

# Systematic review and perspective on the progress of algal biofuels

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**Abstract.** In recognition of the increasing demand of energy and the worsening environmental problems linked with fossil fuels usage, algal biofuel has been proposed as one of the alternative energy sources. It has become one of the hottest topics in renewable energy field in the new century, especially over the past decade. In this review, we summarized the characteristics of different types of algae biofuels. Besides, an in-depth evaluation of the systematic cultivation and practical application of algae have been conducted. Although algal biofuel has a great potential, its unacceptably high cost limits the large-scale industrialization. In order to resolve such restrictions, feasible methods of improving the large scale production and practical application of algal biofuels are proposed. Future efforts should be focused not only on the cost reduction and innovation techniques, but also towards high value by-products to maximize economic benefits. Our results are dedicated to provide valuable references for subsequent research and guidelines on algal biofuels field.

## 1 Introduction

Contemporarily, Human society relies heavily on energy, especially fossil fuels. To be more specific, the demand for fossil fuels is expected to increase by 40% from 2010 to 2040, where the current reserves are insufficient to meet the demand [1]. In addition, the massive consumption of energy is one of the causes of global warming. The world's carbon dioxide(CO<sub>2</sub>) emissions have reached 36.6 billion ton in 2018 and energy usage is responsible for 87% of global greenhouse gas emissions [2, 3]. These effects have lent urgency for the scientists to search for clean, renewable and affordable energy alternatives [4-7].

Biofuel, defined as the fuel manufactured from a wide range of planted organic sources (comprehensively depicted as biomass), offers an alternative path to gain energy. It has a higher overall cost-benefit and meets the sustainable demand [8-10]. Furthermore, it is cleaner with less harmful carbon emission compared to fossil fuel. Typically, the first biofuel generation uses specific food plants as feedstocks [11]. Second generation biofuels rely on non-food plants and non-edible parts of current crops [12-15]. The algal biofuel, considered as the third generation, has significant advantages [16]. In detail, algae has a great growth rate and can be produced on large-scale independent upon agricultural land due to their

strong adaptability. Moreover, they are photosynthetic organisms with a high efficiency of absorbing carbon dioxide(CO<sub>2</sub>). In addition, the metabolic engineering of microalgal genome (the fourth-generation algal biofuel) is currently the hottest frontier research [17-20].

On account of the high potential of algae as the alternative feedstock, this review will evaluate several types of algae as feedstocks for production based on primary biological studies. Besides, different cultivation systems will be also introduced with discussion of the application. These results provide reference for the bio-energy field and contribute to the emergent energy problems for the whole human society.

## 2 Introduction to algal biofuel mechanisms and species

### 2.1 Introduction to algal biofuel mechanisms

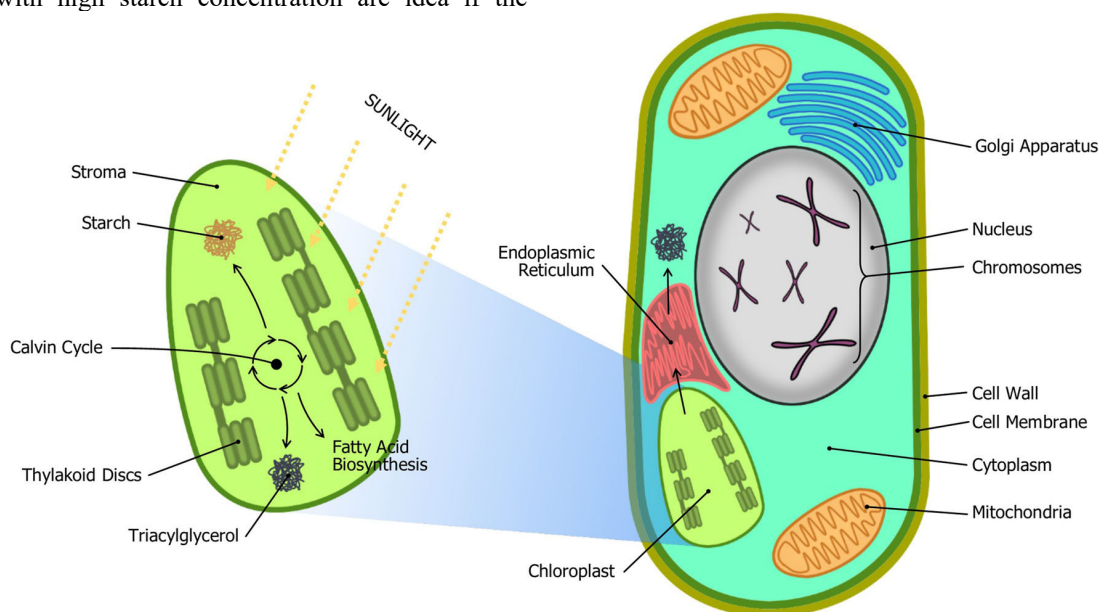
Microalgae are an ancient group of eukaryotic organisms that constitute a range of species, which are known for their phototrophic nature. However, microalgae strains exhibit a high amount of variation. The oil production capacity of microalgae is much higher than oil-producing plants, e.g., 10 times higher than that of oil seed crops [21]. The most important aspect of microalgae strain selection

