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Review

Factors in mass cultivation of microalgae for biodiesel

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ARTICLE INFO

Article history:

Received 28 October 2012

Accepted 1 December 2012

Published 20 January 2013

Keywords:

Microalgae

Biofuel

Photobioreactor

Lipid content

Mass cultivation

ABSTRACT

Biofuel from microalgae is a long term strategy to solve the energy crisis. It is a new area of biological engineering and process engineering that consists of the isolation and characterization of microalgae species, mass cultivation of microalgae, harvesting and post-processing. The successful mass cultivation of microalgae is one of its main challenges. Several factors influencing the mass cultivation of microalgae are discussed, such as microalgae species, metabolic mechanism, culture conditions and the photobioreactor. This paper will help the development of biofuels from microalgae and its photobioreactor.

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1. Introduction

Energy is the basic driving force for the development of society and economy. The rising demand for energy threatens the availability of sustainable energy for future generations. Fossil fuel is a non-renewable resource that is getting exhausted. Developing new means of biofuel production as a renewable green energy is becoming increasingly important [1,2]. The first generation of biofuels were mainly extracted from food and oil crops, which include starch and corn. The use of cellulose, such as crop stalks, as the raw material to extract biofuel is considered the second generation of biofuels. Biofuels from microalgae are considered the third generation of biofuels. It has marked advantages over the previous two generations and it has been widely studied [3–5].

The technology of biofuels from microalgae mainly comprises four areas, which are isolation and characterization of microalgae species, mass cultivation of microalgae, harvesting, and post-processing. It is a new area that integrates biological engineering and process engineering, which still needs much

study. Several factors are important in the development of biofuels from microalgae, among which its mass cultivation plays a key role. Many species have a high lipid content, including Chlorophyta and Bacillariophyta, such as *Chlorella*, *Scenedesmus*, and *Phaeodactylum*. However, the growth rate and lipid content of microalgae under mass cultivation conditions are significantly lower than those grown in the laboratory. This may be due to that optimal conditions are provided in the laboratory, and these may not be the same in mass cultivation, thereby influencing the growth rate and lipid content. Therefore, it is very important to study how to maintain microalgae in a state of high growth rate and lipid content. The study of the factors, such as isolation of microalgae species, metabolic mechanism, culture conditions and the photobioreactor, can improve the development of biofuels from microalgae. Further studies and summaries of previously obtained results are important for optimal microalgae biomass production, optimal photobioreactor design, the development of catalysts for the conversion of lipids to biodiesel and for understanding the pollutant formation chemistry of microalgae-derived biofuels.

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This work was supported by the National Basic Research Program of China (973 Program, 2012CB224803).

DOI: 10.1016/S1872-2067(11)60497-X

2. Microalgae species

2.1. Microalgae

Microalgae are microscopic photosynthetic, free living organisms of several microns (*Chlorella* only 3–5 μm). They thrive in diverse aquatic habitats, which include freshwater, brackish (<3.5% salt), marine (3.5% salt), and hypersaline (>3.5% salt) environments with a wide range of temperature and pH. Algae are typically subdivided into microalgae and macroalgae according to size. Unlike microalgae, macroalgae have cells organized into structures resembling the leaves, stems, and roots of higher plants, and some are as long as 60 m. Microalgae can also be subdivided into two broad categories: the prokaryotic cyanobacteria and the true eukaryotic microalgae. Cyanobacteria, often referred to as the blue-green algae, have chlorophyll *a* and do not possess chloroplasts or any other such organelles. These have a high protein content as much as 70% of dry mass and a low fat content of approximately 5% [6]. The kinds of eukaryotic microalgae are enormous and there are tens of thousands of species, such as Chlorophyta, Bacillariophyta and Xanthophyta. These are exceeding rich in lipid and protein, and are considered the main microalgae species for biofuels.

Microalgae have several advantages as a material for biofuels to replace petroleum-based fuels. Microalgae grow rapidly and many kinds are rich in lipid (20%–50%). The cultivation of microalgae does not entail a land conflict with agriculture for food because it can utilize wastewater and non-potable saline water that cannot be used by conventional agriculture. The fixation of CO_2 by microalgal photosynthesis is one of the most promising method for CO_2 sequestration from flue gas. The microalgae biomass can produce biofuels including green diesel, green gasoline, aviation fuels, ethanol, and methane as well as valuable co-products. The biochemical mechanism of photosynthesis in microalgae is similar to that in plants, but due to their simple structure, microalgae are particularly efficient converters of solar energy. They do not have support and reproductive structures, and when provided with light and nutrients, the microalgal cells can use most of the energy trapped for biomass growth.

Microalgae can either be autotrophic or heterotrophic. The former require inorganic compounds such as CO_2 , salts and a light energy source for growth and the latter are nonphotosynthetic [7–10]. Some microalgae are mixotroph, such as *Chlorella*, which has the ability to both perform photosynthesis and acquire exogenous organic nutrients. Microalgal cultivation not only provides biofuels but also provides greenhouse gas reduction as it utilizes a large amount of CO_2 during the cultivation. Moreover, wastewater can be used to culture microalgae. The heterotrophic or mixotrophic growth capabilities of microalgal strains are attractive attributes since these allow them to grow both in light and in dark conditions. The addition of external carbon sources can help the growth of microalgae and the accumulation of lipids. A potentially serious disadvantage of adding an external carbon source, especially an organic carbon source, is the possibility of increased contamination by unde-

sired microbes.

There are two general resources from microalgae that can be utilized: biofuels and co-products [5,11,12]. Biofuels from microalgae include lipids, H_2 , isoprenoids, carbohydrates, alcohols (either directly or by biomass conversion), and methane (from anaerobic digestion). Lipids, mainly in the form of triacylglycerols (TAGs), are distributed in the cell membrane and intracellular organelle membrane, and parts of these contain carbohydrates, and they are similar to petroleum. Besides lipids, co-products are also valuable. These include proteins and pigments, which can be used in many fields, e.g., pharmaceuticals (therapeutic proteins, secondary metabolites), food supplements, and materials for nanotechnology. There have been many discussions on the use of microalgae biomass to produce fuel and non-fuel co-products. In our opinion, the approach should not be inflexible. A reasonable approach to decide which fuel products and additional co-products to make is to identify the optimal microalgae species according to the environment so that cellular metabolism is geared towards the products, which simplifies the characterization and possible development for products, rather than to be fixed about the type of fuel or co-product.

2.2. Isolation and characterization of microalgae species

Many microalgal species are available from culture collections such as UTEX (Culture Collection of Algae at the University of Texas at Austin, Texas) with about 3000 species and CCMP (Provasoli-Guillard National Center for Culture of Marine Phytoplankton at the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, Maine) with more than 2500 species. However, many species may have lost some of their original properties, such as mating capability or versatility due to being cultivated for several decades in the collections. Therefore, it is important to obtain versatile and robust species for mass cultivation. Further, to collect the information on cultivation and species development for the mass cultivation of microalgae, it is recommended that the isolated species be screened to develop baseline data on regional environmental variability [13]. To provide the largest possible range in metabolic versatility, new species should be isolated from a wide variety of environments ranging from freshwater, brackish waters, marine, and hypersaline environments to a soil environment, as well as from a wide variety of their symbiotic associations with other organisms. Detailed information should be available for potentially valuable new species, such as required time, specific habitat, growth conditions, germination conditions, and temporal succession. In our research, it has been found that many potentially valuable new species are not suitable for mass cultivation for biofuels, although they grow rapidly and are rich in lipid. Sinopec Group collaborates with the Chinese Academy of Sciences in the study of the microalgae including isolation and characterization of microalgae species with high growth rate and lipid content in sea water, freshwater, and coastal zone and desert areas. Some microalgae species are cultured to provide biomass for biofuels. It is also possible that genetic engineering achievements in the laboratory can be applied to these species.

The ideal screening should cover three major areas: growth physiology, metabolite products, and species robustness. The growth physiology of microalgae encompasses a number of parameters such as maximum specific growth rate, maximum cell density, tolerance to environmental variables (temperature, pH, CO₂ levels, etc), and variability between in situ and laboratory performance. Screening of the metabolite products has to include not only the metabolite composition and content, but also the productivity of the cells for metabolites and the valuable cellular contents of proteins, lipids, and carbohydrates. The rapid lipid analysis of strains would be also very helpful. An ideal analytical method should be able to provide fatty acid profiles and identify neutral and polar lipids. Furthermore, metabolites excreted into the growth medium have been largely ignored, but these may prove to be valuable co-products. For the mass cultivation of a selected microalgal species, it is also important to consider robustness, which include parameters such as culture consistency, resilience, community stability, and susceptibility to predators present in a given environment. Moreover, it is necessary to perform small scale simulation of mass cultivation conditions to determine the robustness of the selected species. The project on the investigation of the technology of biofuels from microalgae carried out by Sinopec Group and the Chinese Academy of Sciences has begun the culture of microalgae in photobioreactors larger than 100 L.

2.3. Biosynthesis and regulation of lipids

Triacylglycerols (TAGs) are the major source of biofuels as these are the main stored neutral compound in many microalgae under stress conditions, such as high light or nutrient starvation [14–17]. Some microalgal species naturally accumulate large amounts of TAGs (30%–60% of dry weight), and have photosynthetic efficiency and lipid product potential that are at least an order of magnitude higher than those of terrestrial crop plants. However, the synthesis mechanisms of fatty acids and TAGs in microalgae are not known.

The Kennedy pathway is believed to be the major pathway for the accumulation of TAGs in plants and microalgae. It involves de novo fatty acid synthesis in the stroma of plastids and subsequent incorporation of the fatty acid into the glycerol backbone, and leads to TAGs via three sequential acyl transfers from acyl CoA in the endoplasmic reticulum. The conversion of acetyl CoA to malonyl CoA, catalyzed by acetyl CoA carboxylase (ACCase), is the commitment step and also the first step in fatty acid synthesis. Thus, it has been propounded that enhancing the activity of ACCase would be helpful for the accumulation of lipids in microalgae. However, the results were different, and excessive production of ACCase, which catalyses a key metabolic step in the biosynthesis of lipid, did not lead to increased lipid content. There are several steps between the first step catalyzed by ACCase and the step of synthesizing TAGs in the Kennedy pathway, and the decreased activity of ACCase in these steps may be the reason [18,19]. There are several pathways for TAGs synthesis by microalgae. The relative contributions of individual pathways to overall TAGs formation depend

on environmental and culture conditions. Alternative pathways that convert membrane lipids and/or carbohydrates to TAGs have been demonstrated in plants and yeast, which perform in an acyl CoA-independent way, but these have not yet been found in microalgae [20,21]. It is well known that fatty acids are common precursors for the synthesis of both membrane lipids and TAGs, but there are several aspects that need elucidation, including the distribution of the precursors in the two distinct destinations, the inter-conversion between the two types of lipids, and information on regulation at the genetic level.

It is a challenge to extrapolate information learned about lipid biosynthesis and regulation from laboratory species to species cultured on a large scale. It is also not known if the ability to control the fate of fatty acids varies among microalgal taxonomic groups and even between isolates or strains of the same species, that is to say, whether the basal lipid/TAGs content is an intrinsic property of individual species. Similarly, it is difficult to use information about lipid biosynthesis in plants for microalgae [22]. Thus, the study of fatty acid and lipid synthesis in order to identify the key genes/enzymes and new pathways in microalgae species is very important for biofuels, as is the study of the genes involved in lipid metabolism.

