Chapter 7
Algal Biofuels: An Economic and Effective Alternative of Fossil Fuels

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Abstract Microalgae, cyanobacteria are vital organisms for sustainable production of various value-added products, e.g. food, chemicals and biofuels are also well known to meet out high energy requirements. These organisms can be a sustainable tool for reducing our current dependency on fossil fuels with growing world populations and environmental concerns. In recent times, the huge exploitation of algae as third-generation feedstocks for producing biofuels, e.g. biodiesel, biohydrogen, bioethanol and bioethanol, are underway. The biofuels have similar combustion properties, the energy content that is present in the fossil fuels furthers their transportation, and the storage is well suited with the existing infrastructure. The metabolic and genetic engineering of algal cultures can be manipulated for the advancement in the development of promising strains to produce alternative biofuels. This chapter includes the detailed account of various aspects of biofuel production using valuable algal feedstock, such as open and closed cultivation, stock availability, intercellular components (carbohydrates and lipids, etc.), challenges and future prospective.

Keywords Algae · Cultivation · Feedstock · Value-added products · Biofuel

7.1 Introduction

The depletion of fossil fuels with the rise in global energy demand (70%, whereas only 30% electricity) has led to countless initiations towards finding an efficient alternative for fuel used in manufacturing, transportation, and domestic applications. The use of fossil fuels, oil extraction and natural gas has been considered the society's primary resource of energy which eventually leads to global warming

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(Change 2014). Hence, the bio-based methods can serve as an effective alternative that can convert biomass into economic and sustainable value-added by-products. Biofuels are the fuels obtained from biological sources, e.g. plants, microorganisms and animals (Chowdhury and Loganathan 2019). The first-generation biofuels are obtained from edible agricultural crops and the second-generation biofuels from non-edible feedstock. As the feedstocks are non-sustainable and less economically feasible, the third-generation biofuels obtained from algal biomass gained more importance as it is more viable and sustainable as compared to other sources which have limited economic requirements (Chowdhury and Loganathan 2019). In contrast to land plants, algae are fast-growing microorganisms (100 times faster reproduction capability) that accumulate lipids inside the cells of some algal strains which are used for bioconversion into biodiesel (Mutanda et al. 2011; Pittman et al. 2011). Algal biofuel does not affect the food safety concern, and there is no competition for agricultural land area as it does not require fertile land; further, it can be simultaneously processed with the treatment of wastewater as it also contains good amount of important nutrients, which eventually make the process sustainable (Chisti 2007; Mata et al. 2010). Apart from these advantages, after the extraction of lipids, the residues from algal biomass can be utilized to produce biohydrogen, bioethanol and biomethane (Harun et al. 2010). The genetic manipulation is quite easier with the microbial resources to produce highly competent strains (Khan and Fu 2020). Among various studied microorganisms, algal resources are more commonly used for biodiesel and bioethanol production, as they can easily grow in open ponds, simple Continuous Stirred Tank Reactor (CSTR) system or large photobioreactors (Hulst 2013). Various end-products such as feedstocks, pigments, enzymes and pharmaceuticals are produced during biofuel generation process (Mata et al. 2010). As a result, biorefinery process carries double advantages in waste management and generating useful end-products. Therefore, a detailed study in the field of biofuel production with all the recent technologies will be useful for the researchers focusing on the biorefinery aspects. It includes various aspects of biofuel production using algal feedstocks, an open and closed cultivation system, intercellular components (carbohydrates and lipids, etc.), challenges and future prospective (Fig. 7.1).

7.2 Sources of Algal Biomass

Algae are aquatic photosynthetic organisms that emit oxygen. The structure of algae cannot be easily defined as it does not fit under a single monophyletic group although it is simple structure with no roots, leaves or stem, unlike land plants. They occupy specific habitat as they are a group of universal but individual species. They are found in different forms, e.g. attached with plant substrates, some in motile forms like animals, in suspended water, loosely grown on trees, animals, and soil and also in symbiotic association with another organism such as corals and lichens (Kumar et al. 2019). Algae consist of 40% lipids which is rapidly converted to biofuel, thus it

