Environmental Chemistry Letters (2020) 18:285–297
https://doi.org/10.1007/s10311-019-00939-0

REVIEW

Biofuel production from microalgae: a review

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Received: 21 August 2019 / Accepted: 9 October 2019 / Published online: 18 October 2019

Abstract
The shortage of fossil fuels is actually a major economic issue in the context of increasing energy demand. Renewable energies are thus gaining in importance. For instance, microalgae-based fuels are viewed as an alternative. Microalgae are microscopic unicellular plants, which typically grow in marine and freshwater environments. They are fast growing, have high photosynthetic efficiency, and have relatively small land requirement and water consumption in comparison with conventional land crops biofuels. Nonetheless, selling biofuels is still limited by high cost. Here, we review biofuel production from microalgae, including cultivation, harvesting, drying, extraction and conversion of microalgal lipids. Cost issues may be solved by upstream and downstream measures: (1) upstream measures, in which highly productive strains are obtained by strain selection, genetic engineering and metabolic engineering, and (2) downstream measures, in which high biofuels yields are obtained by enhancing the cellular lipid content and by advanced conversion of microalgal biomass to biofuels. Maximum biomass and high biofuels production can be achieved by two-stage culture strategies, which is a win–win approach because it solves the conflicts between cell growth and biomass accumulation.

Keywords Microalgae · Biofuel · Commercialization · Challenges · Upstream and downstream measures

Introduction

Fossil fuel has played an important role in the industrial era; however, they are non-renewable and less environment-friendly. The statistics from Energy Information Administration (EIA) estimated that the reserves of worldwide fossil fuels will be exhausted in less than 50 years (Plant and Floorspace 2010). By comparison, biofuel is renewable and sustainable alternative energy, which is regarded as the replacement of fossil fuels. It is predicted by mobility model results for the 2 °C scenario (2DS) that the biofuels' market share will account for 37% of total transportation fuel consumption by 2060, and this number indicates a great space for the production and market requirement as compared with the current share of 4% published in 2016 (Oh et al. 2018). In general, biofuels can be classified as three generations such as the first generation, second generation, and the third generation, corresponding to the feedstocks of food sources sugarcane, wheat, corn, soybean, potato, or sugar beet (Bringezu et al. 2007), lignocellulosic biomass and agricultural wastes (Eisentraut 2010), and algae (i.e., macroalgae and microalgae) (Demirbas 2011), respectively.

Among the three types of feedstocks, microalgae-based biofuel is considered as the promising one and has attracted more and more attention in the past years, owing to the characteristics such as fast growing rate, high-efficiency photosynthesis, and high lipid content for some species (Peng et al. 2016a). Generally speaking, microalgae are capable of converting nutrients either in medium or wastewater into biomass and high-value cellular constituents (Bouabidi et al. 2018; Wang et al. 2010). For example, lipid, protein, carbohydrate, pigments, antioxidant biomolecules derived from microalgae can be applied in other sections of CO_2 mitigation, wastewater treatment, cosmetics, dyes, pharmaceuticals, functional food, food additives, feeds for animals and aquaculture, fertilizers, and others (Peng et al. 2013; Ramasamy et al. 2015; Ummalyma et al. 2017; Yun et al. 2014). Under such circumstance, microalgae-based biofuels have received more and

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more concerns; however, the commercialization remains to be expanded because of barriers such as the shortage of feedstock, the blending wall of conventional biofuels, and the most fundamental cause like high production cost. This is derived from various challenges in the processes from the selection of strain, mass-cultivation, harvesting, drying, extraction, conversion, and others (Ho et al. 2014; Rastogi et al. 2018; Bwapwa et al. 2017; Seo et al. 2018).

Although some major challenges for the production of microalgae-based biofuels were presented in previous studies, most are limited to partial sections of the production process. This paper presents a brief review on the progress and challenges of microalgae-based biofuels in the development of upstream and downstream technologies. The main objective of this study was to introduce the progress of technologies involved in upstream such as strain selection, genetic engineering, metabolic engineering for obtaining high concentration of microalgae, and the progress of downstream measures in large-scale cultivation, process control, harvesting, dewatering, extraction, and conversion to biofuels products.

Microalgal species for producing biofuels

Autotrophic microalgae consume carbon dioxide and produce carbohydrates or hydrogen, protein, and lipids, which can be further utilized as feedstock of biofuels, including biodiesel, biogas, biohydrogen, bioethanol, butanol, bio-oil, char, and even power (Cheng et al. 2015; Francavilla et al. 2015; Im et al. 2015; Khandelwal et al. 2018; Kim et al. 2017; Lee et al. 2015; Song et al. 2017; Yun et al. 2016; Zhao et al. 2014) (Fig. 1). Some typical microalgal species can reach high concentrations of targeted biofuels; for example, *Chlorella protothecoides* is considered as a perfect feedstock of biodiesel since they can accumulate 55% of lipid when cultivated heterotrophically under nitrogen limitation (Xu et al. 2006) (Table 1). Microalgae-based biodiesel is generally obtained through two steps of