Under environmental stress conditions, such as nutrients starvation and high light, microalgae quickly stop their division and accumulate TAGs as the main storage compound [14–17]. This is almost the default pathway to synthesize and deposit TAGs to form lipid bodies in cytosol under environmental stress. The pathway of TAGs synthesis may play more active and diverse roles in response to stress. Under stress, excessive electrons that accumulate in the photosynthetic electron transport chain may induce the over-production of reactive oxygen species, which may in turn cause the inhibition of photosynthesis and damage membrane lipids, proteins and other macromolecules. The excess electrons can be consumed during the TAGs synthesis pathway. The synthesis of a C18 fatty acid consumes approximately 24 NADPH from the electron transport chain, which is twice that required for the formation of a carbohydrate or protein molecule of the same mass. This reduces the over-produced electrons in the transport chain under high light or other stresses [23]. Conversely, the TAGs synthesis pathway is usually associated with secondary carotenoid synthesis in microalgae [24,25]. The molecules (e.g. β -carotene and lutein) produced in the pathway are sequestered in cytosolic lipid bodies, which can prevent or reduce excessive light from striking the chloroplast under stresses by peripheral distribution. TAGs synthesis can also utilize phosphatidylcholine, phatidylethanolamine and galactolipids or toxic fatty acids excreted from the membrane system as acyl donors, thereby serving as a mechanism to detoxify membrane lipids and deposit them in the form of TAGs. The nitrogen concentration influences the lipid content of microalgae. A low concentration of nitrogen reduces the protein content in microalgae and increases the lipid and carbohydrate contents. With *Dunaliella tertiolecta* cultured in a nitrogen-starvation medium, the accumulation of total lipid occurred on the fifth day, and was higher than in a normal medium [26]. The accu-

mulation of lipid per cell was more in the nitrogen-starvation medium than in the normal medium, and it happened on the third day and it had achieved a maximum of more than five times by the fourth day. Although the accumulation rates were different in the two media, the composition of the lipid were almost the same [26].

Currently, near-complete genome information of some microalgal species are or will shortly become available. A large scale EST (Expressed Sequence Tag) sequencing of microalgae will provide better knowledge on genes differently expressed under different lipid production conditions to give information about photosynthetic carbon partitioning and lipid synthesis in microalgae. Based on such information, metabolic engineering through genetic manipulation represents yet another promising strategy for producing biofuels. The available approaches include random and targeted mutagenesis and gene transformation. The cloning and transforming of genes that influence lipid synthesis or improve robustness in selected microalgal species that have proven amenable to mass cultivation will enhance the overall performance and sustainable products of TAGs or other lipids. Genetic engineering can be used to regulate lipid metabolism to increase the microalgae content by enhancing the fatty acid synthesis pathway, regulating the TAGs synthetic bypass, inhibiting competitive and degradation pathways, as well as enhancing the lipid composition [18,19,27]. However, genome functional annotations have indicated that some aspects of TAGs accumulation and degradation pathways are species-specific and these are essentially unknown thus far. It is worth noting that the ecological security of microalgae species during genetic engineering should receive more attention.

3. Factors influencing the growth and lipid content of microalgae

Microalgae are potential valuable materials for biofuels. Microalgae with sequenced genomes and transgenic capabilities are suitable for studying cellular processes to provide information on the basic cellular processes and regulation involved in the synthesis of the biofuel precursors, which will be useful for the cultivation. Moreover, microalgae that can grow well in the laboratory may be suitable for large scale cultivation under different environments. However, this is not true of all microalgae. Therefore, there is uncertainty about whether the information obtained from the laboratory model species can be applied to mass cultivation outdoors. Numerous factors influence the cultivation of microalgae, including light, nutrient supply, CO₂, pH, temperature and O₂ [28–31]. It is important to apply the factors that influence the cultivation of microalgae in the laboratory to mass cultivation outdoors. The growth rate and lipid content of microalgae have a close relationship with light, nitrogen, phosphorus, and temperature [15,32,33]. The conditions for growth and lipid accumulation are different with different species. Therefore, the optimum mass cultivation conditions to be used for microalgae should be those that increase growth rate and lipid content, which are the main factors affecting the biofuel process.

3.1. Light

The intensity, wavelength and frequency of light affect the photosynthetic efficiency of microalgae [34]. The light intensity is critical because microalgae grow only when the intensity is higher than the light compensation point. It is better for the light intensity to be lower than light saturation. Microalgae and plants have two photosystems: photosystem I with peak absorption at 680 nm and photosystem II with peak absorption at 700 nm. The absorptivity of light with different wavelengths vary for microalgae and plants. For example, the light absorptivity order of *Chlorella* is red light, followed by yellow and glaucoma light [35]. Light and dark cycle also strongly influence the growth and photosynthetic efficiency of microalgae. It has been suggested that when the frequency of the light/dark cycle increases to higher than 1 Hz, the photosynthetic efficiency of microalgae is improved [36]. This phenomena suggested that adding baffles to a flat photobioreactor helps to enhance the photosynthetic efficiency of microalgae [37,38]. Natural light is always used for the mass cultivation of microalgae because it is free. Solar radiation is plentiful in China. It can reach more than 280 W/m² per year for some zones and 120 W/m² per year for most zones [39]. The natural light conversion efficiency of microalgae can reach 3%–11%, which becomes higher under artificial conditions. Natural light has a full light spectrum, which is good for cultivation. One of the disadvantages of natural light is the difficulty of its control, and it is too high on sunny days especially at noon and too low on rainy days.

3.2. Nutrients

Many elements have to be provided for the growth of microalgae, such as carbon (C), oxygen (O), hydrogen (H), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), sulfur (S), phosphorus (P), and trace elements. The major nutrients are carbon, oxygen, hydrogen, nitrogen, phosphorus, and potassium. The first three are obtained from water and air and the latter three have to be absorbed from the culture medium. During cultivation, N and P become limiting. They both play a role in controlling the growth ratio and lipid production of microalgae. Therefore, the ratio of N and P is often used as an important indicator, with too high a value meaning P restriction and too low a value showing that the supply of N is falling short.

Nitrogen is one of the essential elements for the growth, development, reproduction, and other physiological activities of microalgae. The nitrogen source and concentration also affect the accumulation of lipid in microalgae. Usually, ammonium salts, nitrates, urea, etc. are used as nitrogen sources, but their absorption rates and utilization are different [40]. Experiments showed the absorption and utilization of nitrogen have the following order: ammonia > urea > nitrate > nitrite. This is because ammonia is directly used to synthesize amino acid while the other nitrogen sources have to be converted to ammonia to synthesize amino acid [41,42]. It also has been found that microalgae grow well with urea and nitrate. Using the nitrogen sources of urea, NaNO₃ and NH₄HCO₃, the maxi-

imum growth rate of *Chlorella* was found to be with urea and the maximum lipid content was found to be with NaNO_3 . Therefore, if the cost is not a problem, it is better to choose NaNO_3 as the nitrogen source from a consideration of the productivity and lipid content [43]. On increasing the concentration of N, the growth rate of microalgae first increased and then decreased, which indicated that a high concentration of N inhibited the growth rate [43,44]. A possible reason is that microalgae grow so fast with abundant nitrogen that P is depleted, and thus the ratio of N to P gets unbalanced [45]. In other researches, the maximum growth rate of *Dunaliella tertiolecta* was found to be at the concentration of 23 mmol/L NaNO_3 and the growth rate did not increase with the concentration up to 46 mmol/L [26]. The growth rate of *Dunaliella tertiolecta* was almost the same with the concentrations of 1 mmol/L NH_4Cl and 2.3 mmol/L NaNO_3 and decreased on increasing the concentration of NH_4Cl . Although NO_3^- has to be reduced to NH_4^+ to be used by microalgae, a high concentration of NH_4^+ is not suitable for growth. This which may be due to that the respiration of microalgae is adversely affected by NH_4^+ in too high concentration [26].

N-Stress can increase lipid content. This is usually applied to get more biofuels in the microalgae process. The possible reason is that the content of adenosine monophosphate deaminase (AMPD) increases under N-stress, enhancing the catalysis of adenosine monophosphate (AMP) to inosine monophosphate (IMP) and ammonia. Because most of isocitrate dehydrogenase (ICDH) in mitochondrion is dehydrogenase dependent on AMP, the reducing of AMP would depress or even completely inhibit the activity of ICDH [46]. Citric acid can either be catalysed to acetyl CoA by citrate lyase, or in the citric acid cycle. ICDH is an enzyme in the citric acid cycle, and the inhibition of the activity of ICDH will increase the production of acetyl CoA. Applying stress in the form of limiting nutrients, esp. N and P, can increase lipid content. However, this stress application also curtails the growth ratio, and thus may lower the amount of biomass and lead to a reduced overall lipid production.

Phosphorus is another essential element for the cultivation of microalgae. Phosphate, hydrogen phosphate, and so on, play an important role in the metabolic processes of microalgae, as well as the succession of phytoplankton in aquatic ecosystems. It takes part in many metabolic processes, such as signal transduction, energy conversion and photosynthesis. The metabolic mechanisms of P in the different forms are different in microalgae. Orthophosphate is most easily absorbed and significantly promotes the growth of microalgae [47]. Within a range, the growth rate of microalgae increases with increasing concentration of P, and the opposite occurs when the concentration is too high, which may be due to that the changing N/P inhibits the cell division of microalgae [48].

3.3. CO_2 and pH

CO_2 is one of the reactants and also one of the limiting factors in the photosynthesis of microalgae and plants. The photosynthesis of microalgae requires a certain CO_2 concentration, and the maximum photosynthetic efficiency is often achieved

with CO_2 concentrations from 1% to 5% (by volume). Increasing CO_2 levels can improve photosynthetic efficiency, which is consistent with a higher CO_2 concentration leading to a higher biomass of microalgae [49]. Adding NaHCO_3 to the medium during the cultivation of microalgae not only supply CO_2 to promote productivity, but also can be used as a buffering agent to control the pH [43]. The conversion efficiency of solar energy is improved by supplementing CO_2 to the medium of *Nitzschia closterium* to provide sufficient energy for photosynthesis [50]. The photosynthesis of *Nitzschia closterium* is affected by a high CO_2 concentration in two ways: increasing the binding of CO_2 to Rubisco (Ribulose biphosphate carboxylase oxygenase) sites thereby enhancing carboxylation, and inhibiting the photorespiration activity to increase the net photosynthetic efficiency [49,50]. In addition, the photosynthetic efficiency of photosystem II rises with increasing CO_2 levels and more light energy is captured and transformed to chemical energy [51]. This would explain why high CO_2 levels enhanced the photosynthetic efficiency of microalgae. CO_2 levels in flue gases are relatively high and are able to meet the need for CO_2 of microalgae. So it is very meaningful to utilize flue gases, which would reduce greenhouse gas emission as well as the cost in the economics of biofuels from microalgae. When *Chlorella* sp. (wild type) and its mutant were cultured with a continuous CO_2 -enriched gas (2%, 10% and 25% CO_2) and flue gas (approximately 25% CO_2 , 4% O_2 , 0.008% NO , and 0.009% SO_2), the maximum growth rate was found with *Chlorella* sp. (wild-type) with 2% CO_2 and the growth rate of the wild type was higher than that of the mutant [52]. It was also found that both *Chlorella* sp. (wild type) and its mutant grew faster with the flue gas than with the CO_2 -enriched gas. This was because NO absorbed in the medium can be converted to NO_2^- and then oxidized to NO_3^- , which can be utilized as a nitrogen source. The presence of NO_x in flue gas did not inhibit the growth of microalgae. A demonstration base with 400 m^2 has been built to culture microalgae in Shijiazhuang by Sinopec Group, which directly used the flue gas from a petroleum refinery, and the cultivation of *Chlorella* was successful.

The pH is another main factor influencing the abundance of inorganic carbon (DIC). When the pH is below 5, the majority of DIC is CO_2 . CO_2 and HCO_3^- are equal under the condition of pH = 6.6. It is almost all HCO_3^- when the pH is 8.3 [53]. Therefore, the pH should be controlled during cultivation to enhance the absorbability and utilization of CO_2 by microalgae. The pH can also directly affect the permeability of the cell and the hydronium forms of the inorganic salt, and indirectly influence the absorption of the inorganic salt. CO_2 in the culture is consumed by the microalgae during photosynthesis, thereby increasing the pH of the medium. Therefore, substances like hydrochloric acid and acetic acid have to be added to control the pH to keep the pH from increasing too much to be beyond the tolerance of the microalgae. Compared with hydrochloric acid, acetic acid has the advantage that it not only adjust the pH value but also is used as a carbon source to enhance the growth rate of microalgae [54].

3.4. Other factors

Other factors like temperature and O_2 are also important for the growth of microalgae. The tolerance range of temperature is different for different species. Freshwater microalgae, for instance, *Chlorella* and *Scenedesmus*, are able to adapt to the temperature in the range of 5–35 °C, with the optimum temperature of 25–30 °C, which should be controlled during the mass cultivation of microalgae. For microalgae and plants, O_2 is the necessary gas for respiration and also the released gas from photosynthesis. Much dissolved oxygen released as a byproduct of photosynthesis is accumulated in the medium, which can lead to a high dissolved oxygen content that can threaten the survival of the microalgae. Attention should be paid to the dissolved oxygen content in the cultivation of microalgae, which can oxidize one or more enzymes and affect the electron transmission chain, and inhibit the photosynthesis process.