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is the lipids production ability. Biomass productivity, lipid content, and lipid productivity are the three considering parameters when comparing microalgae in terms of lipid production. Many microalgae strains can be used in biofuel production including phytoplankton, red microalgae, green microalgae, and cyanobacteria.

Biodiesel is usually composed of fatty acid methyl ester (FAME) and sometimes fatty acid ethyl ester (FAEE) molecules, which have a molar mass of about 290 g/mol. The lipid precursors of these molecules are stored in liposomes of the cytoplasm. Liposomes are round organelles that not only store lipids, but are also responsible for lipid transport, protein storage and degradation, etc. The strains with high lipid production are favorable under the goal of biodiesel production. Whereas, strains with high starch concentration are idea if the

desired product is ethanol. Starch, the main source of energy for microalgae, is produced based on chloroplasts photosynthesis. Triacylglycerols (TAG) and other fatty acid derivatives act as a secondary energy source located in the cytoplasm of microalgae. These biomolecules serve as both electron sinks and energy reserves for microalgal metabolism. The figure 1 schematicl shows the procedure of chloroplast photosystem. The light enters the cell absorbed by the chloroplast, triggering the oxidation-reduction reaction. Subsequently, high-energy electrons are produced, which are then shuttled by NADPH into the Calvin cycle. The products of this cycle are used to make precursors for various molecules, including starch and TAG. TAG can be synthesized in the chloroplast and endoplasmic reticulum [22].



**Figure 1.** A schematic of energy reservation in microalgae[22]

## 2.2 Introduction to algal biofuel species

Studies on algal breeding have been carried out throughout the past 10 years [24]. Chlorella is the best studied species of algal, while the number of studies on Chlamydomonas, Nannochloropsis, Scenedesmus, and Spirulina have risen rapidly. The microalgae reported for biodiesel production were Chlorella, Scenedesmus, and Botryococcus braunii, which possess oil levels between 20% and 50% with favorable productivities. Thereinto, Chlorella appears to be the best option for biodiesel production. Chlorella is a spherical unicellular alga with a diameter of 3-8  $\mu\text{m}$  belonging to Chlorophyta, Chlorophyceae, Chlorellaceae. In addition to Chlorella, other algae have rich applications in the biofuel field. The Table 1 lists the lipid productivity of 12 algae species, several of the more common ones for which we give detailed descriptions later. As shown in the table, the highest lipid productivity was found in Chlorella (204.91 mg l<sup>-1</sup> day<sup>-1</sup>) and Botryococcus strains (112.43 and 98.00 mg l<sup>-1</sup> day<sup>-1</sup> for Botryococcus braunii and Botryococcus terribilis, respectively).