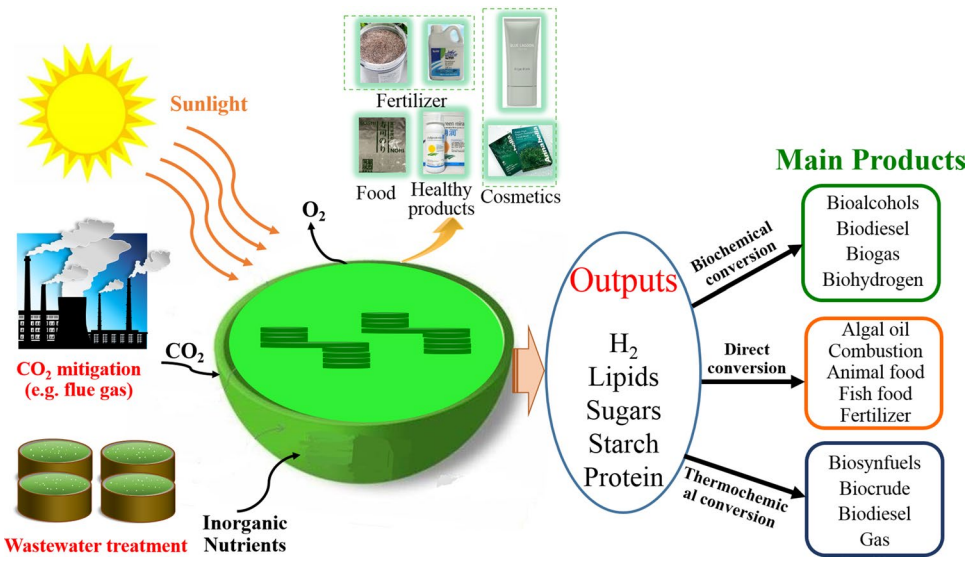

Fig. 1 Applications of microalgae

Table 1 Typical microalgal species for biofuels production

Microalgal species	Targeted biofuels	Microalgal growth and/or biofuels production	Cultivation/reaction conditions	References
C. protothecoides	Biodiesel	55% of lipid	Heterotrophy; nitrogen limitation	Xu et al. (2006)
S. obliques	Biohydrogen	300 μmol H_2/(mg Chl*h)	Indirect process; light biophotolysis; (Fe–Fe) enzymes	Appel and Schulz (1998)
Nannochloropsis salina	Biogas	0.70 L biogas/g	Photobioreactor, large scale, 35 °C	Quinn et al. (2014)
Chlorococum sp.	Bioethanol	38 wt%	Fermentation	Singh and Gu (2010)
Spirulina sp.	Biomethanol		Gasification/anaerobic fermentation	Rodionova et al. (2017)
Microalgal consortium	Biochar	45.0 ± 5.9% dw solid biochar (with energy density 8–10 MJ/kg)	Hydrothermal liquefaction	Roberts et al. (2013)

firstly extract lipids from microalgal cells, and followed by transesterification of lipid fraction using alcohol in the presence of catalysts (Rodionova et al. 2017). A rate up to 300 µmol H_2/(mg Chl h) was reported in *Scenedesmus obliques*, and this enables *S. obliques* to be suitable for producing biohydrogen (Appel and Schulz 1998). The general routes include indirect process with firstly produce biomass through photosynthesis and then covert the biomass (particularly carbohydrates) to biohydrogen via fermentation and/or photofermentation (Benemann 1996, 2000), while the other approach is splitting water to hydrogen and oxygen via processes of direct or indirect water biophotolysis (Rodionova et al. 2017).

As also shown in Table 1, some microalgal species are also reported to produce biogas with productivity of 0.70 L biogas/g VS (0.43 L CH_4/g VS), and the results were obtained in the cultivation of *Nannochloropsis salina* in photobioreactor at large scale at a temperature of 35 °C (Quinn et al. 2014). Moreover, microalgal biomass can be also fermented into biogas, which can be illustrated by that 3.83 g/L obtained from 10 g/L of lipid-extracted microalgae debris of *Chlorococum* sp. (corresponds to 38 wt%) when the microalgal biomass as a substrate via yeast fermentation (Singh and Gu, 2010). In practice, the addition of hydrogen is demonstrated to modify the combustion characteristics of natural gas (Ren et al. 2019). Biomethanol can be produced by some species such as *Spirulina* sp., through gasification or anaerobic fermentation (Rodionova et al. 2017). It was also found that microalgal consortium has capability to take wastewater effluent as growth medium in open ponds, and the biomass can be harvested as the feedstock of biochar, reaching 45.0 ± 5.9% dw solid biochar (with energy density 8–10 MJ/kg) via hydrothermal liquefaction (Roberts et al. 2013).