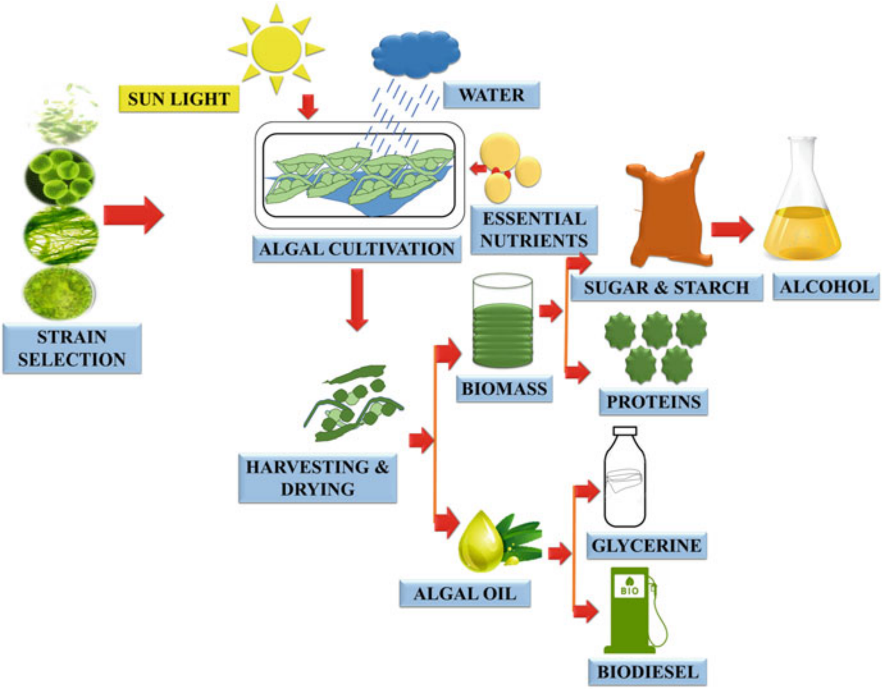

Fig. 7.1 Various aspects of algal biofuel production process

leads to the economic and environmentally friendly process and also algae cultivation is easy, hence making the process globally attractive (John et al. 2011). The classification of biomass obtained from algae can be done as sugar, triglycerides, and proteins that can be used for the production of value-added products. Therefore, biorefinery can be used as an efficient replacement of petroleum refinery (Kumar et al. 2019).

7.3 Micro- and Macroalgae

Microalgae consisting of diatoms, golden and blue or green algae and macroalgae involve green, brown and red seaweeds, bacteria and yeast. All these organisms have specific characteristics to remove greenhouse gas and adsorb CO_2. The biofuel production using microalgae includes various steps such as the selection of species-specific microalgae cultivation, pre-treatment, harvesting including acid transesterification, anaerobic digestion and separation of products (Assacute et al. 2018). Macroalgae are also called as seaweeds, commonly available in the intertidal region and subtidal region of the ocean. Similar to microalgae, macroalgae are also selected, cultivated and harvested, followed by the extraction of biodiesel, enzyme

Table 7.1 Algal culture currently used for biofuel production

Algal culture	Products	References
Chlorella vulgaris	Biofuel and by-products	Cheung et al. (2020)
Natural algae from Taihu lake	Bioethanol	Zhou et al. (2020)
Desmodesmus sp. ASK01 *Chlorella* sp. ASK14 *Scenedesmus* sp. ASK16 *Scenedesmus* sp. ASK22 *Chlorella* sp. ASK25 *Chlorella* sp. ASK27	Biofuel production, feedstock and dairy effluent treatment	Pandey et al. (2019)
Chlorella vulgaris YH703	Biofuel	Yun et al. (2019)
Nannochloropsis oculata *Euglena gracilis* *C. protothecoides* *C. sorokiniana*	Two-stage biofuel production	Nagappan et al. (2019)
Chlorella vulgaris	Microalgal cultivation and wastewater polishing	Chong et al. (2019), Zhu et al. (2019)
Chlorella sorokiniana	Enhanced production of algal biomass and microbial fuel cell power generation	Das et al. (2019)

production, fermentation of alcohol and anaerobic digestion. The complexity of macroalgae is more as compared to microalgae (Assacute et al. 2018) (Table 7.1).

7.3.1 Microalgae

7.3.1.1 Chlorella

It is one of the well-known green microalgae containing high protein content and can be utilized for the consumption by humans and when grown under stress conditions can accumulate lipids in the large amount (Guccione et al. 2014). The cost of cultivation of *Chlorella* is more, due to its open pond growing condition under photoautotrophic situations (Ramaraj et al. 2016). In 2000, the major autotrophic production of algae was initiated, the system used for the process was in glass tubing (500 km) in Klotz, Germany, and approximately 100 tonnes of *Chlorella* was produced annually (Pulz and Gross 2004). *Chlorella* species comprises high starch content which can be exploited for bioethanol production in the presence of 50% sulphur (Brányiková et al. 2011). *C. vulgaris* and *C. pyrenoidosa* are two main species which are used commercially (Brányiková et al. 2011). In addition to high starch content, C*hlorella* biomass is also rich in minerals, carbohydrates and proteins, which can be further utilized for the other value-added products' production after the extraction of biofuel (Brennan and Owende 2010).