4. Photobioreactors

Photobioreactors are the critical equipment in the biofuels from microalgae process, and are one of technical bottlenecks. Before an analysis of the photobioreactor configuration, general design considerations are presented so that photobioreactor designs can be evaluated and compared effectively. The aim of photobioreactor design is to achieve optimal mass transfer, light transfer and transmission with low cost. The following aspects should be considered in the design. A growth system that rely on artificial light is not often considered because of energy efficiency and costs. A design principle for photobioreactor design is to maximize the surface area to volume ratio to provide sufficient light. How to maximize the surface area to volume ratio is the major factor considered in the designs. CO_2 is necessary for photosynthesis and should be supplied in sufficient quantity for the microalgae. A high dissolved oxygen content does not favor photosynthesis, which limits the size of photobioreactors. A major disadvantage of open ponds is the loss of water to the atmosphere by evaporation, especially in water-poor areas. This problem almost does not exist in closed photobioreactors. Controlling the temperature in open photobioreactors is relatively easy due to evaporation, but this is difficult for closed photobioreactors. The cultivation location and mode have to be chosen according to the natural environmental conditions. In all cases, the design principle for photobioreactor designs is to maximize the surface to volume ratio and reduce the costs.

The common growth systems used are open and closed photobioreactors. The advantages and disadvantages of these two systems are compared in Table 1. Each system has tradeoffs between the key design parameters. The full understanding of these would help the development and innovation of photobioreactors. In practice, the energy consumption in the process is considerable, which is the core issue in the innovation of new photobioreactors. Several representative photobioreactors are discussed below.

4.1. Open photobioreactors

Raceway ponds are the most common open systems for

Table 1

Comparison of open and closed microalgae cultivation photobioreactor systems.

Parameter	Open system	Closed system
Biomass concentration	low, 0.1–0.5 g/L, high harvesting costs	high, 2–8 g/L, low harvesting costs
Space required	high	low
Construction costs	low	high
Contamination risk	high	low
Water losses	high	almost none
CO_2 -losses	high	low
Biomass quality	difficult to control	easy to control
Weather dependence	high	low
Repeatability	low	high
Period of culture	long, approx. 6–8 weeks	relatively short, approx. 2–4 weeks

commercial photobioreactors. They are usually constructed either as single or as groups of channels built by joining individual raceways together with a depth of 15–30 cm. Paddlewheels are often used to drive the water continuously around the circuit to use mixing to prevent the cells from depositing, thereby increasing the exposure of the microalgae to light and CO_2 . Other types of mixing systems, such as pumps and airlifts, have also been proposed. Open raceway ponds were used in the treatment of industrial wastewater in the United States, Israel and other countries from the 1960s to 1970s, and were applied to culture microalgae used for health purposes, such as *Spirulina*, in China, the United States, and Japan from the 1980s to 1990s [55,56]. Open pond raceways were the focus in the well known “Aquatic Species Program” conducted by the United States Department of Energy. The 1000 m² ponds were located in New Mexico and good research was achieved in this test site. However, the disadvantages of open pond raceways emerge outdoors, and this should be considered in the design, mainly how this affects the factors including light and medium depth.

First of all, light is the major factor that limits the photobioreactors. Many methods have been tried to increase the exposure of the cells to light. The flow velocity is an important factor to prevent the cells from deposition to increase the exposure of microalgae to light and CO_2 . It is chosen based on the sinking rate of the cells and the medium depth. So the appropriate flow rates for different photobioreactors are necessary for the mass cultivation. A velocity of 10 to 30 cm/s is found effective. Higher velocities are preferred, but it would consume too much energy [57]. Adding equipments to stir the medium is another effective method to increase the flow rate [58]. Providing additional light, especially artificial light, can enhance photosynthetic efficiency, but the energy consumption is high and very expensive. Hsieh et al. [59] employed transparent rectangular chambers (TRCs) to conduct light deep into an open tank photobioreactor to improve the photosynthetic efficiency of microalgae. This is an effective method with low cost. Figure 1 depicts the photobioreactor consisting of an open tank with different arrangements of TRCs. In this proposed photobioreactor, TRCs made of transparent acrylic provided a large area of illumination by conducting irradiance deep into the culture and redistributing light inside the tank. This increased the surface

Fig. 1. Open tank with different sizes of transparent rectangular chambers inserted [59].

area to volume ratio of the photobioreactor, and improved light utilization by the microalgae, especially at high cell density and deep depth. In our research, the TRCs were in an open tank with the same volume, shown in Fig. 1. TRC3 has the highest illumination surface-to-volume ratio and it gave the highest cell density (3.745 g/L) and biomass productivity (0.34 g/(L·d)). The total biomass from TRC3 was 56% more than a standard open tank system without TRCs. Many methods have been considered to enhance the light in an open pond raceway, but there are still no effective solutions.

The medium depth, which is in the range of 15–30 cm, is another key factor influencing the mass cultivation of microalgae in open pond raceways. This has a close relationship with light transmittance. In the initial phase, the density of microalgae is low, and the light can be transported to a great depth to promote the growth and photosynthetic efficiency, which is limited by the density [60]. Enhancing the light supply is possible by reducing layer thickness. For example, a thickness of 1–10 cm should be present for microalgae in deep open pond raceways, which is achieved by raising the bottom by less than or equal to 5 cm so that a thin layer is provided [61]. The photosynthesis by the microalgae can be increased, but the proportion of the bottom raised is not clear. The depth of open pond raceways affects construction cost. With a depth less than 15 cm, the construction and operation of large pond systems become difficult, and when this is more than 50 cm, the cost of construction becomes prohibitive and it is very difficult to get a high cell density.

There are some other problems in the cultivation of microalgae in open pond raceways. It is difficult to control the environmental conditions including CO₂, temperature, light, and pH. For instance, the optimum light intensity of *Chlorella* is 4–30 klx, but there can be up to 80–120 klx at noon. Photoinhibition easily happens under this high intensity, especially when the density of microalgae is low. CO₂ is another limiting factor but it is relatively easy to solve by bubbling. It is difficult to completely apply open pond systems to culture microalgae because these require highly selective environments due to the inherent threat of contamination and pollution from other species.

4.2. Closed photobioreactors

Although open systems have many advantages, they also have many problems in the mass cultivation of microalgae. Thus, closed systems have become the choice for biofuels pro-

duction. The volumetric productivity in closed photobioreactors is more than 30 times higher than that in open pond raceways [62]. Owing to the higher mass productivity, harvesting costs can be significantly reduced. Numerous types of closed photobioreactors have been designed to best control the conditions in mass cultivation. These can be divided into three main categories: tubular, column, and flat plate photobioreactors. Table 2 lists some closed photobioreactors with solar and artificial radiation that have overcome some shortcomings in open photobioreactors. However, these closed photobioreactors are still not very suitable to use for mass cultivation and need more design work.

Light path is an important design factor for closed photobioreactors. In a certain range, a shorter light path leads to more productivity. As shown in Table 2, the maximum productivity was gained in the thin-layer photobioreactor with the shortest light path. Light path, which can be considered as the depth of the medium, directly affects the probability of microalgae exposure to light. Therefore, microalgae grow well where the light path is short, and slowly where the light path is long due to the severe light attenuation. Chlorophyta was cultured in photobioreactors with light paths of 3 and 6 cm. The productivity with the 3 cm light path was higher by 50% than that with the light path of 6 cm [73]. In addition, the light path also affects the lipid content. The lipid content of microalgae cultured with the 3 cm light path was higher than that cultured with the 6 cm light path [73]. However, light intensity that is too high is not helpful for more lipid in the microalgae. When *Pyramidomonas* sp. and *Chlorophyceae* L-4 were cultured under different light intensities, the lipid content was reduced as the light intensity increased, and it decreased from 14.17% to 5.94% for *Pyramidomonas* sp. and from 14.17% to 3.40% for *Chlorophyceae* L-4 [74]. The light path may act in two ways to affect the lipid content. One is that microalgae grow fast with a short light path, which leads to nutrition stress when there is no more supply, and thus the lipid content increases. The other is that photoinhibition often occurs under high light intensity with a short light path, which affects the growth rate and lipid content.

Several typical closed photobioreactors are discussed below.

Tubular photobioreactors can be aligned horizontally, vertically, inclined or as a helix. The diameters of the tubes are generally 2.5–5.0 cm [64,75]. The length of the tubes depends on potential O₂ accumulation, CO₂ depletion and other factors, which limits the scale of tubular photobioreactors. To increase the scale, the tubes have to be arrayed horizontal fence-like,

which improves the utilization of land but increases the operation cost. The washing of the wall of tubular photobioreactors is another difficult problem. This has a closed relationship with light permeability. At present, mechanical cleaning is the main method to do the wash. Although tubular photobioreactors have many disadvantages, they are considered suitable for outdoor mass cultivation due to a large surface area to light that can allow a high density of microalgae. The productivity reached 830 mg/(L·d) in the works in Table 2. In addition to the light path, other factors should be considered when designing the photobioreactor, such as the ratio of culture volume. *Chlorella vulgaris* was cultured in helix tubes with 3.12 cm diameter and straight tubes with 3.2 cm diameter. The maximum productivities of the helix tubes and straight tubes were 40 and 600 mg/(L·d), respectively [63,66]. The light paths and culture conditions were similar in the two photobioreactors, but the maximum productivity of the straight tubes was almost 15 times higher than that of the helix tubes. This was because out of the 230 L culture volume, only 150 L was in the helix tube and under light and the rest was in the storage tank, decreasing the total productivity.

The main types of column photobioreactors are the bubbling, airlift and stirring types. They are made of transparent materials with low costs, such as glass, plastic and polyethylene [76,77]. Column photobioreactors are similar to the conventional fermentation tank, and the main difference is that the former needs an internal or external light. Airlift column photobioreactors use a draft tube to mix the medium. The light utilization efficiency of stirred column photobioreactors is relatively low. Column photobioreactors are generally 2–2.5 m high with 20–50 cm diameter. The simple materials used help to

Fig. 2. Schematic of column photobioreactor.

reduce costs, and mixing by CO₂ bubbling is another way to maximize CO₂ capture and to reduce mixing costs. However, it is difficult to obtain a high cell density in column photobioreactors and this limits the scale. The column photobioreactors used to culture microalgae by Sinopec Group in Beijing are shown in Fig. 2. It was found that when the density is high, light limits the growth of microalgae.

Flat plate photobioreactors are horizontal or vertically aligned [71,78] and is influenced by placement, light path, and dissolved oxygen concentration. They have attracted much attention due to the large surface area exposed to illumination and the high density of photoautotrophic cells observed. When flat plate photobioreactors are horizontal, the surface receives more light to enhance photosynthetic efficiency but they require more space and are liable to photoinhibition. Space and

Table 2

Textural properties of fresh CZ samples prepared by different methods.