Development of upstream measures

In general, the upstream technologies applicable to microalgae mainly include three aspects (Table 2). Firstly, it is better to select some proper strains characterized with robustness, fast growing rate and rich in lipid from wild environment. Secondly, advanced genetic engineering can be adopted to modify and obtain the strains with fast growth rate and high lipid productivity. Thirdly, other measures such as metabolic engineering can be used to enhance the accumulation of lipid and other fuel products. Genetic and metabolic engineering of microalgae could be used to, for example, eliminate photosaturation and photoinhibition, which is expected to significantly increase productivity of outdoor cultures and greatly improve the economics of microalgae oil production. However, it will require long-term research and funding, to overcome current strictures against the release of genetically modified organisms. Thus, for the foreseeable future it would be prudent to limit projections to what can be achieved with wild-type strains (Rodolfi et al. 2010).

Strain selection

As estimated, there are one to ten million microalgal species on the earth and more than 40,000 species have been identified (Wang et al. 2010). Microalgae are featured as having fast growing rate and high lipid content; however, not all of them are regarded as the best lipid producers but depend on particular strain (Mata et al. 2010). In this regard, the fundamental requirement of microalgae-based biofuel is selecting suitable strains with the best combination of microalgal biomass productivity and lipid content in outdoor culture. For example, it would be promising when the strains can substantially accumulate lipids even to nutrient deficiency.

Table 2 Upstream and downstream measures to enhance microalgal growth and biomass production

Type	Approach	Representative techniques/criteria	References
Upstream measures	Strain selection	Robust strain; fast growing rate, high lipid or other fuels' yield	Arroussi et al. (2017) and Seo et al. (2018)
	Genetic engineering	Trans-conjugation; transformation; electroporation; microinjection	Qin et al. (2012) and Ghosh et al. (2016)
	Metabolic engineering	Degradation of nutrients; biosynthesis; preventing lipid catabolism	Dunahay et al. (1996) and Trentacoste et al. (2013)
Downstream measures	Large-scale cultivation	Appropriate strains	Singh and Gu (2010)
	Process control	Optimal cultivation system and conditions	Quinn et al. (2012) and Peng et al. (2016a)
	Harvesting and dewatering	Primary harvesting; secondary dewatering	Mata et al. (2010), Uduman et al. (2010) and Buckwalter et al. (2013)
	Extraction and conversion	Transesterification; fermentation; hydrotreatment; pyrolysis	Skorupskaite et al. (2016), Asada et al. (2012), Plant and Floorspace (2010), Sharma and Singh (2017) and Lee and Lee (2016)

The strains should be robust enough to withstand shear stress generated by mixing or the interference by wild strains or other microorganisms. Moreover, they are flexible to adapt to the changes in physicochemical parameters of the growing environment (Arroussi et al. 2017; Rodolfi et al. 2010; Seo et al. 2018).

The strain of marine or freshwater microalgae can be generally isolated using a micropipette for isolation under a microscope, cell dilution, and cultivation in liquid medium or agar plate (Arroussi et al. 2017). Winkler test screening protocol entailed a new and fast method for mutant strain selection and analysis of algal hydrogen metabolism without applying nutritional stress, and this method was recommended to isolate the hydrogen-producing *Chlamydomonas reinhardtii* strains (Rühle et al. 2008). Four strains, in which two marine and two freshwater strains were screened among thirty microalgal strains, were found with high biomass productivity and lipid content. For example, up to 60% lipid content was achieved in eustigmatophyte *Nannochloropsis* sp. F&M-M24 under nitrogen starvation (Rodolfi et al. 2010). Besides, some locally isolated strains are more likely accustomed in highly variable environment of outdoor cultures, though they may have difficulties in dominating yearround in the fluctuated cultivation conditions (Rodolfi et al. 2010).

Genetic engineering

Genetic engineering is defined as the direct manipulation of organism's genes using biotechnology. It has been applied in the production of microalgae to satisfy the growing needs and increasing quality of human life (Peng et al. 2016a; Rodolfi et al. 2010).

In the past, some researchers adopted a crucial systemic technology to obtain high microalgal biomass concentration for sustainable industrial applications, and to modify the metabolic pathway for producing more anticipated high-value products (Qin et al. 2012). Generally speaking, there are several methods for transformation in marine algae, including trans-conjugation, natural transformation and induced transformation, electroporation (or electropermeabilization), biolistic transformation, glass beads, silicon carbon whiskers method, microinjection, artificial transposon method, recombinant eukaryotic algal viruses, and agrobacterium tumefaciens-mediated genetic transformation (Qin et al. 2012).

Among the methods, gene transfer by electroporation is characterized by simplicity of the procedure and high efficiency with a small amount of DNA and is a common method for various cells and bacteria for over 30 years (Neumann et al. 1982; Zimmermann et al. 1975). In detail, an electrical field (e.g., 1–1.5 kV, 250–750 V/cm) can be imposed on cells to increase the permeability of cell membrane, and then chemicals, drugs, or DNA can be introduced into the cells (Neumann et al. 1982; Sugar and Neumann 1984). The method is applicable to both prokaryotic cells and eukaryotic algae including red algae, green algae, and diatoms. For example, marine alga *Nannochloropsis* sp., suitable for potential biofuel production, has been successfully genetically transformed with several knockout genes involved in the nitrogen metabolism (Kilian et al. 2011). However, this method is constrained in brown algae because of undeveloped protoplast preparation and immature regeneration technologies (Qin et al. 2012).