7.3.1.2 *Botryococcus braunii*

It is a pear-shaped, bloom-forming green microalga that grows in the form of cluster and can be utilized for the biodiesel production, e.g. *B. braunii* which secretes lipid in the extracellular medium. Blooming is responsible for high quantity and quality of biodiesel production (Lassing et al. 2008). The strain selection depends on the production of lipids in high amounts; however, its growth is very slow when not grown under optimized conditions, in contrast to microalgae which have cytoplasmic lipids (Hirose et al. 2013).

7.3.1.3 *Pleurochrysis carterae*

It is a unicellular microalga with an unusual capability of calcification which occurs at subcellular level for the production of calcified scales. *P. carterae* can be utilized commercially for the production of biodiesel because it is a fast-growing microorganism with less contamination risk along with high content of lipid (Rahbari 2009).

7.3.1.4 *Dunaliella salina*

It is a biflagellate green microalga belonging to *Dunalliellacea* family. *D. salina* is mostly seen in marine waters (high salt concentrations). It is also considered as a food source due to high carotenoid and its antioxidant activity. Due to fatty acid methylation, e.g. palmitic and linolenic acids, it can also be used as biodiesel production (Oren 2005).

7.3.2 Macroalgae

7.3.2.1 *Gracilaria chilensis*

It is a red macroalga that produces a higher amount of biomass in comparison to other macroalgae (Wi et al. 2009). Because of high polysaccharides content, *G. chilensis* can be efficiently used for bioethanol production using hydrolysis as well as other value-added by-products production after the extraction of biomass.

7.3.2.2 *Sargassum angustifolium*

Sargassum angustifolium is a brown alga found in Persian Gulf and is utilized for the biodiesel production. After biofuel extraction, it is mostly utilized for sodium alginate production, and the obtained biomass is further used for the production of bioethanol using the fermentation process. The biomass after acid pre-treatment can also be utilized as an alternative of yeast during ethanol fermentation (Ardalan et al. 2018). The pre-treatment process completely damages the intricate structure of macroalgal biomass and evolves nitrogen gas which can be utilized for fermentation that will reduce the nutrients' cost (Yazdani et al. 2015).

7.3.2.3 Sea Lettuce: *Ulva lactuca*

It is a green macroalga and renewable gas fuel which is used for bioethanol production and commonly refers to green tides because of high secretion of nitrogen (eutrophication) or as algal blooms because of high lipid content (Allen et al. 2013). Generally, sea lettuce constitutes very less cellulose that is utilized for biomethane production using anaerobic digestion (Vergara-Fernández et al. 2008). It is mostly grown in the shallow basins, whose topographs protect the washout of algae, and it also keeps nitrogen and urea pollutant from starting the algal growth.

7.4 Nutritional Requirements for the Algal Biomass Production

Algal biomass comprises various metabolites (primary and secondary) such as carbohydrates, proteins, lipids and pigments, which either belong to cell structural components or involved in several metabolic processes (Markou and Monlau 2019). In their composition, mostly carbon is present along with various other elements, such as nitrogen, phosphorus, sulphur and potassium, in varying concentrations. Lipids, proteins and carbohydrates are the three crucial metabolites of the algal biomass with a total biomass content exceeding 80%, which greatly varies between different species (Tibbetts et al. 2015). *Chlorella* and *Scenedesmus* are the species that have high protein content ($\gg$35%), and *Porphyridium* has high carbohydrate content (>40%). Lipids and carbohydrates are the carbon-rich compounds, whereas proteins are nitrogen-rich compounds (approximately 16% nitrogen content). Similarly, other metabolites such as enzymes and pigments contain other nutrients, e.g. iron, zinc and cobalt (Markou and Monlau 2019). Hence, for the proper growth of the algal biomass, all the nutrients are required in specific concentrations as it is directly proportional to the growth rate. Generally, algae have flexibility regarding their nutritional requirements as it can grow within suboptimal conditions of one or more elements (Beuckels et al. 2015; Thrane et al. 2017).

7.5 Energy Requirements for Life Cycle of Algal Biofuels

Tremendous researches have been performed for algal biofuel production, and still, experiments are in process for the further development of the process towards scale-up and commercialization (Markou and Monlau 2019). Although, it may negatively affect the growth due to the relation between cell growth and particular nutrients' intracellular content as they are directly proportional to each other. Therefore, 'subsistence quota' which is an intracellular nutrient content threshold is introduced where algae are repressed and ceased for further growth (Droop 1968). To get the maximum possible lipid or carbohydrate accumulations, the nutrient starvation can be optimized up to the optimum concentrations (Markou and Monlau 2019).

