Progress of Algaculture technology as Biofuel

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Abstract. As the problem of climate change becomes more and more serious, improving the carbon emission situation will be an effective way to solve various climate problems. Algal biofuels can build a complete carbon cycle and reduce carbon emissions. In this paper, the conditions required for microalgae cultivation and how diverse factors play a role in the growth rate and yield of microalgae were reviewed. And show how different factors like pH value, illumination, temperature, medium of culture, and nutrients in the medium affect the content of different substances in the final microalgae to help breed the matching microalgae. Macroscopically, this paper introduces both open raceway ponds and photobioreactors as microalgae cultivation systems and their respective characteristics. Microalgae will produce higher lipid content and hydrogen production capacity in different acid-base environments, respectively, and provide a nitrogen-deficient environment to further increase lipid production. The two-stage hybrid system combining an open raceway pond and photobioreactor has more obvious advantages in terms of growth rate and yield.

Keywords: Biofuel; biomass; algaculture; open raceway pond; photobioreactor.

1. Introduction

With the emergence of more and more severe environmental problems, more and more industries and people began to pay attention to environmental protection-related research and development. One of the most popular environmental issues at the moment is carbon neutrality and carbon peaking. As research that can realize the carbon cycle, biofuel can greatly reduce the carbon emissions produced by the original use of fossil fuels. Biofuels can also reduce the need for electricity to some extent and avoid the scrapping of petrol vehicles. Biofuels are usually produced by biochemical or thermal conversion of biomass, and the carbon produced by biofuels is captured in the next round of growth. Biofuels have now gone through three iterations, and there is a fourth generation of biofuels. The first two generations used agricultural products and domestic wastes as biomass sources.

Algae is the source of biomass in third-generation biofuels, and culturing is where it all begins. The success and efficiency of algae cultivation have been a concern. Algae farming has many advantages, such as less area occupied and high yield. Two main systems exist for algae farming, Including a photobioreactor and open raceway pond. Artificial algal culture seeks more efficient production and higher lipid yield. To do this, it is necessary to construct an environment suitable for algae growth and provide it with conditions such as temperature, humidity, salinity, pH, nitrogen and oxygen content, air pressure, and density. In terms of methods, most media are mainly based on batch culture and continuous flow culture. The use of algae in biofuels has not yet been large-scale, and progress is being made in developing more economical cultivation systems to pass pilot trials. The main development directions are hydrogen production by microalgae and lipid extraction by microalgae. Among them, Chlorella pyrenoidosa has the highest lipid extraction content and is widely used for lipid extractio [1]. Other algae such as Chlamydomonasmoewusii, Scenedesmus oblique, Clostridium butyricum, and Bacillus coagulans are used in anaerobic fermentation [2, 3]. For hydrogen production, photo-fermentation was mainly done using Anabaena variabilis [4]. In the actual process, in addition to the choice of microalgae types, the conditions of the culture environment will also lead to different growth rates and yields. In this paper, several factors and three main breeding systems will be introduced.

2. Factors for Algaculture

The lipids of microalgae provide a crucial resource for biofuels. By forming saturated fatty acids in palmitic acid during fat synthesis, fatty acid synthase can be expanded into stearic acid and some derivatives. To target the lipid content in microalgae, the ecology of microalgae and various influencing factors become particularly important. These factors affect the microalgae's lipid production and growth rate from micro to macro.

2.1. pH

The pH value is a crucial element in the culture of microalgae, which directly determines the growth of microalgae. The acidic environment promoted the microalgae to have a stronger hydrogen production ability. According to Sharma, A., & Arya, S. K.'s report, the pH is between 5.2 and 6.0, which is more acidic, and the microalgae can produce higher levels of glucose and starch. Therefore, the microalgae provided by the acidic environment are suitable for hydrogen production, and the hydrogen produced can be used to provide the corresponding biofuel [5]. On the other hand, the alkaline environment promotes the microalgae to produce higher lipid and fatty acid content. Fang et al. suggested that a culture environment with a pH of 8.2 to 8.7 was conducive to the collapse of algal walls. The pH in the medium can be adjusted by adding carbon dioxide to the medium. The pH setting was 6.5 at the beginning of the culture, and gradually changed to about 8 under the continuous addition of carbon dioxide [6]. This more alkaline environment is thought to be a factor in the production of higher levels of lipids. Sasaki, M. et al proposed the Parachlorella sp.BX1.5, Stress Resistance Dot test with 2 to 11 of pH was performed on different media of BX1.5. Notably, BG11 enhanced the instability of acid-side cells. In the study, microalgae cells oversecreted oil and acid EPS to help microalgae resist the acidic environment. But at the same time, under the condition of a pH of 11, the microalgae with high oil secretion also showed a certain alkaline resistance [7]. By properly adjusting the pH of the environment, algae can produce different biomass feedstocks to meet the demand for a single type of biomass production. More efficient output also contributed to the increase in profits.