Direct gene transfer by biolistic transformation (i.e., micro-particle bombardment) has been demonstrated to be the most efficient method for many diatom strains (Qin et al. 2012; Cheney 1992), and it has been widely employed in the transformation of the nuclear and chloroplast expression systems (Qin et al. 2012). The method is characterized by some advantages; for instance, it is the only effective method that can repeatedly transform chloroplasts, mitochondria and other organelles. It can introduce exogenous DNA into broad cells and tissues of plants, animals, microbes, pollen, and other peculiar acceptors. Furthermore, diversified endogenous vectors can be also used in biolistic transformation through a controllable and mature manipulation procedure (Qin et al. 2012). However, this method is highly reproducible and works under specialized and high-cost equipment (Qin et al. 2012). Particularly, DNA is generally coated with gold particles to target within the cell via pressurized helium gas (Apt et al. 1996; Dunahay et al. 2010; Ghosh et al. 2016).

To date, more than twenty marine microalgal strains have been successfully transformed with the aforementioned transformation methods. For marine cyanobacteria, the genetic transformation was successfully demonstrated in 5 strains of *Synechococcus* (i.e., *Synechococystis, Pseudanabaena*) using the method of trans-conjugation (Sode et al. 1992), or natural transformation (Jiang et al. 2003).

For brown algae, five types of acceptor cells such as juvenile sporophytes, male and female gametophytes, tissue pieces from sporophytes, and parthenogenetic sporophytes can all be transformed by particle bombardment (Jiang et al. 2003; Qin et al. 1999). For diatoms, biolistic transformation was successfully demonstrated in centric diatom *Thalassiosira weissflogii* (Falciatore et al. 1999), *Thalassiosira pseudonana* (Poulsen et al. 2010), *Chaetoceros* sp. (Miyagawa-Yamaguchi et al. 2011), *Cyclotella cryptica* (Dunahay et al. 2010), and the pinnate diatoms *Navicula saprophila* (Dunahay et al. 2010), *Cylindrotheca fusiformis* (Fischer et al. 2010), and *Phaeodactylum tricornutum* (Miyagawa et al. 2010). For green algae, foreign DNA can be also introduced in marine microalga *Dunaliella salina* and freshwater microalga *C. reinhardtii* using the agitation of glass beads (Feng et al. 2009; Kindle 1998).

Metabolic engineering of microalgal pathways

Metabolic engineering is the practice of optimizing genetic processes (i.e., remove and add genes) and regulatory processes on an organism, to alter its metabolic functions in a predetermined manner such as increasing the organisms' production of a certain substance (Shuler and Kargi 2001). The control of metabolic pathways is generally completed by nutritional and environmental regulation in bioprocess engineering (Park et al. 2019). In general, metabolic reactions can be classified into three major types, including the degradation of nutrients, biosynthesis of small molecules (e.g., amino acids, nucleotides), and biosynthesis of large molecules (Dunahay et al. 1996).

It was reported that fatty acid of diatom *C. cryptica* can be increased by the overexpression of the acetyl-CoA carboxylase gene (ACCase) under stressed condition (Dunahay et al. 1996), because ACCase controls the biosynthesis of fatty acid (Ghosh et al. 2016). The oil content of diatoms and green algae was also increased by adding heterologous plant fatty acid synthetase enzymes gene (Blatti et al. 2012; Radakovits et al. 2010). An increase up to 82% total neutral lipid was achieved by knocking down the pyruvate carboxylase kinase expression of diatom *P. tricornutum* using an antisense cDNA construct (Ma et al. 2014).

In addition to pyruvate, the overexpression of endogenous malic enzyme can result in a 2.5-fold increased lipid accumulation of *P. tricornutum* under nutrient-replete conditions (Xue et al. 2014). Some transgenic strategies are also applied to increase the lipid accumulation of green alga and diatom under nitrogen-starved conditions (Hamilton et al. 2014; Wang et al. 2009; Yongmanitchai and Ward 1991). Furthermore, lipid accumulation can be enhanced by preventing lipid catabolism, for example, as compared with the wild-type, 3.3-fold higher total lipid content of the diatom *T. pseudonana* was achieved by transforming antisense constructs (Trentacoste et al. 2013).

Development of downstream measures

The downstream technologies applicable to microalgae mainly include the following aspects, such as large-scale cultivation of suitable microalgal strains, the design of efficient cultivation system and modified cultivation modes to deal with the conflict between biomass and lipid production, energy-saving approaches for harvesting and dewatering, and the efficient technologies of extraction and conversion.

Large-scale cultivation

Large-scale cultivation is the fundamental of commercializing microalgae-derived biofuels, due to sufficient biomass makes biofuels production possible. In general, it is limited by those factors as appropriate strain, farming, process control, and other measures.