7.5.1 Carbon

In the algal biomass, the most abundant component is carbon which accounts for approximately 45–55% of the total biomass and also increased up to 65% under appropriate environmental conditions. Naturally, microalgae are phototrophic organisms which use light energy for producing organic molecules by fixing inorganic carbon (CO_2). They are mostly marine microbes, where the carbon (inorganic) is available in the solubilized form (Markou and Monlau 2019).

The cultivation of microalgae depends mainly on atmospheric carbon dioxide, and this source is prone to carbon limitations as the rate of transfer of carbon dioxide to media is low as compared to the uptake by microalgae. Therefore, it is necessary to supply CO_2 to improve the productivity of the microalgal culture to fulfil the cell's carbon requirements (De Godos et al. 2014). CO_2 can be either found from the pure gas which is commercially available or created from various flue gases (Van Den Hende et al. 2012) after the removal of potentially harmful components, e.g. volatile heavy metals (Huang et al. 2016). Some algal species can also utilize various organic carbon, e.g. glucose and acetate, in the presence or absence of light (mixotrophic/heterotrophic mode) (Morales-Sánchez et al. 2015). The most commonly used organic carbon molecules are monosaccharide, e.g. glucose, volatile fatty acids, e.g. acetic acid, urea and glycerol (Perez-Garcia et al. 2011). Mixotrophic mode for algal growth plays a very important role in wastewater treatment by utilizing the organic compounds and reducing the organic load. For growing mixotrophically, microalgae need wastewater organic compounds in a fermentable form.

7.5.2 Nitrogen

The second most abundant element is nitrogen for the microalgal biomass. Nitrogen is the main component of nucleic acids, amino acids, proteins and other secondary metabolites which comprise around 5–10% of algal biomass. When compared to land plants, microalgae consist of comparatively more protein content (30–60%); hence, nitrogen requirement is also high, which can be utilized in both inorganic and organic forms, e.g. the two important forms are H^3/NH^{4+} (ammoniacal nitrogen) and NO^{3-} (nitrate-nitrogen). Certain species of cyanobacteria are able to fix atmospheric nitrogen (Peccia et al. 2013).

Various harmful effects are caused by ammoniacal nitrogen (in toxic form), i.e. free ammonia because it freely penetrates into the cells and causes serious damages to the photosynthetic machinery or to the various metabolic processes (Markou et al. 2016). Because of being a gaseous molecule, usually, free ammonia inclines to slip out from the cultivation medium, which results in the loss of essential nutrients. To overcome this problem, it is necessary that the cultures in which ammoniacal nitrogen is added should be pH controlled or gradual addition of the nutrient must be done in fed-batch cultivation (Ji et al. 2015; Markou 2015).

7.5.3 Phosphorus

It is also required for the microalgal growth and is present in various important organic molecules, e.g. RNA and DNA, ATP, and approximately 0.5–1% maximum up to 3% is present in the microalgal biomass (Richmond 2004). The cells utilize these in orthophosphate form, and it can also be as organic bounded phosphorus after the mineralization of phosphatase (Dyhrman and Ruttenberg 2006). It is often preventive in algal cultivation systems which are grown on wastewater. Phosphorus may form complexes in the presence of cationic metals and humic substances, and get precipitated in the presence of alkaline pH medium which eventually leads to the low phosphorus bioavailability (Cembella et al. 1982; Li and Brett 2013). The accumulation of a considerably higher concentration of intracellular polyphosphate granules by microalgae for their metabolic processes may be utilized by them when the surrounding phosphorus becomes depleted, and the process is called as luxury uptake (Powell et al. 2009; Shively 1988).

7.5.4 Other Nutrients

Other additional components are also required for proper growth of microalgae, e.g. potassium, magnesium, sulphur, chlorine, iron, calcium, manganese, cobalt, copper, boron and zinc. These nutrients are required in many processes, e.g. oxygen metabolism, ATP reactions for carbon fixation, nitrogen assimilation, electron transfer and synthesis of chlorophyll, DNA and RNA (Markou and Monlau 2019).

7.6 Algal Cultivation Strategies

Various methods have been used for several years for the innovations in algal biomass production. These various cultivation strategies have been developed to increase the productivity of algal biomass, and some of these cultivation strategies are discussed below.