Photobioreactor	Species	Light	Productivity	Ref.
Helical tubular photobioreactor, column diameter 3.12 cm, culture volume 230 L, CO ₂ supplied	<i>Chlorella vulgaris</i>	artificial adiation	maximum productivity, 40 mg/(L·d)	63
	<i>Chlorella emersonii</i>	artificial adiation	maximum productivity, 41 mg/(L·d)	
Helical tubular photobioreactor, diameter 1.6 cm, culture volume 21 L, CO ₂ supplied	<i>Spirulina platensis</i>	artificial adiation	maximum productivity, 400 mg/(L·d)	64
Horizontal tube, diameter 2.8 cm, culture volume 500 L, CO ₂ supplied	<i>Scenedesmus bliquus</i>	solar radiation	maximum productivity, 284 mg/(L·d); average productivity, 11.31 g/(m ² ·d)	65
Vertical tubular photobioreactor, 200 cm high, diameter 3.2 cm, culture volume 1.4 L, CO ₂ supplied	<i>Dunaliella tertiolecta</i>	artificial adiation	maximum productivity, 830 mg/(L·d)	66
	<i>Chlorella vulgaris</i>	artificial adiation	maximum productivity, 600 mg/(L·d)	
Vertical tubular photobioreactor, 63 cm high, diameter 6 cm, culture volume 0.26 L, CO ₂ supplied	<i>Anabaena variabilis</i>	artificial adiation	maximum productivity, 750 mg/(L·d)	67
Vertical tubular photobioreactor, 75 cm high, diameter 7.5 cm, culture volume 3.0 L, CO ₂ supplied	<i>Aphanothece croscopica</i>	artificial adiation	maximum productivity, 770 mg/(L·d)	68
Vertical tubular photobioreactor, diameter 7 cm, culture volume 0.8 L, CO ₂ supplied	<i>Chlorella sp.</i>	artificial adiation	maximum productivity, 640 mg/(L·d)	52
Vertical column, 300 cm high, diameter 16 cm, culture volume 50 L, CO ₂ supplied	<i>Chlorella sp.</i>	solar radiation	maximum productivity, 520 mg/(L·d); average productivity, 360 mg/(L·d)	52
Vertical tubular photobioreactor, 36 cm high, diameter 10 cm, culture volume 2 L, CO ₂ supplied	<i>Chlorella sp.</i>	artificial adiation	maximum productivity, 1.9 g/L; average productivity, 111.8 mg/(L·d)	69
Flat, light path 17 cm, culture volume 60 L, CO ₂ supplied	<i>Chlorella zofingiensis</i>	solar radiation	maximum productivity, 41.3 mg/(L·d) in May; maximum productivity, 58.4 mg/(L·d) in November	70
Flat, light path 10 cm, culture volume 200 L, CO ₂ supplied	<i>Nannochloropsis sp.</i>	solar radiation	average productivity, 240 mg/(L·d)	71
Thin-layer photobioreactor, 224 m ² , 28 m long, light path 0.6–0.7 cm, CO ₂ supplied	<i>Chlorella sp.</i>	solar radiation	average productivity, 4.3 g/(L·d)	72

Table 3

Areal and volumetric productivity of a vertical flat plate glass photobioreactor, as affected by the light path [71].

Light path (cm)	Areal productivity (g/(m ² ·d))	Volumetric productivity (g/(L·d))
1.3	5.5	0.846
2.6	7.25	0.558
5.2	9.25	0.355
10.0	12.10	0.239
17.0	10.05	0.118

light efficiency can be improved when flat plate photobioreactors are vertically aligned, but these have to be made of high cost rigid materials. It has been considered, by analogy with solar panels, for flat plate photobioreactors to automatically tilt to the zenith angle of the sun to maximize the amount of solar radiation intercepted. However, the scalability of such systems have proved difficult. High intensity radiation has been reported to be more efficient for microalgal cultivation. Indeed, the photosynthetic efficiency of vertical flat plate photobioreactors has been higher than optimal tilt reactors, reaching the value of 20% [79]. This is due to the fact that low irradiance levels result in higher photosynthetic efficiency and the too high irradiance levels in optimal tilt reactors inhibited the photosynthesis. The light paths were in the range of 1.5–3.0 cm, which limits the culture volume. Therefore, it is necessary to optimize the relationship among the areal productivity, volumetric productivity, and total productivity for flat plate and other photobioreactors. The length of the light path in a plate reactor determines its areal volume. The volumetric productivity was highest in the shortest light path, and lowest in the longest light path reactor, as shown in Table 3. The highest volumetric productivity was found with the shortest light path of 1.3 cm [71]. Volumetric productivity, therefore, has meaning only in relation to the light path. Areal productivity, in contrast, has an optimal light path at which the productivity or product is maximal. The accumulation of dissolved oxygen limits the scale of flat plate photobioreactors. Closed photobioreactors commonly make use of an external power supply to ensure sufficient mass transfer capacity. To attain the same mass transfer capacity, 53 W/m³ are needed in a flat plate photobioreactor, 40 W/m³ in a bubbling column and 2400–3200 W/m³ in a tubular photobioreactor [80].

Fig. 3. Schematic of the photobioreactor. (1a, 1b) Culture area; (2) Retention tank; (3) Circulation pump; (4) CO₂ storage tank. M—aeration air AS—algal suspension flow. CC—connecting channel; CD—carbon dioxide; DT—distribution tube; RW—rain water [81].

A thin layer photobioreactor is shown in Fig. 3. This photobioreactor is made of transparent materials for maximum solar energy capture, and a thin layer of dense culture flows across the culture area. Doucha et al. [81] reported that the inclination of the area was 1.1%–2.5%, the flow velocity of the microalgae suspension was preferably 30–150 cm/s, and the thickness of the suspension was 5–18 mm. The productivity of the microalgae *Chlorella* sp. was 38.2 g/(m²·d) or 4.3 g/(L·d), which was higher than those in the tubular, column, and flat plate photobioreactors. This productivity was obtained using batch feed cycles in a thin layer photobioreactor with a 224 m² culture area (length 28 m, slope 1.7%), and a 6–7 mm layer of culture [72,82]. The layer of culture is thin, so the area for CO₂ mass transfer is very important, esp. with the use of flue gas. To minimize the loss of dissolved CO₂ in the microalgal suspension, a retention tank and distribution tube with a thick layer should supply CO₂. The productivities presented in the researches indicated good prospect for using thin layer culture technology for the production of biomass as a feedstock for biofuels. However, there are still some shortcomings hindering the development of the thin layer photobioreactor. For example, due to the thinness of the layer, photoinhibition of microalgae easily happens when the density is not high, and the total culture volume is low. The concept of the use of a thin layer to culture a high concentration of microalgae is a new design for a photobioreactor. Rong et al. [83] invented a photobioreactor to culture microalgae that included collecting tank, conveying equipment, and culture area. In this photobioreactor, the medium flow through the culture area with a thin layer, and it is suitable for the growth of high density microalgae on a large scale and low costs. Cong et al. [61] devised an equipment that provides a thin layer for the photobioreactor with a thick layer like open pond raceways, thereby photosynthetic efficiency and productivity were increased. A patent by Gorny et al. [84] in 2011 and exhibited in Fig. 4 provided a flow-through photobioreactor containing at least one thermoplastic multi-wall, and one inner and two or more end caps. The medium flowed in the multi-wall sheets with high photosynthetic efficiency. UV light from the sun was filtered out by the reactor walls, the temperature was controlled, water in the system did not evaporate, and CO₂ from flue gas can be fed to increase the yield.

A variety of closed photobioreactors were introduced above, which can overcome the limitations of open pond raceways.

Fig. 4. Scheme of flow-through thermoplastic multi-wall sheet photobioreactor [84].

However, there are still some drawbacks to culturing microalgae on a large scale. The photobioreactors to culture microalgae for biofuels products require high biomass productivity per area and minimal costs. The best reactor type should have simple structure, easy operation, low costs, low energy consumption and that can be adapted to the local environment.

4.3. Combinations of open and/or closed systems

Some investigators have suggested that combinations of open and/or closed systems are the most effective configuration for mass cultivation. The combinations can be between open and/or closed systems, and between autotrophic and heterotrophic cultivation. Open pond raceways can provide abundant microalgae with a low density, which is then cultured in closed photobioreactors at a high density. Two stage processes are used to increase the productivity and lipid content of the microalgae. A high cell density is obtained in the first step in closed photobioreactors with a controlled environment. Next, the microalgae are exposed to nutrient deprivation by being transferred to open systems to increase the lipid content. Han et al. [85] combined open and closed systems, which included three reactors from the inside to the outside. The inside was a closed photobioreactor with artificial light to keep microalgae in the exponential growth phase. The next was an open photobioreactor with artificial or solar radiation to culture microalgae from the inside reactor. The outside was used to increase the lipid content under nutrient stress. Liu et al. [86] used a similar combination of open pond raceway and flat plate photobioreactor (or bubbling column), in which the biomass of *Nannochloropsis* was increased by 75%. The capital and operating costs for the combined systems are likely to be significantly higher than for one reactor, as well as the land requirements.

4.4. Computational fluid dynamics

To improve the development of photobioreactors, the challenge is the efficient design of large scale production facilities. Computational fluid dynamics (CFD) is attractive for the design of optimal large and integrated systems, by making it possible to estimate the productivities of the photobioreactor before conducting cultivation experiments. There is no need to construct a number of actual photobioreactors. However, there are still more aspects that have to be incorporated in the model, such as temperature change and the concentrations of CO₂ and nutrients. Sato et al. [87] applied a computer model system to evaluate the effects of the amounts of emitted O₂, CO₂ fixation amount and the growth curve of microalgae, and obtained the optimal culture conditions. Slegers et al. [88] gave more evidence for the validity of the simulation. To investigate the effect of location, variable sunlight, and reactor layout on biomass production in single standing and parallel positioned flat panels, a model approach was used to predict the yearly production of *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* in the Netherlands, France, and Algeria. The model approach found that vertically placed and east-west orientated

single panels produced the most biomass and shading and diffused light penetration between parallel panels had a strong effect on the productivity in parallel flat panels. This was in agreement with reports in the literature [88]. Although this approach is highly desirable for its low cost and many other benefits, it is also well known that CFD simulations need to be validated before use, and it is difficult to investigate all the decision variables of an experimental work.

The costs and energy consumption of the process are the key factors in the economics that inhibit the development of biofuels from microalgae. CFD simulations can reduce the cost and shorten the development cycle of photobioreactors. It can also be used to evaluate the energy consumption in the process, and can suggest combinations with other energy sources, such as wind energy and electrical energy (using the microalgae biomass), to form a complete and recyclable culture system. The mass cultivation of microalgae can absorb the CO₂ in flue gases to reduce the release of greenhouse gas. It also utilize wastewater, which help to protect the environment and reduce the cost of biofuels from microalgae.

5. Conclusions

Biofuels can be obtained from a variety of sources, but microalgae is of particular interest as one of the most promising sources of biomass for biofuels. Biofuels from microalgae has been demonstrated to have broad application prospects, but these currently still remain at the exploratory stage. This review underlines several aspects involved in the mass cultivation of microalgae, including microalgae species, metabolic mechanism, culture conditions, and photobioreactors, to help the development of biofuels from microalgae. The following aspects need to be further developed. Microalgae species have to be screened to get good robustness for mass cultivation using a mature system. The metabolic mechanism of microalgae has to be regulated to increase the growth rate and lipid content of the microalgae under mass cultivation conditions with low costs, high efficiency and easy operation. The combination of mass cultivation and the use of flue gas or wastewater to reduce the costs has to be studied, and the metabolic mechanisms of NO_x and SO_x in microalgae need to be investigated. Photobioreactors, which affect the photosynthesis of microalgae for the initial conversion of sunlight into stored energy, should receive more attention, and special photobioreactors for the mass cultivation of microalgae that overcome the problems of the costs and energy consumption are needed.

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Graphical Abstract

Chin. J. Catal., 2013, 34: 80–100 doi: 10.1016/S1872-2067(11)60497-X

Factors in mass cultivation of microalgae for biodiesel

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This review presents the factors that influence the mass cultivation of microalgae for biofuels, such as microalgae species/strains, metabolic mechanism, culture conditions and the photobioreactor.

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影响微藻规模化培养的因素

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摘要: 概述了微藻生物能源技术中的微藻规模化培养的研究现状,从藻种筛选、代谢机理、培养条件和光生物反应器几个不同的角度阐述了影响微藻规模化培养的因素,为微藻生物能源的发展及光生物反应器的研发提供建议与思路。

关键词: 微藻; 生物能源; 光生物反应器; 油脂含量; 规模化培养

收稿日期: 2012-10-28. 接受日期: 2012-11-26. 出版日期: 2013-01-20.

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基金来源: 国家重点基础研究发展计划 (973 计划, 2012CB224803).

本文的英文电子版由Elsevier出版社在ScienceDirect上出版(<http://www.sciencedirect.com/science/journal/18722067>).

1. 前言

能源是社会发展和经济增长的基本驱动力,其需求量日益增加,而化石能源作为一种不可再生资源,面临着巨大的供给压力. 因此,寻求替代能源变得尤为重要. 生物能源作为可再生绿色能源备受关注^[1,2]. 第一代生物能源是以淀粉、玉米等粮食作为原材料提取生物燃料; 纤维素生物能源是以农作物秸秆等作为原材料提取生物燃料,即第二代生物能源; 微藻生物能源,是通过培养微藻提取生物燃料,被称为第三代生物能源,与前两代生物

能源相比,它具有独特的优势而得到广泛研究^[3~5].