Appropriate strain guaranteeing high biomass production

As aforementioned, it is the priority to select promising microalgae species with high oil content and can quickly grow in the culture, and this is one of the essential keys to produce biocrude, biodiesel, and drop-in fuels and further develop economically viable project (Singh and Gu 2010; Arroussi et al. 2017). As reported in Algae 2020 (Will 2009), five key strategies such as "fatter (i.e., algae species with high oil content), faster (i.e., grow more quickly), cheaper (i.e., capital and operating costs), easier (i.e., manipulation at each sub-sets of systems) and fractionation marketing approaches (e.g., biomass co-product marketing strategies)" have been identified as the key factors of driving a successful commercialization of microalgae-based biofuels.

The strains with high lipid contents are promising species feedstock of biodiesel, and this would be the base of successful industrial farming of microalgae (Sadvakasova et al. 2019). Some studies screened a series of local strains (57 strains) from Moroccan coasts and found that diatoms are generally rich in triglycerides (TAG), while the lipid content of marine microalgae *Tetraselmis* sp. and *Dunaliella* sp. reached up to 56% and 50% of dry cell weight, respectively (Arroussi et al. 2017).

Scale-up cultivation

Commercialization of microalgae-based biofuels is dependent on the high biomass concentration and lipid contents through large-scale cultivation of microalgae (Quinn et al. 2012). However, large-scale cultivation faces formidable challenges and the major barrier is the high production cost (Borowitzka 2013), owing to the intensive energy demands required for the complex processes of algal cultivation such as sterilization, mixing, aeration, illumination, gas exchange, and others (Peng et al. 2015, 2016b). For example, the processes such as the sterilization of large volume of cultures and maintenance of sterility for the whole cultivation system, efficient illumination, and deoxygenation approach, and other steps for better process control are difficult and costly. Thus, it is important to cut down the cost of microalgal cultivation that is potentially caused by the above processes, and meanwhile to obtain high biomass concentration and lipid productivity. The routes of offsetting overall costs mainly include co-producing value-added products, optimization of algal cultivation processes, and lowering cultivation cost via the utilization of wastewater or flue gas as nutrients and carbon source (Peng et al. 2013, 2015).

Under the persistent efforts on the improvement and development of technologies in the past years, some successful examples of large-scale microalgae farming have been implemented in companies. For instance, Sapphire Energy was found in 2007 and has built up some multi-year cooperation or agreement on algae-based research projects with other companies, aiming to co-develop algae-to-fuel cultivation systems at commercial-scale in the past few years (SapphireEnergy 2016). A commercial demonstration algae-to-energy facility, the Green Crude Farm, was announced by the company in 2012, and the farm consists of 100 acres of two ponds (at sizes of 1.1 acres and 2.2 acres), and the planned 300 acre facility for the mechanical and processing equipment that are applied in the processes such as harvesting, extraction of algae, and recycle of water (SapphireEnergy 2016).

Algenol is a notable world-class team assorted with state-of-the-art facility and proprietary algae growing systems (Algenol 2018). The company adopts photobioreactors with production yields 2–3 times that of open ponds, and VIPER manufacturing (i.e., Algenol's proprietary photobioreactors) with 40,000 square-foot facility. In addition to produce some high-value products including natural colorants, protein, *Spirulina*, personal care ingredients, biofertilizers, and biostimulants, Algenol also dedicates to biofuels such as bioethanol and green crude oil with proprietary vapor compression steam stripping unit (VCSS) for the purification of the ethanol and hydrothermal liquefaction (HTL) technology to make crude oil, respectively.

Process control during the cultivation of microalgae

Suitable cultivation system for microalgal growth

Microalgae are capable of growing in natural habitats of oceans, rivers, lakes, and ponds or artificial systems of open ponds and photobioreactors (Wang et al. 2010). Open ponds can be natural waters (e.g., lakes, shallow lagoons, ponds) or artificial ponds such as circular ponds, raceway ponds, shallow big ponds and tanks, and container-based systems (e.g., hanging plastic bags) (Pulz 2001; Singh and Gu 2010; Tredici and Materassi 1992). In general, open ponds are characterized by some advantages of low cost, simplicity, and easier to operate. However, they also have some major limitations; for example, they require large areas of land, more water evaporation, low biomass productivity, low utilization of CO_2 and light, poor mixing, and easier to be contaminated (Peng et al. 2013, 2015).

By comparison, closed systems like photobioreactors can be sorted as vertical column photobioreactors, flat panel photobioreactor, tubular photobioreactor, internally illuminated photobioreactors, spectral shifting, membrane photobioreactors, and plastic bag photobioreactors (Wang et al. 2012). They are capable of offering good control over key operational parameters of microalgal cultivation, including temperature, length of light path, pH, species control, and others (Peng et al. 2016b). Thus, photobioreactors provide a higher possibility of achieving much higher growth rate, microalgal cell density, and volumetric biomass productivity compared to open ponds (Wang et al. 2012). The high capital and operational costs result from complex configuration, illumination, cooling, mixing, deoxygenation, and other operational requirements. These limit the application of photobioreactors at large-scale microalgal farming (Peng et al. 2013, 2016b).