7.6.1 Open Pond Photobioreactor

Considering the commercialization of algal biofuel with minimum energy input and economic approach, phototrophic cultivation method seems to be the most favoured process due to abundantly available sunlight at no cost. Along with this, phototrophic alga is capable to capture flue gas CO_2 and can effectively act as a superior carbon bowl which offers an advantage to the cultivation process. A perfect cultivation system must have the following characteristics: (1) efficient lighting area, (2) optimum liquid–gas transfer, (3) easy to operate, (4) low-level contamination maintenance, (5) economic, and (6) smallest land requirement (Xu et al. 2009). Open pond and closed-photobioreactor cultivation method has limitations when used in those countries where appropriate sunlight concentration is not available throughout the year (Lam and Lee 2012).

7.6.2 Raceway Pond System

It can be considered as the most effective method for the cultivation of a huge amount of algal biomass, mainly because of economic and easy operation process. This pond system (Fig. 7.2) is generally formed of a closed-loop, oval-shaped recirculation channel, in which circulatory mixing is done by paddle wheels in order to prevent the algal biomass from sedimentation. The CO_2 source is sprayed at the raceway pond bottom. There are some reports stating that the artificial light is incorporated in the raceway pond system, although it is practically not possible and also affects the economic efficiency of the process (Singh et al. 2011). This pond (depth 0.2–0.5 m) is made up of concrete earth covered with plastic bags (white), considering algae to take satisfactory sunlight exposure (Brennan and Owende 2010; Chisti 2007).

However, the raceway ponds have various advantages, e.g. low energy requirement and cost of process however, there are also some limitations such as more water loss because of evaporation and more chances of microbial contamination because of continuous exposure (Schenk et al. 2008). Therefore, the consistent cleaning and maintenance of the raceway pond are required to maintain the optimum conditions for proper algal growth.

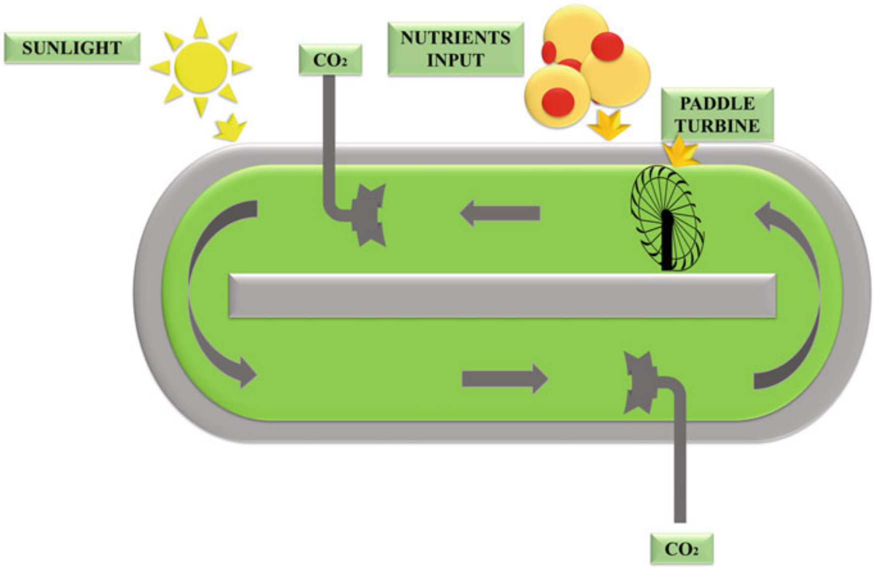

Fig. 7.2 Raceway pond system for algae

Considering the proper working of raceway pond system, the production of maximum algal biomass and large lipid content are not only the points to be considered, whereas other factors such as high growth rate, easy cultivation and increased survival capacity in extreme environments are also need to be considered. The best example is *Chlorella*, which can efficiently grow in a nutrient-rich medium. Similarly, *Spirulina* and *D. salina* can grow effectively at high bicarbonate/pH concentrations and highly salinity medium, respectively (Borowitzka 1999; Brennan and Owende 2010).

7.6.3 Closed-Photobioreactor

Later, considering the restrictions of the raceway pond system, closed-photobioreactors (Fig. 7.3) were designed to confirm the efficient optimal conditions for maximum biomass yield. The cultivation parameters in a closed-photobioreactor system are controlled strictly, and chances for contamination are lowered in the cultivation system, which helps in the cultivation of single strain of algae for long duration, and water resources can be reused multiple time (Brennan and Owende 2010; Chisti 2007). The flexibility of the cultivation system is more than the raceway pond because it can be used for the optimization of biological and physiological characteristics favouring the proper growth of algal strains (Mata et al. 2010). This process has gained interest among the researchers because optimization of physical

