future, such as Algae Tec, EcoFuel Laboratories, IBV Biotech, Algaetech International, AlgaeBiotech, Sapphire Energy, etc. (Katiyar and Arora 2020). For the biofuel productivity, Solix Biofuels is able to produce 3000 gallons of biofuel, whereas Seambiotic is able to produce 100 to 200 gallons of biofuel (Rajesh Banu et al. 2020). Moreover, ExxonMobil and Synthetic Genomics cooperated to develop renewable and lower-emission algae oil by genetic modification of the algae strain. The oil content was found to be more than doubled and could lead to the generation of 10,000 barrels of algae biofuel per day by 2025 (Tang et al. 2020a). Till now, the production of microalgae biofuel is still facing

difficulties and on the way to commercialization due to lack of advancements in downstream processing techniques. In short, microalgae-based lipids can be a possible feedstock for functional foods and fuels in view of the fact that microalgae are one of the most sustainable and environmentally friendly ways to generate high-energy-density biofuels and bioproducts.

Challenges

The economics of microalgae lipid production rely on the expenditures related to the upstream and downstream processing (Katiyar and Arora 2020). The microalgae-based lipid production in large scale needs many cost consideration in the aspects of capital cost, labour, land use, power and water (Show et al. 2015). Till now, the production of microalgae oil in industrial scale is limited because the production cost is high, hindering the pathway to industrialization and commercialization. To give an example, the production cost of microalgae biofuels is approximately 1.50–2.50 US\$/L, higher than the edible crops (0.45–0.55 US\$/L) and lignocellulosic biomass (0.80–1.20 US\$/L) (Chen et al. 2015). In the USA, the petroleum cost (1.10 USD/L) is cheaper than the estimated cost of 10,000 tonnes of microalgae biomass with 30% lipid (Rajesh Banu et al. 2020). The harvesting of microalgae biomass contributes 30% of the output cost (Kim et al. 2013) and has become a burden to the manufacturer. Brentner et al. (2011) also reported that the required power to harvest microalgae biomass was highest by ultrasonication (110%), followed by centrifugation (90%), press filtration (79%) and supercritical CO₂ (66%). Therefore, an energy-efficient lipid extraction technique is needed to ensure the microalgae lipid production is economically sustainable. The effectiveness of pretreatment and lipid extraction methods will affect the production of biomass and lipid yield by the microalgae (Gonçalves et al. 2013). Currently, the production of microalgae lipid does not meet the economic aspects yet due to high operating costs.

Furthermore, the other challenge in microalgae lipid production in large scale lies in the selection of suitable microalgae strain with high lipid productivity (Peng et al. 2020). Currently, the main issue faced by the researchers is the microalgae species with high biomass production have low oil productivity or low biomass production with high oil productivity, hindering the extraction of large amount of lipid from the microalgae. For example, lipid content of *Botryococcus braunii* biomass was high but impeded by low growth rate. On the other hand, *Scenedesmus* sp. and *Chlorella* sp. with the relatively high growth rate had the low lipid content (Deng et al. 2009; Nayak et al. 2019; Show et al. 2017). Moreover, to produce microalgae lipid in industrial scale, a cost-effective cultivation system is needed. Spruijt et al.

(2015) had compared the expenses needed for closed system, photobioreactor and open pond system which operated on the standard scale of 1000 m². The finding revealed the operating cost for photobioreactor was less than open pond system because less labour was needed and capital costs were low. However, the electricity needed for photobioreactor was higher than open pond systems. Similarly, in 2011, Norsker et al. (2011) showed the biomass production cost for tubular photobioreactor and open pond system was 4.15 €/kg and 4.95 €/kg dry weight cost, respectively. Therefore, it can be inferred that photobioreactor is more cost-effective as compared to open pond system but the production costs for both microalgae cultivation system are still not economical. The major disadvantages of photobioreactor are the difficulty to scale up as compared to open pond systems and requiring high energy input despite the high biomass productivity.