微藻生物能源技术主要涉及四个领域: 藻种筛选、微藻规模化培养、微藻采收和微藻生物质加工处理. 这是将生物工程与过程工程相结合的一个新领域,需要进行大量的研究及实践论证. 微藻生物能源技术所涉及的各个环节中,“规模化培养”是瓶颈,大规模地获得高油脂含量的微藻生物质是微藻生物能源技术能否产业化的最关键的难题. 很多微藻具有较高的油脂含量,通过筛选,已经得到多株高油脂含量的藻种,主要为绿藻和硅藻,例如小球藻 (*Chlorella*), 栅藻 (*Scenedesmus*), 褐指藻

(*Phaeodactylum*). 但是, 这些藻种在规模化培养中的油脂含量和生长速率显著低于在实验室培养的. 这可能是由于在实验室时, 为微藻提供了最适环境条件, 使其处于最佳生长状态. 而在规模化培养中, 培养条件未能达到最佳生长条件, 影响了油脂含量和生长速率. 在规模化培养中, 如何实现微藻快速生长而又不影响油脂含量成为阻碍微藻生物能源发展的瓶颈. 因此, 需要综合考虑藻种选育、代谢机理、培养条件、光生物反应器等多方面因素, 才能研发出适合微藻规模化培养的成套技术, 推动微藻生物能源的发展.

2. 藻种

2.1. 微藻

微藻是一类在显微镜下观察到的、具有光合作用的、自由生活的有机体, 其大小为几微米, 如小球藻约为 3~5 μm , 它们广泛存在于水生态系统中, 如淡水、盐水 (<3.5% 盐度)、海水 (3.5% 盐度) 和在一定温度、pH 范围内的水环境. 微藻属于藻类, 藻类还包括巨型藻 (或海藻), 其细胞组织已经分化成类似于高等植物的根、叶、颈, 有的长达 60 m. 微藻又可分为原核微藻和真核微藻. 原核微藻以蓝藻 (*Cyanobacteria*) 为主, 又名蓝细菌 (*Cyanobacteria*)、蓝绿藻 (*blue-green algae*), 属于传统的藻类学范畴, 含叶绿素 a, 不形成细胞器, 能进行光合作用, 细胞中蛋白质含量高, 可达干重的 70%, 脂肪含量低, 5% 左右^[6]. 真核微藻种类比较多, 例如绿藻 (*Chlorophyta*)、硅藻 (*Bacillariophyta*)、黄藻 (*Xanthophyta*), 细胞中蛋白质和脂肪的含量较高, 是生物能源藻种的主要来源.

微藻作为生物能源的原料具有独特的优势: 它生长快、油脂含量高 (20%~50%); 其培养不需消耗陆地农耕产品和占用耕地; 可以在污水及盐水中生长, 不需占用生活用水及灌溉用水; 可以利用废气中的 CO_2 进行光合作用; 可以用来生产多种燃料, 其副产品价值也很高. 除此之外, 光合作用的生理机制与植物相似, 但与陆生植物相比, 由于其构造简单而具有突出的光能转化效率, 不需要维持和复制各种组织结构, 藻细胞可以将其获得的大部分能量转化为生物量. 然而, 微藻的优势不仅仅因为具有高效的光合效率成为生物能源的主要研究对象, 更重要的是, 它不像陆生植物需要生产特殊的油类来养育种子, 每个微藻细胞就像一个油脂制造厂, 尽其可能地将获得的能量转化为油脂.

微藻能够进行多种营养模式也是非常具有吸引力的特性^[7~10], 它们既可利用光能和无机盐进行光能自养

(autotroph), 又可利用有机物进行异养 (heterotroph), 还可同时进行这两种营养方式, 即兼养 (mixotroph). 部分微藻, 例如小球藻, 可以利用现成的有机物进行异养或兼养, 这样生长更快. 微藻营养方式的多样性既可将微藻培养与 CO_2 减排相结合, 又可利用污水培养微藻, 因此微藻是非常具有潜力的生物能源研究对象. 异养或兼养的生长特性是微藻非常突出的特点, 因而在有光和无光的条件都能生长, 可以通过添加碳源的方法缩短微藻的培养周期, 并促进油脂的积累. 添加碳源, 尤其是有机碳源, 一个非常不利的潜在因素是可能促进了某些微生物的生长, 增加了微藻受污染的几率.

微藻中可以利用的资源有燃料和副产品两大类^[5,11,12]. 可能的燃料类型包括油脂、氢、类异戊二烯、碳水化合物、酒精 (直接或生物量转化) 和甲烷 (厌氧发酵). 油脂具有较高的能量和开发潜力, 主要以甘油三酯 (TAGs) 的形式存在, 常分布在细胞膜及胞内细胞器膜上, 部分油脂中含有碳水分子, 使其与石油有相似之处. 除了油脂外, 微藻副产品的价值也很高, 包括蛋白质、色素, 可用于医药品、食品、保健品、特殊的动物饲料、纳米材料等. 微藻在用于生产燃料和副产品的问题上存在较大争议, 我们认为, 这不应该是固定的模式, 应根据藻种的生理生化特性, 将细胞内代谢物与产品相结合, 再根据培养条件选择合适的模式体系, 进行过程优化, 从而就可能将单一特性与最终产品相匹配, 而非单纯的将策略集中于燃料产品或非燃料产品.

2.2. 微藻的筛选鉴定

藻种对于微藻生物能源产业来说至关重要, 它们的特性直接影响着后继的规模化培养、采收和生物质加工处理. 目前, 能源微藻的藻种包括绿藻、硅藻、金藻和裸藻等, 其中绿藻的研究和应用较为广泛. 多种微藻可以从藻种库中获得, UTEX (The Culture Collection of Algae at the University of Texas at Austin, Texas) 有约 3000 种, CCMP (The Provasoli-Guillard National Center for Culture of Marine Phytoplankton at the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor, Maine) 有 2500 多种. 然而, 由于部分藻种在藻种库中保存的时间比较长, 有的甚至有几十年, 藻种原有的一些特性可能已经丢失, 例如繁殖能力、营养多样性. 为了得到功能齐全、生命力旺盛的藻种进行微藻生物能源的开发, 需要从环境中分离新的、本土的藻种, 通过分离、筛选和鉴定保存具有潜力的藻种. 根据新分离的藻种可以获得地域环境的变化对微藻生理指标影响的基础数据, 为后期的规模

化培养提供参考依据^[13]。为了尽可能最大限度地获得多种多样的新陈代谢功能,藻种需要从一个涵盖足够大的、多样性的环境中采集,例如淡水、盐水、海洋和盐度超高的环境以及土壤,甚至是与其它生物体共存的环境。新藻种的采样和分离过程中,应详细记录采集时间、地点、环境因素和培养条件,还应说明微藻在自然环境中的演替。并不是所有的藻种都适合规模化的人工培养,我们在实践中发现很多高油脂含量的藻种在规模化培养中会出现各种问题。中国石化与中国科学院合作,在全国范围内采集、分离油脂高、生长速率快的藻种,采集的范围包括自然海域、内陆淡水区域、沿海地区和荒漠地区等,已分离得到多株符合要求的藻种,并对其中具有开发潜力的藻种进行基因测序,通过基因工程的手段进行改良,为微藻生物能源产业化提供优质藻种。

藻种的筛选主要包括生理、代谢产物和生命力。藻种生理指标包括一系列参数,例如最大生长速率、最大细胞密度、环境因素变化的耐受力(温度、pH、盐度、溶氧及CO₂水平等),及野外与实验室操作的可变性。藻种代谢产物的筛选不仅包括代谢成分和含量,还包括这些代谢产物作为生物能源的潜力,需要测定细胞内油脂、蛋白质和碳水化合物的组成并对其经济性进行评估,其中藻种油脂的快速分析可以大大简化工作,理想的分析方法应该可以区别中性和极性油脂,并能提供脂肪酸的结构。很多藻种可将代谢产物分泌到培养基中,这部分代谢产物常常被忽略。研究表明,它们具有较高的价值。因此,藻种筛选时应应对细胞外分泌物进行分离鉴定。此外,还应考虑藻种的生命力,包括培养密度、适应性、稳定性及特定环境中对掠食者的敏感性等等。研究表明,微藻在实验室与规模化培养中存在差异,为了确定藻种的生命力,小试模拟微藻规模化培养是必需的,因此需要通过小试模拟规模化培养进一步筛选适宜的藻种。在中国石化与中国科学院合作的“微藻生物柴油成套技术的研发”项目中,通过建立完善的实验室藻种评价体系,从自然界获得的藻种中高效筛选出生长速率快、油脂含量高的藻种,尤其是一些高耐受性的藻种,然后通过实验室小规模扩大培养进一步确定藻种的生长活力和耐受性,最后通过100L级以上的规模化培养确定微藻生物能源产业的藻种。

2.3. 油脂的合成和调控

甘油三酯(TAGs)是中性油脂的主要组成部分,也是微藻生物能源的主要来源,它是多种微藻在胁迫环境下的主要储存物质,例如高光、营养缺失^[14–17]。某些微藻在

自然条件下也可以在体内积累大量的TAGs(占干重的30%~60%),在光合效率和产油脂的潜能上比陆生植物要高很多。然而,在生理生化和分子生物学水平上,关于调控微藻脂肪酸和TAGs合成的研究较少。

TAGs合成的主要途径(Kennedy Pathway)为在基质中合成脂肪酸,将脂肪酸转化为甘油骨架,最后通过三步连续的乙酰辅酶A转酰基作用,在内质网上形成TAGs。乙酰辅酶A羧化酶(ACCCase)催化乙酰辅酶A转化为丙二酰辅酶A是脂肪酸生物合成的第一步,也是限速的一个关键代谢步骤。因此,有些学者认为,提高ACCCase的活性有利于提高微藻细胞内的油脂含量,然而实验结果却并非如此。通过转基因的方法使微藻细胞内的ACCCase活性大大提高,但油脂积累并没有显著增加,可能的原因是虽然ACCCase是油脂合成中的关键酶,但仍需要多步反应才能合成TAGs,在后续的多步反应中该酶活性逐渐被消耗,并不利于TAGs的积累^[18,19]。微藻中可能存在多种TAGs合成途径,而且各个途径对于TAGs合成的贡献依赖于环境或培养条件。在植物和酵母中发现了另一种将膜脂或/和碳水化合物转化为TAGs的方式,这种方式不依靠酰基辅酶A^[20,21];还未在微藻中发现这种转化方式。脂肪酸通常是膜脂和TAGs的合成前体,微藻细胞中这些前体在这两种途径中是如何分配的、这两种类型的油脂是如何相互转化的以及这个过程中基因是如何调控的都需要深入研究。如果微藻中油脂/TAGs的含量可以表现为种类或同类的特异性,不同藻种甚至同株藻种在某一环境中表现出特定的油脂/TAGs含量,那么就很难将由实验室藻种得到的油脂合成的调控机理推广到规模化培养中^[22]。类似地,将植物中油脂生物合成的调控信息用于藻种改良也存在一定困难。因此,研究脂肪酸和油脂合成的关键基因/酶和合成途径对微藻生物能源产业很重要,还需研究它们在微藻新陈代谢中的作用,以及培养环境的变化对基因/酶表达的影响,阐明规模化培养中油脂合成的调控机理。

在环境胁迫下,例如氮、磷、硅营养胁迫,以及盐度、温度和光强胁迫^[14–17],藻细胞迅速停止分裂,并且积累TAGs作为主要的能量储存物质。TAGs的合成、积累、沉淀并形成细胞质油脂体是微藻在环境胁迫下默认的合成途径。在胁迫条件下TAGs的合成途径可能更多样化。例如,胁迫条件下,光合作用电子传递链中积累的过剩电子可能诱导活性氧大量产生,这些活性氧反过来抑制光合作用和破坏膜脂、蛋白质和其它大分子,此时合成的TAGs可以消耗过剩的电子而缓解光氧化的胁迫。