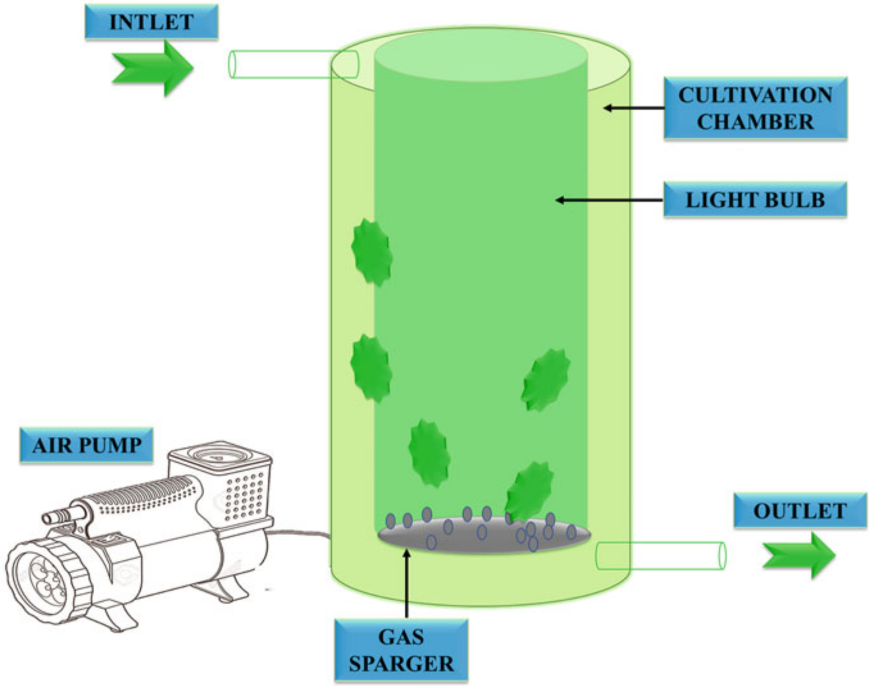

Fig. 7.3 Closed-tank photobioreactor

parameters such as CO_2 concentration, cultivation pH, intensity of mixing, temperature, and level of nutrient can be carried out easily in closed-photobioreactor system (Brennan and Owende 2010). Various closed-photobioreactors have been reported such as air-lift tubular, flat plate and column. Apart from having several advantages the air-lift tubular photobioreactor also have some limitations such as use of huge electricity volume to control heavy-duty pumps for the adequate mixing and optimal gas–liquid transfer rate, which may lead to a negative energy balance in the algal biofuel production, when precautions have not been taken for the reduction of energy input. However, the energy input has not included the energy used for other processes such as artificial lights at night-time, algal biomass harvesting and drying, water treatment, extraction of lipid, and conversion to biodiesel (Stephenson et al. 2010; Razon and Tan 2011). If these factors have also been included, the overall energy balance for algae cultivation will become even more negative. An air-lift tubular photobioreactor is costlier as compared to the column type and flat-plate which make them more reasonable for commercialization.

7.6.4 Hybrid Cultivation System

The hybrid cultivation system is a more advanced form of the bioreactor for the cultivation of algal biomass which was designed after reviewing the challenges of the open ponds and closed photobioreactors (Huntley and Redalje 2007). Brennan and Owen (Brennan and Owende 2010) reported that the hybrid cultivation system mainly work in the process where hybrid two-stage cultivation was required.

The first stage in which the closed-photobioreactor cultivation conditions are controllable to attain large biomass volume in the nutrient-sufficient cultivation medium, and in the second stage, open ponds which allows environmental stress such as nutrients lacking environments is applied on the cultivated algae to increase the lipid production. However, the hybrid cultivation system strongly affects the financial feasibility of the process towards the commercialization of algal biomass to biofuels (Kunjapur and Eldridge 2010). This kind of combined cultivation system generally involves higher processing costs as compared to any single bioreactor.

7.7 Harvesting and Drying of Algal Biomass

Harvesting of algal biomass is defined as separating the algal culture from water for producing biofuels. This process contains two different steps, first harvesting in bulk for the separation of microalgae from the suspension of bulk using gravity sedimentation, flocculation, and flotation; and second, thickening for the concentration of microalgae slurry via centrifugation and filtration (Brennan and Owende 2010; Chen et al. 2011). The harvesting process is challenging because of cell small size, i.e. generally 1–20 μm and water suspension (Lam and Lee 2012; Suali and Sarbatly 2012). Although the algae are grown in closed-photobioreactor, the mass ratio of algal biomass and water is very low (Chen et al. 2011) which usually lies between the range of 0.00035 and 0.027 assumed to produce around 0.05 and 3.8 gm/L per day of biomass during cultivation for 7 days. During the scaleup of a closed photobioreactor, approximately 73 tonnes of water needs to be processed while harvesting 1 tonne algal biomass (Lam et al. 2019).