Chlamydomonas Reinhardtii is a eukaryotic unicellular

organism with a large cup-shaped chloroplast, accounting for 40%-60% of the cell volume, surrounding the nucleus. Two flagella located at the top of the cell and photoreceptor eye spots located on the side of the cell. Rhinophytes are characterized by simple culture conditions, short growth cycle, high photosynthetic efficiency, and clear genetic background. Scenedesmus obliquus is a green alga. In recent years, it has been a commonly used test organism due to its relatively high oil production, sensitivity to toxins, easy availability, small size, rapid reproduction, and ability to evaluate the effects of chemicals on multiple generations immediately. Spirulina is a unicellular aquatic plant. The cell walls are very thin and soft composed of easily digestible polysaccharides instead of indigestible toxic substances such as tannins. Spirulina cells do not have a true nucleus, i.e., they are also called cyanobacteria. Ankistrodesmus falcatus is a single-cell green alga with extremely tough cell walls. Therefore, the tough cell wall is a barrier when extracting lipids. Botryococcus braunii is a widespread microalga located in temperate and tropical freshwaters all

over the world. This single-celled alga has the potential to produce large amounts of liquid hydrocarbons.

**Table 1.** Growth kinetics, lipid content, and lipid productivity of different microalgae strains [23].

Local strains	Specific growth rate $\mu_{\text{oln}} (N_y/N_x)/(t_y-t_x)$ (day <sup>-1</sup> )	Biomass productivity $P_{\text{dwt}}$ (g l <sup>-1</sup> day <sup>-1</sup> )	Lipid content ( $L_c$ ; % dwt)	Volumetric lipid productivity $P_{\text{dwt}} \times L_c \times 10^3$ (mg l <sup>-1</sup> day <sup>-1</sup> )
<i>Ankistrodesmus falcatus</i>	0.57	0.34	16.49±0.44	56.07±1.75
<i>Ankistrodesmus fusiformis</i>	0.39	0.24	20.66±2.07	49.58±5.74
<i>Kirchneriella lunaris</i>	0.25	0.14	17.30±1.12	24.22±1.81
<i>Chlamydomonas</i> sp.	0.30	0.24	15.07±0.95	36.17±2.61
<i>Chlamydocapsa bacillus</i>	0.75	0.32	13.52±0.65	43.26±2.40
<i>Coelastrum microporum</i>	0.13	0.11	20.55±0.99	22.61±1.26
<i>Desmodesmus brasiliensis</i>	0.28	0.13	17.99±0.42	23.39±0.63
<i>Scenedesmus obliquus</i>	0.21	0.16	16.73±1.37	26.77±2.53
<i>Pseudokirchneriella subcapitata</i>	0.27	0.08	28.43±5.40	22.74±4.97
<i>Chlorella vulgaris</i>	0.53	0.73	28.07±4.31	204.91±6.37
<i>Botryococcus braunii</i>	0.14	0.25	44.97±4.00	112.43±11.52
<i>Botryococcus terribilis</i>	0.13	0.20	49.00±1.48	98.00±3.42

### 3 Systematic culture of algae

After selecting suitable algae for bioenergy production, the key step to affect their energy output is large-scale culture. Combined with the physiological needs of algae, algae culture is mainly divided into autotrophic and heterotrophic. The influencing factors include light, nutrition, temperature, PH and so on [25]. At present, the market adopts autotrophic culture more.

#### 3.1 Light autotrophic culture

The key to light autotrophic culture is the criteria for adequate light. With light, carbon dioxide and water, microalgae can synthesize organic matter and produce the energy for growth through photosynthesis, where oxygen is usually a by-product [26]. Autotrophic culture mainly adopts open photobioreactor and closed photobioreactor.