C18 脂肪酸的合成需要消耗约 24 个由电子传递链生成的 NADPH, 几乎是合成同样量的碳水化合物和蛋白质所需能量的两倍。因此, 在高光或其它胁迫下, 合成 TAGs 可以起到释放过量电子保护光合作用电子传递链活性的作用^[23]。在微藻中, TAGs 的合成途径往往伴随着二级类胡萝卜素的合成^[24,25]。类胡萝卜素合成途径中产生的分子(如 β -胡萝卜素, 叶黄素)被运输到细胞内脂质体, 这些脂质体分散在外围可以作为遮光剂, 减少过剩的光照对叶绿体活性的影响。TAGs 的合成可能还需要卵磷脂、半乳糖酯或从膜系统中排泄的有毒脂肪酸, 如酰基供体, 因此具有膜脂解毒并以 TAGs 的形式将其沉淀的作用。培养液中氮浓度是影响微藻油脂含量的一个重要因素, 当氮浓度较低时, 藻细胞内的蛋白质减少, 脂肪和碳水化合物增加; 伴随着 TAGs 的积累, 而且这种积累会随着氮的减少或受限而加速。*Dunaliella tertiolecta* 在正常和缺氮培养基中培养时, 第五天出现了明显的油脂积累, 而且在正常培养基中的积累量大。然而, 在缺氮的培养条件下单位藻细胞密度的油脂积累更快, 第三天就出现了明显的积累, 在第四天时达到最大, 是正常培养基中藻细胞的 5 倍。虽然在两种培养条件下藻细胞油脂积累的速率不同, 但脂肪酸的组成几乎没有差别^[26]。

目前, 部分微藻的全基因组信息已经或很快可以得到, 在不同培养条件下, 产油微藻的 EST(Expressed Sequence Tag) 序列可以提供不同培养条件下微藻的基因表达以及与 cDNA 微阵列和/或蛋白质组的信息, 这些都将成为调控微藻光合作用碳分配和油脂合成提供理论基础。基于这些信息, 在微藻生物能源产业化过程中, 通过基因工程改良藻种新陈代谢是一条非常有效的途径, 可行的方法包括随机的、特异的突变和基因转化, 增强微藻各方面的生理指标。利用基因工程的方法对微藻油脂代谢进行调控有利于提高油脂含量、降低微藻生物能源成本, 例如增强脂肪酸合成途径、调控 TAGs 合成的旁路途径、抑制脂肪酸合成的竞争途径以及抑制脂肪酸分解等。除此之外, 还可改变油脂的组成, 提高产品及副产品价值^[18,19,27]。值得注意的是, 如果在微藻规模化培养中选择转基因藻种, 要对其生态安全性进行评估, 防止造成生态入侵。

3. 影响微藻生长和油脂含量的因素

微藻是非常具有研发潜质的一类生物原料。首先, 它具有基因序列和基因改造功能, 适合细胞过程的研究, 可以得到关于细胞代谢过程和燃料前体合成调控的信

息, 为后期培养提供有力依据; 其次, 微藻适应范围广, 可以在不同环境下培养。然而, 大量研究表明, 并不是所有在实验室中生长良好的藻种都适合规模化培养。对于一些已熟知的物种, 将实验室模式中得到的经验应用于规模化培养中比较容易; 对于微藻而言, 却并不确定, 也就是说, 并不能简单地将实验室微藻的培养经验完全应用于规模化培养。在实验室条件下研究影响微藻生长的因素, 并结合实际规模化培养中出现的问题具体分析, 对微藻规模化培养非常重要。影响微藻生长的因素很多, 主要有光、营养盐、CO₂、pH、温度和 O₂、以及其它胁迫因素等^[28–31]。微藻的生长及脂类物质的含量与光照、氮、磷和温度等培养条件密切相关^[15,32,33]。不同微藻生长和积累油脂的培养条件不尽相同, 应该根据不同藻种确定最佳培养条件, 提高生长速率和油脂含量。在藻种确定的条件下, 培养条件是影响微藻油脂含量的决定因素, 也是规模化产油微藻培养的主要工艺控制因素, 在本领域有大量专利文献报道。

3.1. 光

光的三因素, 光强、光质和光周期都会影响微藻的光合效率^[34]。光强, 即光的强度, 它在光补偿点以上才会有生物量的积累, 当光强超过光饱和点后, 即使光强再增加, 光合效率也不会增加, 而且光强过高对光合效率还有抑制作用, 因此在培养过程中应使光强处于这两个转折点之间, 并且尽量接近光饱和点使微藻处于最大光合效率的状态。光质, 指光的波长, 微藻和植物的光合系统中有两个系统, 即光系统 I 和光系统 II。光系统 I 的吸收峰位于 680 nm, 光系统 II 的吸收峰位于 700 nm, 微藻和植物对不同波长光的吸收量是不一样的。例如, 小球藻对红光的利用率最大, 其次为黄光和青光^[35]。光周期, 即光暗交替, 对光合作用效率也有影响。研究表明, 藻细胞在光区与暗区交替达到一定频率(常高于 1 Hz)时, 就会产生“闪光效应”, 从而有利于提高微藻对光的利用率^[36]。利用这个现象对光生物反应器进行改良, 在板式等封闭式光生物反应器内壁上加入一些挡板, 可以提高微藻的生产率^[37,38]。许多光生物反应器的创新都在于如何高效地利用闪光效应。在微藻规模化培养中, 常以自然光作为光源, 其优点首先是可以降低成本, 而且我国太阳能资源丰富, 年总辐照量可到达 280 W/m² 以上, 大部地区可达到 120 W/m²^[39]; 微藻对自然光的利用率可达到总辐照量的 3%~11%, 如果再加上人工条件, 利用率则更高。其次, 自然光光质好, 全波长。自然光的缺点就是光强难控制, 中午光强大, 微藻易发生光抑制, 阴雨天光照不足, 影响

微藻生长。

3.2. 营养盐

营养盐是微藻生长的基础,微藻生长必需的元素有碳、氧、氢、氮、钾、钙、镁、铁、硫、磷等十几种,此外还需要一些微量元素。碳、氢、氧、氮、磷和钾是微藻生长所需的大量元素,前三种可以从水和空气中获得,后三种需从营养盐中得到。在培养过程中氮磷往往会成为限制因素,影响藻细胞的生长和细胞内组分的合成,氮/磷比常作为微藻氮磷受限制的重要指标,该比值过高表示磷受限制,比值过低表示氮受限制。

氮是微藻生长、发育、繁殖等生理活动不可缺少的重要元素之一,不同的氮源及浓度对油脂的积累也有重要的影响。通常可以被微藻利用的氮源有铵盐、硝酸盐及尿素等,但在吸收速度与利用程度上有所差异^[40]。研究表明,微藻利用氮的能力的顺序为:氨氮>尿素>硝态氮>亚硝态氮,这是因为氨氮可以直接通过转氨基作用合成氨基酸,而其余氮源都需要通过酶的催化转为氨氮再被细胞利用。但是很多研究表明,利用尿素和硝酸盐培养微藻也可以达到非常好的效果^[41,42]。在研究尿素、硝酸钠和碳酸氢铵对小球藻生长及油脂含量的影响时发现,尿素对生长的促进作用最大,而硝酸钠对油脂的积累最有利,综合考虑生物量产率和油脂含量,宜选择硝酸钠作为优化氮源,但成本较高^[43]。随着氮浓度的增加,微藻的生长速率表现出先升高后下降的现象,高浓度的氮对生长有一定抑制作用^[43,44]。这可能是由于高浓度氮源促进微藻的大量繁殖,磷逐渐被耗尽,氮/磷比例失调,而藻细胞吸收过量的氮源,不利于细胞的分裂^[45]。在不同浓度的有机氮和无机氮培养基中培养 *Dunaliella tertiolecta*,藻细胞在 23 mmol/L 的 NaNO_3 中生长速率最高,当 NaNO_3 浓度增加到 46 mmol/L 时,藻细胞的生长速率并没有增加;分别在 1 mmol/L 的 NH_4Cl 与 2.3 mmol/L 的 NaNO_3 中培养时藻的生长速率相当,当增加 NH_4Cl 的浓度时生长速率有所降低。该研究结果表明,虽然藻细胞需要将 NO_3^- 还原为 NH_4^+ ,但过高的 NH_4^+ 不利于细胞的生长。这可能是由于过高浓度的 NH_4^+ 影响了藻细胞的呼吸作用^[26]。

低氮浓度有利于微藻油脂的积累,这也成为目前规模化培养中提高微藻油脂含量的常用工艺。可能是由于当氮含量较低时,细胞内的腺苷一磷酸 (AMP) 脱氢酶的含量增加,它将 AMP 大量转化为肌苷一磷酸 (IMP) 和氨,而线粒体中异柠檬酸脱氢酶 (ICDH) 多为 AMP 依赖性脱氢酶,细胞内 AMP 浓度的降低将减弱甚至完全抑制该酶

的活性^[46]。藻细胞内的柠檬酸可被柠檬酸裂解酶催化生成乙酰辅酶 A,也可进入柠檬酸循环,ICDH 是柠檬酸循环中的催化酶,在氮浓度含量较低时,ICDH 活性的降低促使柠檬酸更多的被催化生成乙酰辅酶 A,从而使细胞内脂肪的积累量增加。

磷是藻类生长发育所必需的元素。常用的磷源有磷酸盐、磷酸氢盐。磷在藻细胞代谢过程以及在水生态系统中都起着重要作用,参与信号传递、能量转换和光合作用等生理活动,也是水体中浮游植物竞争产生优势种群的原因之一。微藻对不同形态的磷酸盐有着不同的代谢机理,其中正磷酸盐最容易被吸收,且对生长促进作用显著^[47]。在一定范围内,微藻的生长随着磷浓度的增加而提高,当磷浓度过高时,不但不会促进微藻的生长,反而对微藻的生长起抑制作用。这可能是由于过高的磷改变了氮/磷比,从而抑制了细胞分裂^[48]。

3.3. CO_2 和 pH

CO_2 是微藻和植物光合作用的底物,又是光合作用的限制因子之一。微藻进行光合作用需要一定浓度的 CO_2 ,其体积比例为 1%~5% 时可达到最大光合效率;空气中的含量虽然较低,但通过鼓气也可以达到较高的光合效率。 CO_2 浓度的提高能显著地提高微藻对数生长期的光合效率和光合作用饱和光强,这与 CO_2 浓度的提高能得到较高的微藻生物量相一致^[49]。微藻培养过程中可适量添加 NaHCO_3 ,以缓解 CO_2 供应不足,作为碳源促进藻细胞生物量的增加;同时还可起到缓冲剂的作用。但是,一次性添加 NaHCO_3 浓度不宜过高,否则藻液 pH 偏碱性,微藻生长就会受到限制^[43]。补充 CO_2 有利于新月菱形藻对光能的利用和转化,提高光合效率,为光合作用碳循环提供充足的能量,进而促进新月菱形藻代谢产物积累^[50]。 CO_2 浓度升高可能在两个方面影响新月菱形藻的光合作用:(1) 增加了 CO_2 对 Rubisco (Ribulose biphosphate carboxylase oxygenase) 结合位点的竞争,从而提高羧化速度;(2) 通过抑制光呼吸作用提高净光合效率^[49,50]。另外,增加 CO_2 浓度有利于微藻光系统 II 光化学效率的提高,把捕获的光能以更高的效率转化为化学能,为光合作用碳同化提供充足的能量^[51]。这可能也是补充 CO_2 有利于提高光合效率的原因之一。工业烟道气中 CO_2 的含量较高,完全能满足微藻对 CO_2 的需要,若能将其应用到微藻规模化培养中,既降低了微藻培养成本又可用于减排 CO_2 ,非常具有意义。研究表明,微藻是利用烟道气生长的,用不同浓度的 CO_2 (2%, 10% 和 25%) 气体和烟道气 (25% CO_2 , 4% O_2 , 0.008% NO 和 0.008%

SO₂) 培养野生型和突变型小球藻, 结果表明, 通入 2% CO₂ 时小球藻的生长最快, 而且突变型比野生型小球藻生长速率要高^[52]. 该研究还发现, 通入烟道气时, 两株小球藻生长比通入不同浓度的 CO₂ 时生长速率高. 这可能是由于烟道气不仅提供了碳源, 还提供了氮源. 中国石化在石家庄建成了 400 m² 的微藻培养基地, 是国内首套利用微藻直接减排炼油厂烟气中 CO₂ 的示范装置. 在此基地中, 小球藻的小试培养已取得成功, 为下一步 CO₂ 减排奠定了基础.

pH 是影响水环境中无机碳 (DIC) 各形式丰度的一个重要因素. 在 pH=5 以下, DIC 大部分为 CO₂; pH=6.6 时, CO₂ 与 HCO₃⁻ 基本持平; 当 pH=8.3 时, DIC 绝大部分为 HCO₃⁻^[53]. 因此在微藻培养过程中应控制 pH, 便于 CO₂ 的吸收利用. pH 还能直接影响微藻细胞膜的渗透性及无机盐的离子形态, 间接影响细胞对无机盐的吸收. 研究表明, 小球藻在高光照、高 pH 时, 藻细胞对磷酸盐的吸收增加; 而在低光照、低 pH 时, 藻细胞对磷酸盐的吸收量减少. 随着光合作用的进行, 培养液中的 CO₂ 不断被消耗掉, 使得培养液的 pH 不断上升, 甚至超出微藻所能耐受的范围, 成为微藻生长的限制因子, 此时需要添加一些酸类物质来调节 pH, 如盐酸和乙酸. 比较而言, 乙酸具有很大的优势, 既可调节微藻培养液的 pH 值, 也可以作为碳源被微藻利用, 因此对微藻的生长具有双重作用^[54].