In addition, during the lipid extraction from microalgae biomass, the liposoluble intracellular pigments such as chlorophyll will be coextracted into crude oil and thus affect oil quality and limit its large-scale application. Chlorophyll is insoluble in water but readily dissolved in organic solvents including ethanol, acetone and chloroform (Hosikian et al. 2010), and these solvents are used commonly for lipid extraction. To date, there are few studies regarding the removal of chlorophyll from microalgae oil and the developed methods were inefficient. Chen et al. (2012) used bleaching method to remove the chlorophyll and carotenoids in *Scenedesmus* sp., and this method successfully reduced the pigments contents. However, this method was not useful for large-scale application and the bleaching agents used were not suitable to produce food, nutraceuticals and pharmaceutical products. Li et al. (2016) removed the chlorophyll in microalgae, *Scenedesmus* sp. biomass via saponification reaction, followed by the oil extraction. The end product was transparent orange *Scenedesmus* sp. oil with 96% removal rate of chlorophyll in biomass. However, the pigments composition of extracted oil became carotenoids based and the concern of the feasibility of this method with other microalgae species and other cultivation stages was raised. Hence, an effective chlorophyll removal technique is needed to produce the high-quality and pure microalgae oil.

Therefore, a new biotechnological approach with the successful implementation is needed to replace existing methods. Bioengineering strategies and genetic engineering to improve the cultivation, harvesting and extraction process will likely be the main targets to be emphasized (Fu et al. 2019). The further understanding of lipid metabolism and the genes involved through the “omics” approach is needed to increase or overproduce the lipid from microalgae. The emerging of highly advanced techniques such as transcriptomics, proteomics and next-generation sequencing combined with metabolic engineering will enhance our knowledge in accelerating the microalgae lipid production (Saini

et al. 2020). Another aspect to be discussed is the discovery and exploring of more new food-grade or green solvents with high selectivity to extract bioactive compounds, especially lipids from microalgae. Furthermore, the use of nanomaterials can be applied in enhancing the efficiency of extraction of lipids from microalgae biomass, for example use of nanomaterials as catalyst in the pretreatment of biomass (Singh 2017; Srivastava et al. 2017). To incorporate the microalgae lipid into the foods or nutraceutical products, more research, especially preclinical and clinical trials, is needed before distributing them to the market for human consumption. The safety of the consumers is the main priority. In addition, biorefinery can be seen as a method to decrease the production cost of microalgae lipids in large scale. Beside lipids, microalgae also produce other value-added compounds, such as pigments, antioxidants, carbohydrates and proteins (Chew et al. 2017). Through the biorefinery process, the recovery of lipids and other beneficial biocompounds could be enhanced via process integration. The lipid extracted from the biomass can be utilised as feedstock for biofuel or nutraceuticals production. The recovered carbohydrates, pigments and proteins can be utilized as animal feeds or ingredients in food and personal care products (Rajesh Banu et al. 2020). Unfortunately, an appropriate and detailed characterization of the microalgae biomass composition is inadequate. Future research should aim to characterize the composition in each microalgae strain to produce different co-products along with biofuel and lipid-based bioproducts in microalgae.

To produce the microalgae lipid in large scale, a cost-effective cultivation system with high biomass production is required. Pandey et al. (2020) had created a novel two-step cultivation system to facilitate the lipid production and wastewater treatment by *Chlorella pyrenoidosa* simultaneously. The maximum biomass yield and lipid productivity achieved were 2.44 g L⁻¹ and 77.41 mg L⁻¹ day⁻¹, respectively. The estimated production cost and profit earned by this system were \$79.03 per barrel and \$9.59 million year⁻¹. In short, the commercialization of the biofuel and microalgae lipid-based products cannot be realized yet because of the high production expenses. To produce the microalgae lipid in large, economical and sustainable scale, the microalgae with high lipid productivity together with high biomass content must be identified. An economically feasible cultivation system is to culture the microalgae in large scale. Lastly, an efficient, environmental-friendly and inexpensive harvesting and lipid extraction methods should be employed.

Conclusion

In summary, the successful commercialization or industrialization of the microalgal oil as biofuel or bioproducts depends on the ideal microalgal growth, efficient lipid extraction and

purification as well as conversion of the microalgae oil to biofuel, biodiesel or food as health supplement. At present, there is none of the downstream processing methods, including extraction methods which have been confirmed as a suitable, effective and cost-effective method for microalgae oil production. The effectiveness of lipid extraction methods, lipid recovery rate or purity and the production cost are still the important downstream processing struggles in the microalgae industry. Till now, the research work on microalgae in different aspects is not satisfactory, optimal and limited to a laboratory scale. Therefore, the challenges must be tackled comprehensively for successful industrialization, sustainable and economical production of microalgae biofuels and bioproducts. This goal can be accomplished by continuous research and development efforts by academia–industry linkages.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

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