There are two kinds of open bioreactor, horizontal and inclined, which cannot supply energy independently. The horizontal photobioreactor is placed horizontally on the ground to realize the culture medium circulation by stirring the paddle wheel or rotating arm. The inclined

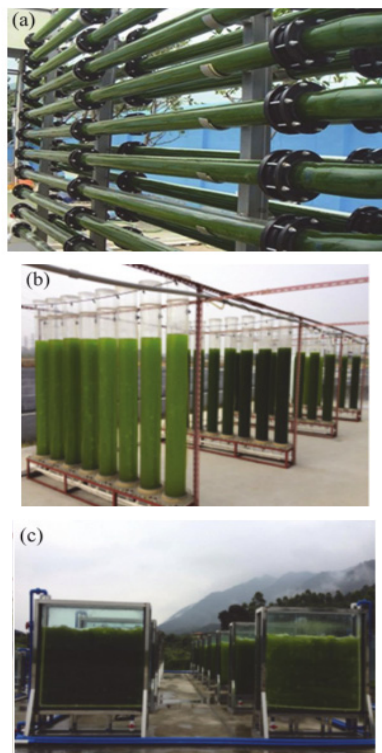
photobioreactor is placed on an inclined surface and the culture medium forms turbulence on the surface through the power of the pump [27]. Currently, efficient open photoreactors are horizontal open runway pools (seen from Fig. 2), 98% of commercial algal biomass is produced runway pools [28]. The open bioreactor is simple in structure and easy to operate. The maintenance and culture costs of building open reaction tanks are relatively low. However, the reactor is easy to be polluted and affected by the environment because of the open culture, resulting in low stability. The parameters of microalgae culture process cannot be accurately monitored, i.e., both the birth efficiency and the added value of its products are low.

Closed bioreactors are mainly divided into column type, tube type and flat type (illustrated in Fig. 3). The most commonly used reactor is tubular reactor[29]. Closed photobioreactors are mainly constructed of transparent materials to allow light energy to pass through as well as facilitate direct external observation and detection of internal reactions. Compared with the open photobioreactor, the main advantage of this closed photobioreactor is the realization of continuous production (at least in certain degree) [27]. Moreover, the growth

parameters of microalgae are easy to monitor with stable environment owing to closed characteristics. However, the construction and culture cost is higher for the closed case. Furthermore, the culture tank is difficult to clean and to be utilized for large-scale cultivation of microalgae. At present, most of the algae are used to produce high value-added products [30].



**Figure 2.** Open bioreactor and open raceway ponds (microalgae cultivation by Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences) [28].



**Figure 3.** Types of closed bioreactor: (a) tubular photobioreactors; (b) air-lift columnar photobioreactors; (c) plate-type photobioreactors [28].

### 3.2 Microalgae heterotrophic culture

The heterotrophic culture uses organic matter as the source of energy and does not need light. As a consequence, the reaction device is capable of adopting the traditional fermentation device (depicted in Fig. 4). In other words, it does not need the complex culture device with smaller area than the open reaction tank. The growth rate and culture density of microalgae are higher than those under autotrophic conditions. *Tetraselmis suecica* biomass yield is 2-3 times higher under completely dark

heterotrophic culture than under autotrophic conditions [31]. Many microalgae have higher oil productivity under heterotrophic conditions, their lipid content can reach up to 80% of dry weight. Microalgae heterotrophic culture is not restricted by environmental factors with a low cost. Nevertheless, heterotrophic culture requires a large amount of oxygen. In addition, oxygen supply is an important factor that limits the scale culture of microalgae [32, 33].



**Figure 4.** Devices of microalgae heterotrophic culture. (<http://www.qibebt.ac.cn/>)

### 3.3 Wastewater culture microalgae

The existing culture tank requires to invest a lot of nutrients and energy consumption. Although the new photobioreactor is being developed, the culture cost of microalgae is still very high. In order to reduce the culture cost of microalgae, it has become a hot topic to cultivate microalgae by sewage and treat the sewage simultaneously. In most domestic and agricultural wastewater, there are different concentrations of organic matter, nitrogen and phosphorus can be removed from sewage and obtained by microalgae biomass at the same time [34, 35]. Recently, the Guangzhou Energy Research Institute of the Chinese Academy of Sciences has established a research site in Foshan City, Guangdong Province, to use dairy wastewater to cultivate microalgae and treat sewage by microalgae biomass [28]. In the future, the technology of using sewage to cultivate microalgae and obtain bioenergy will become more and more mature to realize sustainable development.