3.4. 其它因素

其它因素, 如温度、O₂, 都会影响微藻的生长. 不同的藻种对温度的适应范围不同, 淡水藻 (例如小球藻、栅藻) 能适应的温度范围为 5~35 °C, 最适温度为 25~30 °C, 在培养过程中应尽量使其处于最适温度范围内. 温度有时也会成为提高微藻油脂含量的胁迫因素. O₂ 是微藻呼吸作用必需的气体, 也是光合作用释放的气体, 它对微藻细胞而言是非常重要的. 微藻在进行旺盛的光合作用时会在培养液里积累大量的溶氧, 如果这些溶氧不能及时排放就会形成高溶氧, 会对微藻的生长甚至生存形成严重的胁迫, 因此在规模化培养中要注意溶氧的释放. 溶氧对微藻的影响主要有两方面: (1) 可能会氧化碳同化过程中的一种或数种酶及电子传递链供体受体, 对微藻形成胁迫; (2) 光合作用是产氧的过程, 过多的溶氧会促使光合反应朝逆向进行, 对光合作用产生抑制作用.

4. 微藻光生物反应器

微藻光生物反应器是微藻规模化培养过程中的关键设备, 也是目前微藻规模化培养的技术瓶颈之一, 研发

适合的微藻光生物反应器是推动微藻规模化培养技术发展的首要任务. 微藻光生物反应器的设计需考虑多方面的因素, 以达到传光、传质、传动效率的提高和降低成本的目的. (1) 光是限制微藻规模化培养的主要因素. 从经济角度出发, 微藻规模化培养常使用自然光, 因此微藻光生物反应器应具有高的表面积/体积比, 增加透光率及微藻对光的利用效率. (2) CO₂ 是微藻生长的重要物质, 在微藻规模化培养中消耗量大, 需通过合理的设计提高 CO₂ 的传质效果和利用率. (3) 溶氧的积累不利于微藻的生长和光合作用的进行, 在封闭式反应器表现的比较突出, 在一定程度上限制了封闭式反应器的大小和规模. (4) 在开放式反应器中水分蒸发比较严重, 尤其是在水资源不丰富的地区, 而封闭式反应器水分蒸发相对较少, 因此需要根据微藻规模化培养地点的自然条件选择合适的反应器和培养模式, 尽量降低水的消耗; 开放式光生物反应器由于水分的蒸发, 温度比较容易控制, 但是对于封闭或半封闭式光生物反应器, 温度控制是关键技术, 在某些特殊地区及不同季节应有相应的控温措施. (5) 微藻光生物反应器设计时还需考虑 pH、营养盐的添加以及清洗等问题, 在有外加驱动力的情况下还应考虑剪切力对微藻的伤害. 总之, 对于光生物反应器的研发设计, 如合提高“表面积/体积比”和“降低动力成本”是最主要的考虑因素.

微藻光生物反应器按其培养模式可以分为开放式和封闭式. 表 1 对两种培养模式进行了比较. 可见, 两种培养模式各有利弊, 只有充分了解两种培养模式下光生物反应器的优缺点, 才能对其进行改良和创新, 研发出适合微藻规模化培养的光生物反应器. 在实践中, 目前无论是哪种模式, 规模化培养过程中的“能耗”都相当可观, 是主要成本因素之一, 也是未来光生物反应器创新中必须解决的核心问题. 下面介绍几种具有代表性的微藻光生物反应器.

4.1. 开放式光生物反应器

跑道池是典型的开放式光生物反应器, 应用也很广泛. 跑道池在实际中一般设计面积较大, 单独或几个联合形成一个整体, 藻液厚 15~30 cm, 常以叶轮转动的方式推动藻液在池内循环、混合, 防止藻细胞沉淀吸附并增加微藻与光的接触概率. 为增加藻液的流动, 许多学者进行了多方面尝试, 诸如用拖动挡板、连续流动槽、气升、液体喷射等多种手段. 20 世纪 60~70 年代, 美国、以色列等国家将跑道池应用于工业污水处理; 80~90 年代起, 中国、美国、日本等将其应用于生产保健品, 例如螺

旋藻^[55,56]。1980~1996年美国启动的“水生生物物种”计划,研究以跑道池系统为微藻培养的主要方式,在New Mexico成功建成1000 m²的跑道池,并取得了一定的成果。在实际应用中,跑道池的不足逐渐显现出来,因此在设计跑道池时应合理确定影响微藻生长的参数,主要包括光和液厚。

首先,光是跑道池培养微藻主要的限制因素,设计时可采用多种方式提高跑道池等开放式光生物反应器的透光性,例如增加藻液的流速、添加补充光源或提高表面积/体积比。流速是跑道池中防止藻细胞沉降以及使其快速得到光照和CO₂的重要手段。藻液的流速取决于液厚和细胞的沉降速度,根据不同的液厚及培养藻细胞的特性,确定适合的流速。在一定范围内,藻液的流速越高越好,可以增加藻细胞接触阳光的几率,也可以防止贴壁和沉降,但这样会增加能耗,一般跑道池中流速在10~30 cm/s较合适^[57];另外,还可以通过辅助的搅动装置,来增加藻液的湍流,加速藻细胞上下运动的速率,以达到增加光强和CO₂浓度的作用^[58]。在跑道池中增加人工光源也能改善光照的问题,但能耗过高,从经济上是不可取的。相比增加人工光源,开放式光生物反应器中增加透明导光箱是相对经济、有效的方法之一。如图1所示,在一个开放式培养池中,用一个矩形的透明箱将光传导到藻液深处,通过透明箱的散射作用,增加微藻的光合效率和光能利用率,尤其在液厚比较大或藻细胞浓度比较高的情况下,效果尤为突出,这种方法相当于提高了表面积/体积比^[59]。该研究还表明,在透明导光箱体积一定的条件下,透明箱的个数越多培养效果越好。用一开放池培养小球藻,如图1所示,开放池中透明箱的体积相同,其中TRC3得到的细胞浓度和生产率最大,细胞浓度可以达到3.745 g/L,生产率可以达到0.34 g/(L·d),比未添加该装置时生物量提高了56%。尽管许多学者在跑道池等开放式光生物反应器的光照问题上提出了很多方法与建议,但目前仍没有行之有效的方案解决上述问题。

其次,跑道池的液厚是影响微藻生长的另一个因素,它直接影响光透率和微藻的光合效率,藻液厚度一般为15~30 cm。在接种初期,微藻浓度比较低,光可以到达较深的位置,为微藻的光合作用提供充足的光照;随着微藻的生长,浓度越来越大,光衰减严重,虽然通过搅拌可以使下层的藻细胞运动到上层,但远不能满足微藻对光的需要,很难得到较高的生物量,增加了后期微藻采收和加工处理的难度及成本^[60]。为了增加微藻的光合效率,可以通过减小液厚来实现。以跑道池为例,将跑道池某段的

底部提高,高度差小于或等于5 cm,那么藻液在经过这个区域时液厚就会变薄,形成厚度为1~10 cm的薄层区,藻液经过这段薄层区时可以进行旺盛的光合作用,即使藻液浓度比较高,也能满足微藻对光的需要,有利于提高藻液浓度,在一定程度上可以缓解跑道池等开放式光生物反应器的光照问题;但底部提高这部分区域的比例仍不清晰^[61]。跑道池内的液厚还影响建造成本。当深度小于15 cm时,底部的斜坡就很难做到合适的角度,而且在运行中也容易出问题;当深度过大,例如高于50 cm时,建造和运行成本会增加,而且难获得较高浓度的微藻。

跑道池在培养中还存在一些其它的问题,如在开放式培养过程中,微藻易受外界环境的影响,培养条件控制难度大,包括CO₂、温度、光照、pH等。以小球藻为例,小球藻对光强的适应范围为4~30 klx,而正午光强可达到80~120 klx,微藻浓度低时易发生光抑制,微藻浓度高时光照不充足。CO₂是相对光照比较容易解决的一个限制因素,通过鼓气或与烟道气结合补气,都能满足微藻生长的需要。虽然可以采用各种方法克服跑道池等开放式光生物反应器的不足,但很难完全应用开放式光生物反应器规模化培养微藻,以获得足够的生物量进行生物质加工处理。

4.2. 封闭式光生物反应器

开放式光生物反应器在微藻规模化培养中虽然具有很多优势,但实际培养中也存在很多的问题。部分研究者认为,封闭式光生物反应器是微藻规模化培养的发展方向,并开展了大量的研究,研发出了多种封闭式光生物反应器。封闭式光生物反应器单位体积的生产力是跑道池的30多倍^[62],大大简化后期微藻的采收和加工处理。管式、柱式和板式光生物反应器是典型的封闭式光生物反应器,在此基础上还衍生出很多新型光生物反应器,在一定程度上克服开放式光生物反应器的不足。表2列举一些封闭式反应器培养微藻的研究结果,无论是自然光还是人工光源都可得到比较高的生产率,但封闭式光生物反应器在微藻规模化培养中也存在不足,需要合理设计进行弥补。

光径是封闭式光生物反应器的一个重要设计因素,在一定范围内,反应器的光径越小越有利于微藻的生长。如表2所示,光径最小的是薄层反应器,得到的生产率最大。反应器的光径,也可以理解为藻液厚度,直接影响藻细胞随机接触光的几率,光径越小,微藻吸收的光照越多,生长越快;反之由于光衰减严重,微藻之间的遮挡比较严重,从而导致微藻生长比较缓慢,也容易滋生病虫

害. 在直径为3和6 cm两种不同光径下培养两种绿藻, 3 cm光径下比6 cm光径下得到的细胞干重高, 最大可提高50%^[73]. 光径不但影响微藻的生长, 还影响油脂含量. 在3 cm光径下培养时, 绿藻的总脂含量随培养时间的延长明显增加; 而6 cm光径下培养时, 绿藻的总脂积累时间滞后, 直到培养后期总脂含量才出现了明显的增加^[73]. 两株南极绿藻 (*Pyramidomonas* sp. 和 *Chlorophyceae* L-4), 在不同光强下培养, 其总脂含量随着光照强度的升高显著降低, 其中 *Pyramidomonas* sp. 的总脂含量由14.17%下降到5.94%, *Chlorophyceae* L-4由14.17%下降到3.40%^[74]. 光径对微藻油脂含量的影响可能有两方面的原因: (1) 光径小微藻生长速度快, 未进行营养补给或营养补给不及时, 就会形成胁迫, 提高油脂含量; (2) 由于光径小, 藻细胞获得的光照强度大, 容易发生光抑制, 影响微藻生长和油脂积累.

下面对几种典型的封闭式光生物反应器进行介绍.

管式光生物反应器根据形状及放置方式可分为垂直管式反应器、水平管式反应器、倾斜管式反应器、 α 型管式反应器等, 孔径范围一般为2.5~5.0 cm^[64,75]. 管内容易形成高溶氧, 限制了管子的长度, 不利于提高培养总体积. 为了提高总体积, 同时提高空间利用率, 常采用集光管的排列方式, 减少了土地占用面积, 增加了光的利用率, 但运行难度大、成本高. 在管式反应器培养微藻的过程中, 管壁的清洗非常困难, 藻细胞贴壁后严重影响光透性, 目前以机械清洗为主, 暂无其它有效的清洗方法. 虽然管式反应器存在很多不足, 但用它培养微藻可以提高生产率, 得到较高浓度的微藻, 在表2中列举的管式反应器中, 最大生产率可达到830 mg/(L·d). 在管式反应器中, 除了光径, 其它因素对微藻培养的影响也十分重要, 需合理设计各种参数才能提高生产率, 如培养体积比例. 用螺旋管和直管培养 *Chlorella vulgaris*, 两者光径和培养条件相当 (螺旋管反应器直径3.12 cm, 直管反应器直径3.2 cm), 直管反应器中的最大生产率 (600 mg/(L·d)) 是螺旋管反应器中的15倍 (40 mg/(L·d))^[63,66], 其主要原因是由于螺旋管反应器培养体积为230 L, 处于螺旋管中进行光合作用的藻液体积为150 L, 其余80 L藻液在储液箱中, 严重影响了整个反应器的生产率.