As the water requirement is quite extensive, it is necessary to search an effective harvesting process for the commercialization for the production of algal biofuel. Various researches have been reported for the current harvesting techniques and algal biomass drying which consume a significant energy input for producing algal biofuel (Sander and Murthy 2010). The study evaluated two types of thickening methods for algal slurry without previous bulk harvesting: filter press and centrifugation which resulted in 88.6% and 92.7%, respectively, for the total energy input of the process. Hence, it can be considered as only centrifugation or filtration for algal biomass harvesting is still difficult for the commercial application, due to high energy utilization and high processing price. Whereas, bulk harvesting processes, e.g. flocculation, suggest another approach for algal biomass harvesting with low energy input and economic viability.

7.8 Biofuel Conversion

The biomass feedstock conversion into biofuels and additional value-added by-products is a challenging and costly process. Before the conversion into biofuel of other by-products, harvested and dried algal biomass is broken into different components such as lipid, protein, carbohydrate and mass residues using fractionation or extraction methods. Additional processing methods are required to further change the components into various liquid, solid and gaseous biofuels and other products.

Algal biomass can be utilized for producing various fuels like syngas, hydrogen, ethanol, methane, diesel, butanol, jet fuel, acetone and charcoal by using various chemical conversion methods such as fermentation, photobiological, gasification, anaerobic digestion, liquefaction, pyrolysis and transesterification (Azizi et al. 2018; Gavrilescu and Chisti 2005). Hydrothermal liquefaction (HTL) of microalgae biomass was found to be an efficient conversion technology, where wet biomass is treated under high pressure and temperature and the water present in the wet biomass has been considered as the reaction mixture (Han et al. 2019). Dilute acid pre-treatment was stated as one of the effective process for the active utilization of algal biomass (Dong et al. 2016).

7.9 Improvement of Algal Biofuels Using Biotechnological Strategies

In algal and cyanobacterial species, the cell size, composition of lipid and starch content varies; hence, it is assumed that not all the species can be used for biofuel production. Among them, some are suitable for the production of biofuel because they can naturally synthesize and also accumulate an adequate amount of starch and lipids, which can further be enhanced by genetic manipulations (Urtubia et al. 2016). Various algal and some *Chlorella* species, such as *Synechocystis* sp. (PCC 6803) and *Phaeodactylum tricornutum*, have been studied extensively for biotechnological engineering to improve their biofuel producing potentials (Xue et al. 2017; Yang et al. 2019). They have high potential for the biotechnological applications due to simple genomes, efficient for easy transformation, appropriate strains diversity and availability of complete genome sequences (Majidian et al. 2018). These properties can help in the biosynthesis of starch, increasing the ability of carbon capture, biohydrogen production, lipids enhancement and accumulation (Yunus and Jones 2018).

Genetic engineering process involves overexpression of those enzymes which have their involvement in fatty acid and lipid biosynthesis (Takemura et al. 2019;

Tan and Lee 2017; Yunus and Jones 2018), assembly and biosynthesis of TAG (Fukuda et al. 2018; Xin et al. 2017), targeting the blocking competitive pathways and biosynthesis of lipid/starch catabolism (Kao and Ng 2017; Shin et al. 2019). Several reports are also available regarding the gene-targeted transcription factors which are involved in the regulation of lipid biosynthetic pathways (Ajjawi et al. 2017) or enhancement of reducing agent NADPH availability to improve the content of fatty acid in *P. tricornutum* (Xue et al. 2017).

Overexpressing the key genes responsible for carbon fixation pathways in microalgae for the enhancement of the ability of carbon capture and accumulation of lipid is an efficient strategy to capture CO_2 excess for enhanced lipid accumulation (Huang et al. 2017; Oh et al. 2018). Carbonic anhydrase that exists in various algal strains is a well-known enzyme for CO_2 and bicarbonate interconversion catalysation. The reports on genetic engineering of carbonic anhydrase have been proven as an active participant in CO_2-sequestration. In *Nannochloropsis oceanica*, the carbonic anhydrase is considered as an important constituent of the carbon concentrating mechanism (Gee and Niyogi 2017; Tan et al. 2018) (Table 7.2).

7.10 Economic Aspects of Algal Biofuels

The techno-economic analysis is a primary assessment tool that is used for the estimation of the process cost and also for the determination of the economic viability of the algal biofuels (Hall 1986). This analysis assimilates the engineering process and thermodynamic modelling, processing cost, analysis of sensitivity and risk calculation (Bowyer et al. 2018; Langholtz et al. 2016). The price of biofuel ranges from $0.44/L to $8.76/L (Benemann et al. 1987; Richardson et al. 2012) which is assessed from the available literature, independently generated laboratory-scale experiments, growth rate-related assumptions, lipids yield, nutritional and energy necessities (Chia et al. 2018). The estimated cost of equipment and the economic process were done by Aspen Process Economic Analyzer (PEA) software (Rahimi and Shafiei 2019). The economic viability of microalgae suggested that the present cost per litre of algal biofuel is comparatively higher than conventional fossil fuel. Therefore, further studies can be performed to minimize the cost in the near future (Chowdhury et al. 2019).