## 4 Algal biofuel products

As the third generation biofuel feedstock, algae can grow nearly in all kinds of water and can be mainly used to produce biodiesel, bioethanol, biohydrogen and biomethane [36].

### 4.1 Bioethanol

Bioethanol is ethanol originated from a biological source. It is a renewable energy and able to be an alternative to gasoline. Compared to fossil fuels, bioethanol emits much less greenhouse gas when it burns, which is also widely utilized in industries [36].

Both macroalgae and microalgae can be used to produce bioethanol, as they have the ability to store carbohydrates. After harvested, algae biomass is converted

to carbohydrates by physical methods or enzymatic hydrolysis of cell walls. The next step is fermentation, where separate hydrolysis and fermentation (SHF) as well as simultaneous saccharification and fermentation (SSF) are the most common methods [37-39]. The products of fermentation are finally distilled to remove impurities and ensure the concentration of ethanol. Moreover, microalgae can produce ethanol directly in dark and anaerobic conditions [40-42]. Algenol has developed the technology to produce ethanol directly from the photosynthate of cyanobacteria. In addition, cyanobacteria is capable of converting the carbon fixed by photosynthesis to ethanol by introducing new genes [43]. In macroalgae, some kinds of green and red seaweeds have good ethanol yields [44]. Specifically, *U. pertusa* is able to produce 0.48 g ethanol/g algae with the fermentation of *Saccharomyces cerevisiae* ATCC24858 [45]. The ethanol yield of red seaweeds is relatively lower than green ones, but there are still efficient species. Ethanol yield of *G. verrucosa* will be 0.48 g ethanol/g algae [46]. As for microalgae, there are few kinds which have lower ethanol yield than macroalgae. *Chlorococum* sp. produces 3.83g of ethanol from 10g of lipid-extracted microalgae debris [47] while the number for *Chlorella vulgaris* is 0.4g ethanol/g algae [48].

#### 4.2 Biodiesel

Biodiesel from algae is environmentally-friendly since it has less emission of greenhouse gases (e.g., CO<sub>2</sub> and NO<sub>x</sub>), i.e., it is a good alternative to fossil fuels [36].

Both microalgae and macroalgae can be used to produce biodiesel. After dewatering the harvested algae, cell walls of algae need to be ruptured to release lipid. To rupture the cells, microwave, ultrasonic, autoclaving, freezing and organic solvent are all common methods. Thereinto, microwave and ultrasonic method are relatively efficient to extract algal oil [49, 50]. Transesterification is the traditional method to produce biodiesel. According to the types of catalyst, the production methods can be divided into four categories, acid catalysis, alkali catalysis, enzyme catalysis and supercritical methanol method [51]. Alkali catalysis is usually applied for industrial production with attributions of non-pollution and recyclable. With regard to other methods, they usually require strict reaction conditions or cost a lot, i.e., difficult for them to be introduced to practical production. Although the algae have been researched, there are few that have considerable yields. For example, biodiesel yield of *Spirulina* sp. can reach 86% by in situ process [52]. Another potential type of algae is *Chlorella pyrenoidosa* which yields 95% by in principal [53].

#### 4.3 Biohydrogen

Biohydrogen is a renewable and non-polluting method. It is also an important industrial gas which can be widely applied in metal, chemical, electronic and many other industries [54].

In algal biomass, biohydrogen is mainly produced by

fermentation and photosynthesis. According to the light conditions, the fermentation process can be divided into photofermentation and dark fermentation. There is little hydrogen produced in dark fermentation. Photosynthesis also occurs in two mechanisms, the direct photolysis and indirect photolysis [55]. There are some kinds of microalgae to produce hydrogen by dark fermentation that have shown potential yields. Most studies tend to take advantage of the combined thermal-chemical process, as this technique is easy to operate and less fermentation inhibitors would be generated [56]. The hydrogen production of *Chlorella vulgaris* can reach 190.90 mL H<sub>2</sub>/g volatile solid(VS) [57]. *Spirogyra* sp. can produce 146.3 mL H<sub>2</sub>/g algae [58]. In addition to produce hydrogen from one species of algae, it seems that mixing several kinds of algae may have a better hydrogen yield. The production yield of *Chlorella pyrenoidosa* powder and rice residue pulp in the ratio of 5:1 will reach 201.8 mL H<sub>2</sub>/g VS [59].