2.2. Illumination

There are many different expectations for the illumination of microalgae. Different algae can absorb different wavelengths of light, which is the amount of energy they need to grow. The source of the growth rate is fluorescent lamps and the blue light of 380 nm to 500 nm and 600 nm to 700 nm of red light. But for hydrogen, algae culture is closely related to subsequent processes. Therefore, different processing methods have very different options for the light source of microalgae. For fluorescent lamps, light is between 5 and 10 percent. In direct light, the algae need a high intensity of light to play the characteristics of the high conversion rate of green algae. On the other hand, the light cycle of the light should be cultivated in the 16-18 hours [8]. In Singh, S., & Singh, P.'s research, chlorella was tested with a light intensity of 400 and increased by 200 each time until 2400 μmol m^{-2} s^{-1} at red wavelength, respectively. It was found that the light intensity of 400 μmol m^{-2} s^{-1} was too weak to support the growth of microalgae. On the other hand, the light intensity of 2400 μmol m^{-2} s^{-1} is too strong to avoid photo-suppression. The article studied many different algae including green algae, blue algae, red algae, and brown algae. Chlorophyll a and b in green algae help them absorb blue and red light more efficiently. Moreover, different microalgae have different cultivation light intensities. Enteromorpha species is 20 to 40 μmol m^{-2} s^{-1}. Chlamydomonas species can range from 50 to 1500μmol m^{-2} s^{-1}, or from 150 to 5000 μmol m^{-2} s^{-1}. Scenedesmus species are about 180 to 540 μmol m^{-2} s^{-1} [9].

2.3. Temperature

There are references to algae culture temperature and region, as well as climate-related. For example, in temperate countries, the temperature is between 10 and 25 ºC. On the other hand, it has

been suggested that although microalgae can adapt and survive at 15 to 85 ^{o}C, a mixture of cultures in the medium between 15 and 34 ^{o}C can still have an effect. Among them, hydrogen production of microalgae culture at 28 to 32 degrees Celsius is the best and can have a high hydrogen production rate. "Temperature shift" improves hydrogen production by exploiting the effect of temperature on enzymes in microalgae. Cellulomonas promotes the production of cellulolytic enzymes at 35 ^{o}C and achieves hydrolysis at 45 ^{o}C. This method can increase the reduced sugar content and finally get 4.79 mmol H_2/g reducing sugar [10].

2.4. Medium of Culture

The selection and preparation of medium are also important for the cultivation of microalgae. In itself, as the cradle of microalgae, the quality of the medium is very important. The quality is mainly determined by the degree of contamination of the culture, the quality of the medium, the concentration of the substrate, and the seed cultivation of the medium. Among them, seawater is considered in many studies, because the composition of seawater is complex and it is difficult to ensure a low pollution level. Archita Sharma et al. showed that the increase of glucose in substrate concentration directly affected the final hydrogen production rate of algae. In addition, water retention time and microbial construction also affect the growth rate and yield of algae. Hydraulic retention time is often associated with microbial communities. The study showed that adjusting the hydraulic retention time from 8 hours to 6 hours resulted in more than 90 percent of the glucose being degraded into biogas and fatty acids. As glucose decreases, microbial diversity also changes significantly. However, from the point of view of hydrogen production, microalgae can obtain a higher hydrogen production rate under such conditions. The steady state of the medium also affects the later microalgae cultivation, and the hydrogen concentration of the microalgae cultivation environment needs to be adjusted. The continuous production of hydrogen in the cultivation process of microalgae will lead to the increase of sucrose and the decrease in hydrogen production rate. Hydrogen partial pressure can help to eliminate excess hydrogen in the medium, and the results of the paper show that this way effectively improves the hydrogen production rate by 15 times. The article also mentions that seed cultivation, by using the right microflora, mostly clostridium and Enterobacter, can help inoculate the microalgae so that they can grow steadily. Many studies have shown that it is effective to take microbiota and glucose as substrate and adopt batch culture [5].

2.5. Nutrients

The cultivation of microalgae requires a large and continuous supply of nutrients. Traditionally, nitrogen phosphorus, and more trace elements have been needed to provide resources for cell culture. The conservation of matter is reflected in the fact that nitrogen and phosphorus, which make up proteins and ribonucleic acid, are usually necessary for the cell growth of microalgae. Thus, nitrogenous nutrients and phosphates, are delivered to the microalgae along with carbohydrates or glucose. Among them, years of research have pointed out that organic nitrogen is more nutritious than inorganic. Among other trace elements, an element like Fe can effectively activate the activity of different enzymes in microalgae, thereby increasing the hydrogen production rate of microalgae.