In the present scenario, it is necessary to design a suitable cultivation system for microalgal growth in particular for large-scale microalgal farming. Based on the advantages and disadvantages of open ponds and photobioreactors, some companies prefer to adopt photobioreactors than open ponds systems or natural formations, but it is an attractive option of cultivating microalgae in open ponds in the regions where has sufficient lands and has no competition with arable lands (Singh and Gu 2010). It was reported that most microalgae systems today can produce a range of 2500–5000 gallon of oil per surface acre in raceway ponds with 30% oil content (Singh and Gu 2010).

Cultivation modes to solve conflicts between biomass and lipid production

Lipid synthesis is usually induced by environmental stresses such as salinity, temperature, nutrients, and pH (Peng et al. 2015). Among these factors, nitrogen limitation is regarded as the most effective approach to enhance lipid accumulation of microalgae, whereas at the expense of cell growth (Park et al. 2019). Some studies have demonstrated the feasibility of enhancing strains from low or medium initial lipid content to super high level; for example, the lipid content of *Dunaliella tertiolecta* was dramatically improved from the initial level of 21 to up to 70% under saline stress (Arroussi et al. 2015).

To solve the conflict between cell growth and biomass accumulation, some efforts are made to select genetically strains or screen local strains with fast growth rate and high lipid content (Arroussi et al. 2017; Sadvakasova et al. 2019). High biomass concentration of microalgal cultures can be achieved through simplified cultivation, including the optimization of medium composition (Liu et al. 2007), process optimization and control on light utilization, oxygen accumulation mitigation, and contamination prevention (Liu et al. 2007; Pulz 2001; Li et al. 2008), and improvement of cultivation systems (Wang et al. 2012). Currently, two-stage process has been demonstrated to be a win–win strategy for obtaining both high cell density and biomass concentration (i.e., carbohydrates, lipid or hydrogen) at lower operative

and investment costs (Ra et al. 2015; Nagappan et al. 2019). In the process, microalgal cells are cultivated in photoautotrophic conditions in the first stage, and then transfer the biomass to a heterotrophic reactor where cells use organic carbon to synthesize starch and lipids (Caprio et al. 2016). A hetero-photoautotrophic microalgal growth model was also studied for improved organic-rich wastewater treatment and microalgal lipid yields, and therefore offering a sustainable way to produce microalgae-based bioenergy and byproducts (Zhou et al. 2012).

Energy-saving approaches for harvesting and dewatering

Harvesting and dewatering of microalgal biomass are regarded as a major bottleneck to microalgae-based biofuels due to their energy-intensive feature, and the processes may account for 20–30% of the total production costs (Mata et al. 2010; Uduman et al. 2010). Thus, finding energy-saving approaches is very important to offset the production cost of microalgae-based biofuels. In general, microalgae can be harvested by two steps of bulk harvesting and followed by thickening (or dewatering) (Chen et al. 2011). As shown in Table 3, technologies of primary harvesting generally consist of coagulation and flocculation, flotation, filtration, screening, ion exchange, gravity sedimentation, precipitation, centrifugation, and other techniques (Cheng et al. 2011; Heasman et al. 2000; Hwang et al. 2013; Laamanen et al. 2016; Singh and Patidar 2018; Uduman et al. 2010; Buckwalter et al. 2013). The secondary dewatering methods include filtration, drying, and others such as microwave and fluid bed (Buckwalter et al. 2013; Laamanen et al. 2016; Shelef et al. 1984). Advanced approaches such as a combined method of pulsed electric field (PEF) and hydrothermal liquefaction (HTL) has been proposed as a promising and suitable pretreatment for wet extraction of microalgal residual biomass. It was reported that PEF could accelerate the formation and extraction efficiency of amino acids up to 150% in 60 min, and this promised a higher biocrude yield by 6 wt% (Vlaskin et al. 2018). Besides, other methods are also applied in specially cell wall disruption using high energy ultrasonic or microwave-assisted extraction (Ranjan et al. 2010; Balasubramanian et al. 2011), or using supercritical fluid extraction at high temperature and pressure (Crampon et al. 2013), ionic liquids with very low melting point usually below 100 °C (Kim et al. 2012).

Selection of harvesting technology is directly relevant of the efficiency and cost, while the above technologies have their own advantages and disadvantages. For example, coagulation and flocculation are feasible for small size of microalgae (e.g., < 5 μm) and enable them to be larger sizes flocks (1–5 mm) (Park et al. 2011). Other technologies such as centrifugation have drawbacks such as being energy intensive, having high capital and operating costs, and resulting in shear stress to algal cells (Grima et al. 2003; Harun et al. 2010), while filtration is expensive and easily to be fouling (Vonshak and Richmond 1988). As reported, the combination of flocculation with flotation was demonstrated to be capable of achieving a high rate of solid–liquid separation (Rubio et al. 2007). In the commercialization utilization, Global Algae Innovations developed "ZOBI Harvester" which is renowned for an automated membrane filtration system with 100% harvest efficiency and no need for secondary dewatering in the combined processes of harvest and dewatering (Equipment 2018). This system has been commercialized at 20,000 L/h with an energy use of 0.04 kWh/m^3, and it is capable of reaching a 30 times reduction in harvest and dewatering energy and 4 times reduction in water flow (EnergyGovOffices 2016). The automated harvesting system is scalable (with the size range of 5–200,000 gpm) and easy to operate. Particularly, it harvests microalgae at ambient pressure without exposure to high shear stress or centrifugal forces (Equipment 2018).