#### 4.4 Biomethane

Biomethane is a renewable energy that can be used for heating and transportation [60]. All kinds of microalgal biomass have great potential in producing methane. The main reaction mechanism is anaerobic digestion, which can be divided into four stages Hydrolysis, acidogenesis, acetogenesis, methanogenesis. The effluent of the anaerobic digestion system, which is rich in nutrients, can function as the fertilizer for microalgae while reuse for itself in the mean time [61]. However, there is a more potential technique to produce biomethane and biohydrogen at the same time. It is the two-stage anaerobic fermentation (TSAF), which is a combination of first-stage biohydrogen fermentation and second-stage biomethane fermentation. This technique is of great efficiency for algae to produce gaseous biofuels [62]. For example, *Sargassum* sp. is able to produce 541 mL CH<sub>4</sub>/g VS as well as 91 mL H<sub>2</sub>/g VS [63] and 434.38 mL CH<sub>4</sub>/g VS and 5.15 mL H<sub>2</sub>/g VS by the treatment at 150 °C for *Chlorella* sp. [62]. With regard to *Laminaria japonica*, its biohydrogen production will reach 87.8 H<sub>2</sub>/g VS and biomethane production can reach 463.5 CH<sub>4</sub>/g VS [64].

### 5 Suggestions

The existing technology of algae energy development and production is relatively mature, but the high cost still limits its development. Thus, the cost of biofuel production must be further reduced and the production must be scaled up. New approaches should be proposed to improve the production process, in order to increase the output of fuel energy (e.g., biodiesel) and generate higher economic benefits.

A promising strategy in algal breeding is the application of techniques developed in metabolic engineering and synthetic biology. However, this approach requires an oil gel algae model with high oil production capacity, known genetic information, and established molecular manipulation methods. Therefore, in order to find out the most suitable algae specie, one is ought to

explore the molecular information of algae and use genetic engineering technology to operate at the molecular level. So far, the fourth generation of biofuels aims to improve the production of algae biofuels via genetic engineering [65]. For biological hydrogen production, for the sake of improving the activity and yield of hydrogenase, algae strains need to withstand high concentration of oxygen and high efficiency of hydrogen production [66]. Therefore, more efficient algae varieties can be cultivated by mutagenesis breeding and genetic modification techniques. Nevertheless, genetic engineering may also pose a potential threat to natural ecosystems, i.e., it is crucial to monitor possible risks in a timely manner.

For large-scale culture, new techniques are proposed to improve the efficiency of the culture pool (e.g., the recent algal adherent culture technology, which reduces the culture cost and increases the production of biomass [67, 68]). On the other hand, the cultivation of algae through sewage to form a large-scale is still hot research. This can not only replace the factories to treat sewage alone, but also reduce the size of algae culture by using different nutrient elements in sewage. Whereas, the state-of-art technology is still not mature enough. In the future, the investigation should be carried out to select algae species with higher yield and value by-products in different sewage (livestock sewage, domestic sewage, industrial sewage, etc.).

## 6 Conclusions

In summary, biofuel have attracted a great deal of interest due to its renewable, biodegradable and environmental-friendly nature. Algae are attractive feedstock for biofuel production with advantages of great growth rate, strong environmental adaptability and high lipid content. In this review, the cultivation and biofuel applications are compared and discussed for several types of algae. We find that sustainable and large-scale usage of algae biofuels is far from enough, where high cost remains the key challenge. In order to industrialize algae biofuels desirably, more research on innovation and efficiency is needed in the future. Further research orientation could focus on mutagenesis breeding and genetic modification techniques for algae cultivation. Moreover, to maximize the total benefits both economically and socially, research about algal biofuel by-products is also demanded.

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