In the Parachlorella sp.BX1.5 research, BG11-N and BG11-P are experimentally studied based on BG11. The two are medium lacking nitrogen and phosphorus in BG11, respectively. The influences of several culture conditions on the composition production of microalgae were studied. The report showed that microalgae grown at a CO_2 concentration of 2 percent produced faster and higher yields than those grown at 0.04 percent. According to the experimental results provided by the institute, the yield reduction of BG11-P without phosphorus is greater than that of BG11-N without nitrogen. In Figure 1, it is clear to see that two percent of the carbon dioxide content supported the growth of microalgae in about 150 hours, while 0.04 percent of the carbon dioxide took nearly 500 hours [7].

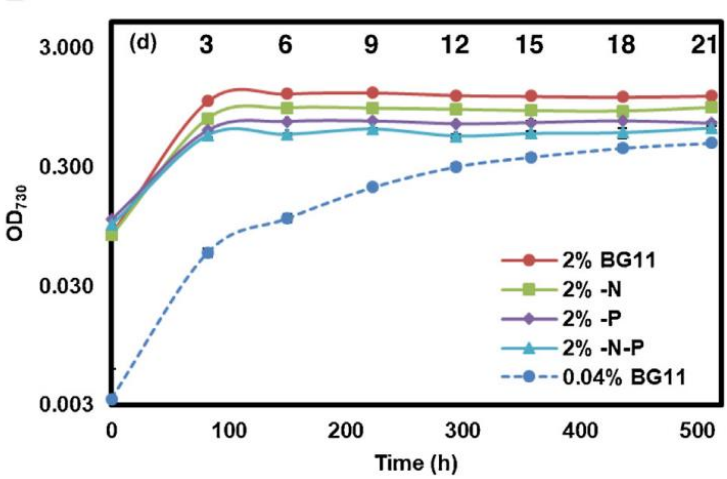


Fig 1. Microalgae turbidity of BX1.5 [7].

In addition, selective overproduction of acidic extracellular polysaccharides in BG11-N has been suggested. In lipid production results for different medium conditions, oil droplets and average diameter for cells reduced to 4.42 μm in BG11-N. Due to inadequate cell staining of BG11-N, extracellular polysaccharides may be inhibited. When microalgal cells were cultured, the addition of 2 percent BG11-P media produced larger cells with a diameter of about 6.45 μm. And BG11-P cells contain a lot of oil. When observed by transmission electron microscopy, it was found that after adding 0.04 percent carbon dioxide, the thylakoid membrane was diffused in the cytoplasm, and obvious starch accumulation was formed. At a 2 percent CO_2 concentration, microalgae cultured in nitrogen-deficient BG11-N showed a significant increase in lipid production, with more than 70 percent of the cell cross-section being lipid storage. In phosphorus-deficient BG11-P, it was about 60 percent, and the thylakoid membrane was more pronounced. It was found that starch content and oil yield were negatively correlated. At 0.04% CO_2 in the air. The experiment confirmed the complete conversion of lipids to fatty acid methyl esters. Finally, the dry weight of BG11 in 1L cells is 5.7 g, the dry weight of BG11-N is 1.77 g, the dry weight of BG11-P is 1.4 g, and the dry weight of BG11-N-P is 1.6 g. It shows that sufficient phosphorus and nitrogen can provide more biomass for microalgae. And these lipids are 16%, 27%, 32%, and 31%, respectively. These data show that media lacking phosphorus or nitrogen can effectively increase lipid content [7].

3. Algaculture System

The cultivation of microalgae has been completed from the perspective of microscopic experiments, and the next step is to make the cultivation of microalgae larger and more effective. To this end, algal cultivation systems and lipid induction techniques have been developed and applied to improve the growth rate and yield of microalgae.

3.1. Open Raceway Pond

The open pond system, which is an effective solution to inducing lipids, provides an open breeding environment for algae by mimicking the ecological conditions of a pond or lake. This open culture system has lower costs and higher area utilization. By combining with the raceway pond system, breeding quality and yield can be further improved. The raceway pond system closes the loop by building circulation pipes and helps mix carbon dioxide. However, such systems are relatively sensitive, more prone to contamination, and less productive. In Figure 2, during the 33-day microalgae cultivation at the open raceway pond, the first four days had higher rates of microalgae growth, with the harvest on day 13. Then the microalgae began to grow on day 16 and harvested 7 days later, with a density of microalgae reaching 2.3×10^{-6} cells/mL. In the third cycle, the culture was subjected to nutritional stress on days 27 and 28. 4 days later, the concentration was 2.8×10^{-6} cells/mL, and lipid accumulation occurred [11].