Table 3 Approaches for harvesting and dewatering

Types	Approaches	Examples	References
Primary harvesting	Sedimentation	Gravity/centrifugal sedimentation	Uduman et al. (2010)
	Flocculation	Chemical/biochemical/electro-flocculation	Mubarak et al. (2019)
	Centrifugation	Decanter; centrifuge; hydrocyclones	Knuckey et al. (2006)
	Flotation	Electro-/dissolved air/suspended air flotation	Pragya et al. (2013) and Chen et al. (2011)
	Others	Screening; ion exchange; ultrasonic separation; electrophoresis techniques; magnetic separation	Cerff et al. (2012), Cheng et al. (2011), Heasman et al. (2000), Hwang et al. (2013), Laamanen et al. (2016), Singh and Patidar (2018) and Uduman et al. (2010)
	Drying	Steam/spray/drum/freeze/oven/sun drying	Shelef et al. (1984)
Secondary dewatering	Filtration	Forward osmosis; Belt/micro-/ultra-/rotary/pressure/cross-flow/vacuum drum filtration	Buckwalter et al. (2013)
	Others	Microwave; fluid bed	Buckwalter et al. (2013) and Shelef et al. (1984)

Efficient technologies of extraction and conversion

Improving the extraction efficiency of microalgal biomass

The dried microalgae biomass will be further used to extract microalgal lipids through mechanical or non-mechanical methods, such as cell homogenizers, autoclave, ultrasounds, bead mills, and spray drying, and freezing, the utilization of polar and non-polar solvents, osmotic shock, acid, base, and enzymatic reactions, and supercritical carbon dioxide ($SCCO_2$) (Halim et al. 2011; Mata et al. 2010). However, most of the extraction methods work at the laboratory scale but to be challenging at large-scale extraction because of the volume and complex (Lam and Lee 2012). For instance, some previous studies (Jesus et al. 2019) found that large quantities of solvent consumption are the largest expense when extracting lipid from wet microalgae using green solvents such as 2-methyltetrahydrofuran and cyclopentyl methyl ether. They also compared various methods of lipid extraction and demonstrated that the Hara and Radin method was the most effective for extracting 1 kg of fatty acids from *Chlorella pyrenoidosa* (at 65.71% moisture) using hexane/isopropanol (3:2 v/v). However, the green solvents prices are not competitive as compared with fossil-based solvents. Moreover, a solvent-free osmotic shock pretreatment method was used to extract lipid and subsequently produce methane from microalgae *D. salina* and *Chaetoceros muelleri*, a lipid recovery efficiency of 21% and 72% was obtained, respectively (González-González et al. 2019). Furthermore, some recent methods including microwave-assisted extraction, supercritical fluid extraction, use of ionic liquids, and switchable hydrophilicity solvents are also recommended for economical lipid extraction (Deshmukh et al. 2019).

Enhancing the conversion of microalgae to biofuels

Microalgal biomass can be converted to renewable fuels (e.g., power, heat, and fuels) and energy source through various technologies including (1) transesterification (Skorupskaite et al. 2016), (2) thermochemical such as combustion, pyrolysis, gasification, thermochemical liquefaction, and (3) biochemical/biological conversion in terms of anaerobic digestion, fermentation, and photobiological hydrogen production (Asada et al. 2012; Plant and Floorspace 2010; Sharma and Singh 2017) (Fig. 2).

Direct combustion is used to convert microalgal biomass into hot gasses for energy production, which can power a turbine and turn a generator to produce electricity, under the condition of oxygen, furnace, boiler, or steam turbine at around 1000 °C (Lee and Lee 2016; Suganya et al. 2016). For example, biomass for power (e.g., electricity) and heat can be achieved by combustion direct-firing in a boiler, where high-pressure steam is produced and introduced into a steam turbine, and flows over a series of turbine blades to make the turbine and electric generator rotate and therefore the electricity is produced (ClimateTechWiki 2006). A