7.11 Challenges and Future Perspective

Algal culture biomass for the biofuel production with marine resource is quite challenging due to its complexity and harvesting problems. The process also involves various pre-treatment processes, fermentation from microorganisms, and risk of contamination; hence, reduction in the biofuel cost is one of the main challenges (Balan 2014). If the cost-related problems are solved, biofuels can be

Table 7.2 Different genomic approaches for the improvement of the production of biocomponents from algal cultures

Algal species	Observations	References
Chlorella sp.	• Gene downregulations under UVR stress condition in various metabolic pathways for energy conservation, carbon resource reallocation and oxidative damage countering • Whole genome transcriptome analysis of Antarctic *Chlorella* sp. (growth temperature: 4 and 33 °C)	Poong et al. (2018a, b)
Chlamydomonas sp.	Gene involved in polyunsaturated fatty acids (PUFA) encoding in the ICE-L transcriptome, synthesizes enzymes, cell membrane transport and molecular chaperon proteins	Liu et al. (2016)
Chlorella vulgaris	Fatty acid and TAG biosynthetic machinery upregulation was obtained under oil-accumulating conditions	Guarnieri et al. (2011)
Chlamydomonas acidophila	• Cadmium exposure enhances expression of transposon in a green alga • The induction of genes for oil biosynthesis is done under heavy metal stress	Puente-Sánchez et al. (2018)
Chlamydomonas reinhardtii	The third fatty acid of TAG is originated from phosphatidylethanolamine or diacylglyceryl-*O*-4′-(*N*,*N*,*N*,-trimethyl)-homoserine betaine lipid species, and the candidate genes were provided by the comparative transcriptomic analysis which is also included in DAG acyltransferase and DGTT1phospholipase A2 homologue	Légeret et al. (2016)
Dunaliella acidophila	High constitutive gene expression methods involved in oxidative stress and response reactive oxygen species	Puente-Sánchez et al. (2016)
Dunaliella parva	During carbohydrate metabolism, glycolysis and the TCA cycle could be affected by nitrogen limitation and later hinder energy production	Shang et al. (2017)
Dunaliella tertiolecta	The de novo assembly integration suggested the long alterations in the expression patterns (13 861 protein-coding transcripts) with the growth phenotypes	Shin et al. (2015)
Aurantiochytrium sp.	The glucose utilization rate was accelerated by gibberellin and also involves in the fatty acid metabolites synthesis	Yu et al. (2016)
Nannochloropsis oculata	Organic carbon and nitrogen obtained from the pigments and protein breakdown were mainly channelled into fatty acid synthesis.	Tran et al. (2016)
Tetraselmis sp.	Organelle-specific responses varies with the temperature variations	Shin et al. (2016)
Nannochloropsis oceanica	N-condition TAG synthesis involves in • Upregulation of putative diacylglycerol acyltransferase (*DGAT*) genes (seven) • Downregulation of other *DGAT* genes (six) • Rise in the Kennedy pathway genes	Li et al. (2014)
Phaeodactylum tricornutum	Betaine lipids are reported as the main source for the development of triglyceride, sedoheptulose accretion during *Phaeodactylum tricornutum* nitrogen starvation	Popko et al. (2016)

represented as "future fuel" for the upcoming years for transportation purposes. The advancements in the modern era with new abilities, companies, and job prospects are likely to appear in the future of biorefineries that require further inventions in the above-discussed points (de Jong and Jungmeier 2015).

7.12 Conclusion

Fossil fuel resource combustion is the major cause of global warming as it releases high atmospheric carbon dioxide. In contrast to this, the utilization of organic waste resources as nutrients can be helpful in the development of a sustainable environment via biofuel production. Therefore, biofuel from marine sources serves several advantages of consuming maximum carbon dioxide, providing high energy production, using cost-effective fuel sources. The algal biomass is found to be an efficient alternative of fossil fuel for the development of sustainable environmental as the process is economic and also produces various value-added potential by-products. Further, biotechnology-based methods offer an effective path for enhanced CO_2 capture and higher biofuel production. Therefore, it will be beneficial to use advance gene manipulation techniques, e.g. using synthetic biology and artificial intelligence for further enhancement of the production of algal biofuels on industrial-scale.

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