柱式光生物反应器主要类型有鼓泡式、气升式和搅拌式, 制造材料通常使用玻璃、聚乙烯等透光性能好而且成本低廉的材料^[76,77]. 与传统的发酵罐类似, 主要区别在于柱式光生物反应器需要在内部或外部设置光源. 鼓泡式和气升式的区别在于后者内部设有导流筒, 底部通气

带动液体循环达到混合的目的. 搅拌式光能利用率比较低, 由于先天结构的问题, 搅拌器占用反应器一定的空间, 设置内光源有困难, 而外光源又受表面积的限制, 藻细胞生长速率慢. 柱式光生物反应器一般高2~2.5 m, 直径20~50 cm, 反应器建设成本较低, 培养时使用的柱子可以循环利用, 但光利用率不稳定, 藻浓度对光透率的影响大, 在培养高浓度微藻方面存在困难. 中国石化在北京微藻培养基地采用柱式光生物反应器培养微藻, 如图2所示, 接种初期微藻生长快; 随着藻浓度的增加, 光成为主要的限制因素, 影响微藻的培养浓度.

板式光生物反应器, 主要有两种水平和竖直放置方式^[71,78]. 放置方式、光径和溶氧都是影响板式光生物反应器培养效率的重要因素. 水平放置时, 反应器接触光的表面积大、微藻光合效率高, 但是空间利用率低、光利用率低, 而且容易发生光抑制; 竖直放置时, 可以提高空间和光的利用率, 但对反应器材质强度要求比较高, 成本较高. 有些研究者认为, 板式光生物反应器的放置角度应该是随太阳而变化的, 虽然具有倾斜角度的平板可以获得更多的光照, 但是在实际培养过程中, 垂直板式光生物反应器中藻细胞的光合效率比有角度的反应器中的要高20%. 这可能是由于具有角度的板式光生物反应器能获得较多的光照, 光强过高, 不利于微藻光合作用的进行^[79]. 板式光生物反应器的光径 (内部厚度) 一般为1.5~3.0 cm, 限制了培养体积, 需权衡体积产率、面积产率和反应器产率的关系. 研究表明, 在一定范围内, 板式反应器的培养效率随着光径大小的减小而增加, 如表3所示. 随着光径的减小, 板式反应器的体积产率逐渐增加, 在1.3 cm时达到最大值0.846 g/(L·d), 而板式反应器的面积产率随着光径的减小先增加后降低, 这主要是由于虽然反应器单位体积的产率增加了, 但由于光径减小, 单位面积上培养的微藻总体积减少, 不利于提高板式光生物反应器的生产率^[71]. 溶氧是板式光生物反应器存在的另一个问题, 高溶氧限制了反应器的培养体积, 常用外加动力或换气的方法控制溶氧, 且同时达到传质、混匀和散热的目的. 达到相同的传质效果, 板式光生物反应器的能耗较低, 为53 W/m³, 而气泡柱式和管式光生物反应器的分别为40和2400~3200 W/m³^[80].

薄层光生物反应器属于封闭或半封闭式光生物反应器, 如图3所示. 通过外加动力将藻液注入倾斜流道内, 在初始动力和重力的作用下, 藻液在流道内形成有利于光合作用的薄层区. 该反应器倾斜槽的斜率1.1%~2.5%, 藻液流速30~150 cm/s, 藻液厚5~18 mm^[81].

在面积 224 m^2 , 长 28 m , 倾斜率 1.7% 的薄层光生物反应器上培养小球藻, 藻液厚 $6\sim 7\text{ mm}$, 小球藻的平均干重浓度可达到 $38.2\text{ g}/(\text{m}^2\cdot\text{d})$ 或 $4.3\text{ g}/(\text{L}\cdot\text{d})$ ^[72,82], 高于管式、板式和柱式光生物反应器中的产率. 薄层光生物反应器在流道内液厚较薄, 因此不适合在流道中补充 CO_2 , 特别是当与炼厂 CO_2 减排相结合时. 为了提高补充 CO_2 的利用率, 一般选择液厚较大区域进行补气, 在薄层光生物反应器中, 流道与流道连接处以及循环液体收集槽内液厚较大, 而且从流道中流回的藻液光合作用旺盛, CO_2 消耗快, 适合在这些位置补充 CO_2 . 但该装置从研发到现在十年多内并没用实现大规模培养, 说明其还存在一些不足. 首先是液厚, 液厚应随藻液浓度的变化而变化, 培养初期藻液浓度较低时, 应当适当增加液厚, 随着藻液浓度的增加, 逐渐减小液厚; 其次就是总生物量较低, 由于薄层液厚较小, 总培养体积偏低, 虽然可以得到高浓度的藻液, 但总生物量较低. 尽管薄层光生物反应器存在一些不足, 然而薄层的理念为培养高浓度微藻开辟了新思路, 在弥补已有薄层光生物反应器不足的基础上, 很多研究者研发出了多种新型微藻光生物反应器. 荣峻峰等^[83]发明了一种微藻培养装置, 该装置包括藻液存储单元、藻液输送设备和藻液导流面. 输送设备将存储单元的藻液送至导流面上, 藻液在导流面上形成较薄的液层, 通过参数控制液层的厚度及流速, 微藻在导流面上进行光合作用, 最后藻液通过导流面流回存储单元, 形成一个循环通路. 该装置适合于大规模、低成本、高效率的培养微藻. 低落差开放池也利用了薄层的思路, 为跑道池等开放式光生物反应器提供薄层区域, 当藻液经过此处时充分吸收光照, 能提高微藻的光合效率, 增加跑道池等开放式光生物反应器的培养浓度^[61]. 2011 年美国一专利也体现了薄层的思路^[84], 如图 4 所示, 储存箱内的藻液在重力的作用下流入下面具有倾斜角度的平板 (multi wall sheets) 中, 在平板上形成薄层区进行光合作用, 最后流入藻液收集箱中 (如果藻液浓度过高, 需要提供外加动力推动藻液流动), 平板上面有透明的覆盖物, 可以过滤掉紫外线, 也可以防止水分蒸发, 底部表面涂反光材料, 提高微藻光合效率.

以上介绍了多种微藻光生物反应器, 这些光生物反应器克服了开放式跑道池的某些不足, 有利于实现微藻的规模化培养, 但又存在一些新的问题, 阻碍了其在微藻规模化培养中的应用, 有待改进. 在满足微藻生长对光、 CO_2 、营养盐等需求的前提下, 适用于微藻规模化培养的光生物反应器应趋向结构简单、操作简便、成本低廉、

过程能耗低的方向发展, 并且根据培养地点自然环境的不同研发和采用合适的光生物反应器.

4.3. 多种光生物反应器结合

单一光生物反应器往往不能满足微藻规模化培养的需要, 很多研究者将相同或不同的反应器结合, 形成互补以扩大培养规模. 如开放式与封闭式反应器之间的结合、自养与异养的结合. 开放式光生物反应器培养体积大但微藻浓度低, 封闭式光生物反应器能提高微藻浓度但培养体积较小. 将两者结合起来可以相互弥补不足, 有利于提高微藻规模化培养的体积和浓度, 其结合方式有两种: (1) 将开放式反应器作为藻种供应装置, 为封闭式反应器提供低浓度藻种, 充分发挥封闭式反应器可以得到高浓度微藻的优势; (2) 将开放式与封闭式反应器糅合在一起, 通过封闭式反应器提高微藻的浓度, 最终提高生物量. 这两种结合一个重要的意义是减少微藻在开放式反应器中的时间以减少受外界污染的危险. 微藻在营养胁迫下会增加细胞内油脂含量, 那么将封闭式与开放式反应器结合可以得到高油脂含量、高浓度的藻液. 首先在封闭式反应器中得到高浓度的微藻, 然后再将其转入开放式反应器中进行营养胁迫, 增加藻细胞的油脂含量. 韩春梅等^[85]根据微藻生长的不同阶段采用不同的反应器, 将开放式与封闭式反应器相结合为同心圆反应器, 由内到外共有三级反应器: 第一级是以人工光源为主的封闭式反应器, 微藻保持在对数生长期, 为后两级培养提高藻种; 第二级是以自然光为主、人工光为辅的封闭式反应器, 微藻处于对数生长期或稳定期, 用来提高微藻生物量; 第三级是开放式或封闭式反应器, 对微藻进行营养胁迫以提高油脂含量. 刘天中等^[86]将跑道池与板式 (或气泡柱式) 光生物反应器相结合, 将跑道池中的部分藻液泵入板式 (或气泡柱式) 反应器的底部, 再通过上部的管道流回跑道池进行循环, 可以将微拟球藻 (*Nannochloropsis*) 生物量提高 75% .

4.4. 计算流体力学 (CFD) 模拟

微藻光生物反应器的研发是微藻生物能源产业生物质来源的关键环节, 传统的研发手段耗时且费用高. 随着计算机技术的进步, CFD 技术由于耗时短、成本低和效率高成为各类反应器设计、优化和规模化的方法, 具有非常好的应用前景. Sato 等^[87]利用计算机模拟微藻生长, 计算出微藻培养过程中溶氧释放、 CO_2 消耗以及微藻生长状态, 还优化出适合不同藻种生长的环境条件, 这种模拟方法比参考文献中的数据更具有针对性. 为了适应不同地区的培养环境, 利用计算机模拟地理位置、太

阳变化和板式光生物反应器放置角度三个因素对单个或平行放置的板式反应器微藻生物量产率的影响^[88]. 藻种为 *Phaeodactylum tricornutum* 和 *Thalassiosira pseudonana*, 地理位置为荷兰、法国和阿尔及利亚, 单个板式反应器培养时, 在荷兰培养时微藻年生物量最大. 多个反应器联合时, 遮挡和光散射(板与板之间)是主要的影响因素, 还发现反应器的放置方位对生产率的影响也很大.

光生物反应器的设备成本及过程能耗是微藻生物能源经济可行性的一个主要评价指标, 也是制约微藻生物能源发展的一个重要因素. 利用计算机模拟光生物反应器及培养过程, 既可以降低光生物反应器的研发成本, 缩短研发周期, 又可以对光生物反应器的过程能耗进行评估, 在可行的基础上还可以进一步模拟与其他能源相结合的效果, 如风能、电能(利用微藻生物质发电)等, 形成一个完整的、循环的培养体系. 另外, 微藻规模化培养的经济性, 还应考虑其带来的社会效益. 首先可用烟道气中的 CO_2 培养微藻, 降低温室气体的排放; 其次可利用废水培养微藻, 将废水中的有机物和无机物转化为有价值的生物质. 这样既能保护环境又能降低废气废水处理的成本, 不但降低了微藻规模化培养的成本, 而且赋予

其社会价值, 将大大推进微藻生物能源的发展.

5. 结束语

微藻生物能源具有广阔的应用前景, 但目前仍处于探索阶段, 其中微藻规模化培养是微藻生物能源技术中的关键环节. 需要系统研究藻种选育、代谢机理、培养条件以及光生物反应器等因素对微藻规模化培养的影响. 我们认为, 在微藻规模化培养过程中, 以下几个方面需深入研究: 建立规模化培养藻种的选育规程, 通过逐级筛选获得理想的优质藻种; 研究藻种的代谢机理, 通过控制培养条件和开发高效、低成本、易实施的油脂诱导工艺促进微藻生长及提高油脂含量, 同时开发其它具有价值的副产品; 与工业烟气减排和污水处理结合, 研究微藻对烟气中 NO_x 和 SO_x 的代谢规律, 建立工业烟气减排和污水处理与微藻培养结合的工业示范基地, 把解决微藻规模化培养的经济问题与环境保护相结合; 围绕“设备成本与过程能耗”两个核心问题, 开发微藻规模化培养的低成本培养模式, 研发培养高浓度微藻的光生物反应器, 开展连续规模化培养的实际验证, 突破“规模化培养”这一影响微藻生物能源技术能否产业化的最关键的难题.