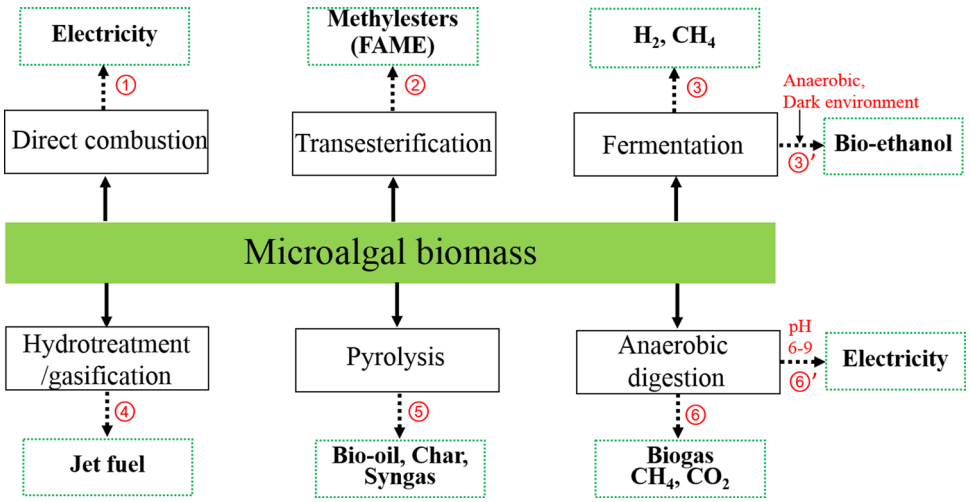

Fig. 2 Conversion technologies of microalgal biomass to renewable fuel. *Note*: The black solid frames indicate the major technologies and techniques applied for the conversion of microalgal biomass to the renewable fuels, respectively. The green dotted frames are the targeted microalgal-based fuels or end products. The number represents the procedures or conditions in which, (1) direct combustion: energy can be obtained under conditions of oxygen, furnace, boiler/steam turbine, at 1000 °C; (2) transesterification: direct/conventional transesterification; (3) fermentation: dewatering → milling → liquefaction → saccharification → fermentation → distillation → H_2, CH_4; and the other route (3)′ anaerobic/dark environment → ethanol, CO_2 → purification → bioethanol; (4) hydrotreatment/gasification: dehydration → pyrolysis → combustion → gasification → water–gas shift reaction → jet fuel; (5) pyrolysis: conventional/fast/flash pyrolysis → bio-oil, char, syngas; (6) anaerobic digestion: hydrolysis → fermentation → acetogenesis → methanogenesis → biogas CH_4, CO_2: and the other route is (6)′ digested at pH 6–9, and methane is produced for generating electricity

limited amount of oxygen or air is required for the burning of organic material to produce carbon dioxide and energy, which drives a second reaction that is the conversion of further organic material to hydrogen (H_2) and additional CO_2. Some CH_4 and CO_2 would be produced when the third reaction occurs in the way of carbon monoxide (CO) and residual water (Rao et al. 2017).

Methyl esters (FAME) can be produced by direct (single-stage) and conventional (two-stage) transesterification, and the chemical equation is that triglyceride and methanol react under the role of catalyst, to produce glycerol and methylesters (Adeniyi et al. 2018; Lee and Lee 2016). By comparison, hydrotreatment or gasification process of microalgal oil into jet fuel is completed by hydrotreating fatty acid and esters (Kuepker 2015) with the steps of dehydration, pyrolysis, combustion, gasification, and/or water–gas shift reaction (Rao et al. 2017). Pyrolysis is the thermal decomposition of biomass in the absence of oxygen, and to produce liquid fuel (bio-oil), solid fuel (biochar), and gaseous fuel products (H_2, CH_4), the process can be classified as conventional pyrolysis, fast pyrolysis and flash pyrolysis (Lee and Lee 2016; Sirajunnisa and Surendhiran 2016).

Anaerobic digestion is a process of obtaining methane from the delipidized algal biomass with carbon and nitrogen content via the consecutive stages of hydrolysis, fermentation, acetogenesis, and methanogenesis (Lee and Lee 2016; Sirajunnisa and Surendhiran 2016). This method converts organic biomass into biogas (~60% CH_4 and ~40% CO_2), and small-scale biogas digesters have been applied throughout many developing countries such as China, India, Nepal, Thailand, South Korea, and Brazil (ESMAP 2005). Fermentation aims to convert the cellulose sugar or starch of microalgal biomass into bioethanol, with consecutive stages of dewatering, milling, liquefaction, saccharification, fermentation, distillation and eventually the fuel products of bioethanol is obtained (Lee and Lee 2016).

Conclusion

With the rapid process of economic development and energy consumption, as well as the crisis of limited fossil fuel resource, and the increasing requirement of environmental protection, more and more concerns have been paid on the development of environmentally friendly fuels such as biofuels to solve the conflict. Microalgae-based biofuels have been regarded as one of the promising feedstocks for the new generation of biofuels. However, its commercialization faces the biggest challenge of high production cost, which results from the high capital and operational costs in terms of complex configuration, illumination, cooling, mixing, deoxygenation, and other operational requirements. Many efforts have paid on exploring enormous advances in the development of upstream and downstream technologies, to offset the cost of obtaining high biomass concentration and high content of anticipated fuels. Some appropriate microalgal strains have been screened or modified to guarantee high microalgal biomass production, and win–win strategies such as two-stage of cultivation have been demonstrated the possibility of obtaining both high biomass production and lipid content or other fuels. Furthermore, advanced technologies applied in harvesting and biomass-to-fuels conversion make microalgae-based fuels more promising. However, some significant challenges remain in the scale-up of microalgal farming systems, and the constraints should be tackled in the future.

Acknowledgements The authors are grateful for the financial supports provided by Natural Science Foundation of Hainan Province, China (Grant No. 518QN212), National Natural Science Foundation of China (41766003), and the Youth Fund Project of Hainan University (hdkyxj201706).

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