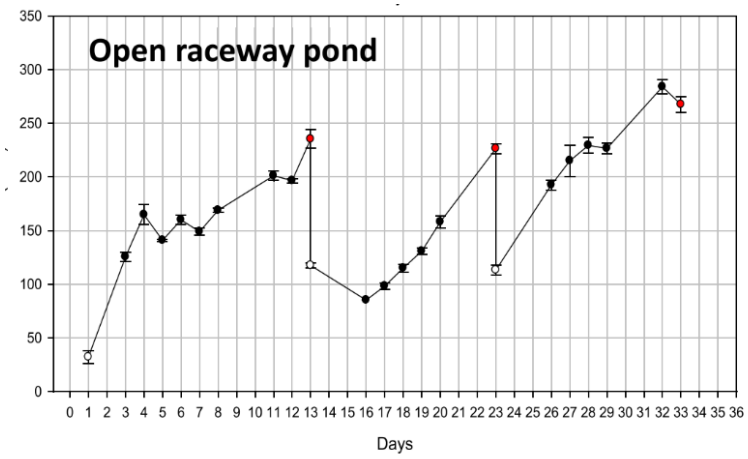


Fig 2. Microalgae density in ORP culture [12].

3.2. Photobioreactor

Photobioreactors improve the control of the reaction system by constructing a completely closed reaction system. Photobioreactors using artificial light sources can provide more precise conditions to improve the quality and production rate of algae cultivation. Many kinds of Photobioreactors have been studied, such as tubular, plate, foil, porous, and so on. Some of the advantages of Photobioreactors are higher specific surface area, better lighting, and more powerful gas exchange properties. However, at the same time, the residual carbon dioxide in some Photobioreactors will cause overheating, and eventually lead to the imbalance of gas content in the Photobioreactors, temperature changes, and other effects. In terms of economic benefits, the high complexity and specialized components of Photobioreactors make the current cost higher than the yield benefits of algal culture. In Figure 3, the photobioreactor was constructed with PVC and polyethylene flexible tubes. A lift is used to pressurize the air and has a pressure outlet point.

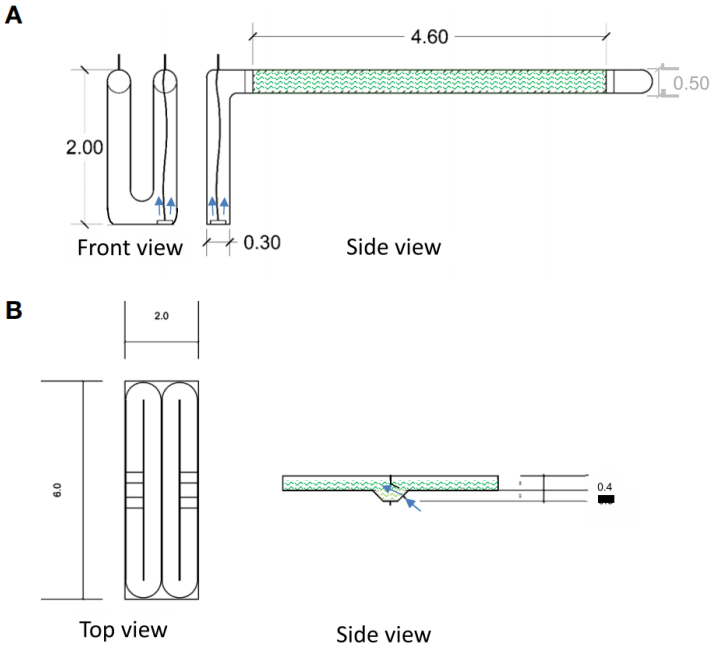


Fig 3. Photobioreactor structure [11].

In the 33-day experiment shown in Figure 4, the microalgae in the photobioreactor showed exponential growth from the sixth day, and a culture amount of 600 L was harvested on the thirteenth day. In the second growth cycle, the growth of microalgae was more stable and the harvest was on the 23rd day. Cell density increased more rapidly in the third growth cycle, but decreased at day 32 and was harvested at day 33 [11].

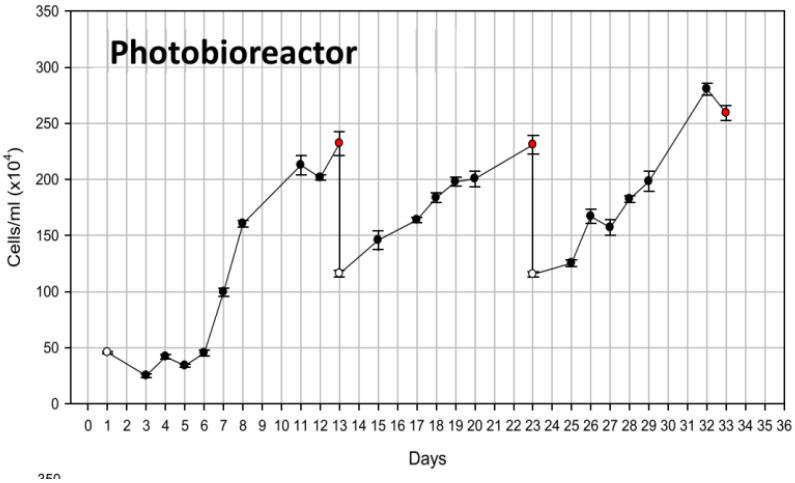


Fig 4. Microalgae density in Photobioreactor Culture [11].

3.3. Two-Stage Hybrid System

Hybrid systems combine OPS and PBR to balance the advantages and disadvantages of both. The advantage of OPS is that the open system can directly use sunlight as a light source, and it is simpler and lower cost. The advantage of PBR is that more targeted control of environmental conditions leads to faster algae growth and better algae quality. Therefore, hybrid systems take advantage of both advantages and combine them to cultivate a batch of algae quickly and with high quality by PBR and then use OPS for subsequent cultivation. Hybrid systems effectively circumvent the low quality and slow output of OPS, as well as the high costs associated with building a large PBR. In Figure 5 of this hybrid system, the bioconcentration of microalgae reached 3×10^{-6} cells/ml in only eight days, and 900 L of algaculture was extracted from a lipid induction tank. The new medium was added to the PBR for the second round of cultivation. During the cycle, the second harvest was on day 15 and the third harvest was on day 18, with a density of microalgae cells to be 1.9×10^{-6} cells/ml. Six microalgae harvests were completed over a 33-day incubation period [11].

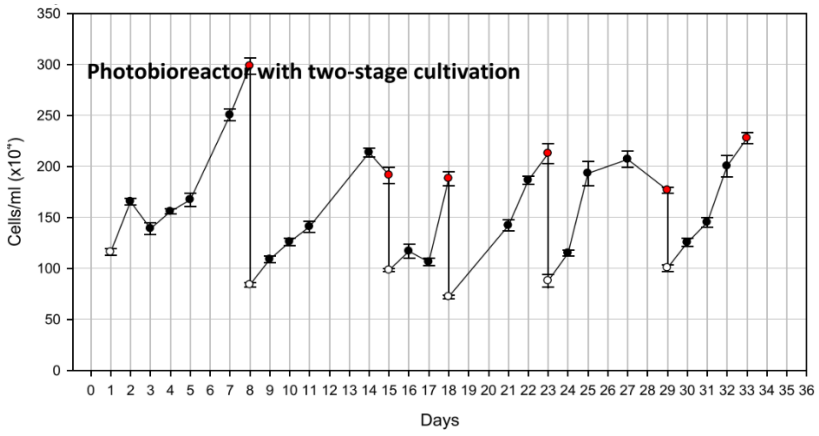


Fig 5. Microalgae density in Two-Stage Hybrid System Culture [11].

4. Summary

To achieve sustainable development goals, reduce carbon emissions, and slow down global warming, biofuels have become one of the ways that human beings must explore. For this reason, the possibilities contained in the third generation of microalgae biofuels are worth exploring. This article introduces the cultivation conditions of microalgae biofuels and the influence of different systems. There are many factors involved in the culture of microalgae, and there are very different conditions depending on the demand for the final product of microalgae. For hydrogen production, microalgae require an acidic environment, as well as high levels of constant light. However, for lipid-producing

microalgae, they prefer more alkaline environments and less intense fluorescent lighting. In addition, adequate nitrogen, phosphorus, and carbon dioxide content can provide greater microalgae production and growth rate in the addition of a medium substrate. On the other hand, microalgae cultured in a nitrogen-deficient medium contained more lipids and oils, with a higher lipid content. Macroscopically, the two-stage hybrid system combined the photobioreactor and the open raceway pond to significantly improve the yield and growth rate of microalgae. As the third-generation biofuel, the technology of microalgae culture has gradually matured and contributed to the development of biofuel with its shorter growth cycle and larger yield.

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