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## ORIGINAL RESEARCH ARTICLE

# Research progress of low dielectric benzoxazine resin

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### ABSTRACT

In this paper, the recent progress of bio-diesel from microalgae in recent years was reviewed. The research history of microbes from microalgae and the breeding and culture of microalgae were studied. Methods and techniques, and compare the microalgae with biofuel prepared from other energy crops. It is pointed out that microalgae is a kind of renewable energy with broad application prospect, and the future of microalgae production Direction of development.

**Keywords:** *microalgae; biodiesel; photobioreactor; engineering; microalgae; biorefinery*

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### Introduction

With the global climate deteriorating, the temperature continues to rise, control the greenhouse effect, the protection of human survival of the natural environment has become an urgent need to solve the problem. According to the global 'low carbon economy' this strategic decision, and as the global demand for resources continues to expand, as well as the collection of oil, mineral resources continue to decline, looking for renewable clean energy has become the current hot spots. Microalgae Biodiesel is expected to be the only clean energy alternative to oil resources with its unique advantages. In this paper, the microalgae breeding, culture, and its bio-oil extraction and biological refining and other aspects were discussed.

### Microalgae Biodiesel

Microalgae Biodiesel is a proven and well-known renewable environment-friendly fuel, the main component of fatty acid methyl ester (FAME) so called fuel methyl ester<sup>[1]</sup>, through chemical and biological enzyme From alcohol and other alcohols and oil in the main component of triglyceride ester exchange reaction occurs, thereby reducing the viscosity of the oil to improve the flow of oil and vaporization performance, to achieve as a fuel use requirements. Its performance and 0 # diesel oil similar, can replace 0 # diesel, for various types of tractors, river and vehicle diesel engine. Its calorific value of about 10 000 kcal / kg, can be mixed with 0 # diesel, no need to change the existing diesel engine<sup>[2]</sup>. The technology for the production and use of biodiesel has been in existence for many years due to its excellent environmental characteristics, good combustion performance and reproducibility, and is superior to the performance of petroleum diesel.

At present, biodiesel is mainly plant and animal fatty acids and 'engineering microalgae' and other aquatic plants algae and microbial<sup>[3]</sup>. For example, in the United States, mainly soybeans as raw materials, and waste edible oil is Japan's main bio-diesel production of raw materials, in recent years, China has also increased the intensity of the development of biodiesel industry, between the standardization and standardization of biodiesel production by Petrochemical Science and Technology Institute and other units drafted China's first national biodiesel standard, has been approved by the State Administration of Quality Supervision approved since May 1, 2007, the biodiesel national standards mainly related to B100 biodiesel (biodiesel content of 100 %) Of the composition, content, lubrication performance, alkane and other aspects of the detailed specifications, including a total of 17

technical requirements<sup>[4]</sup>. However, compared with the animal carrier, microalgae as a biodiesel carrier has a significant advantage, its superiority.

We can see that in the United States under the conditions of meeting the traffic fuels, microalgae as a raw material for the preparation of biodiesel land area (microalgae can use tidal flat, saline, desert and sea water, saline water and desert areas such as groundwater Large-scale cultivation), high oil production. And compared with other plants and animals, its growth rate is fast, the growth cycle is short, high oil content, and can be used as a natural culture of sea water for a large number of breeding.

At present, the production of biodiesel is mainly through the use of seawater as a marine microalgae culture medium and supplemented by commercial nitrate, phosphate fertilizer and several other nutrients cultured microalgae, and then through a series of physical and chemical methods to obtain biodiesel<sup>[6]</sup>.

## Microalgae biodiesel research history

As early as the 20th century, 60 years of microalgae as biodiesel raw materials research has already begun. In the 1970s, the Middle East war and other factors led to tight international crude oil supply. The United States, Japan, Australia and other Western countries in order to reduce dependence on imported crude oil, vigorously funded microalgae culture oil project<sup>[7]</sup>. Although the use of microalgae oil production process was feasible at the time, but the cost of producing oil than the oil prices at that time several percentage points higher.

The exploration of microalgae fats was then focused on obtaining functional oils such as DHA<sup>[8]</sup>. In recent years, with the development of modern industrial biotechnology, has been more high oil production capacity or its antibacterial ability of oil-producing microalgae resources, improve the efficiency of microalgae oil production. At the same time since the 20th century, 90 years since the rapid development of the world economy, greatly increased demand for oil, the extensive use of oil led to energy shortages, environmental degradation and other issues, the world began to vigorously develop microalgae oil production.

## Breeding of Micro - algae and Its Research Progress

Microalgae algae is one of the most primitive organisms, usually single cells, filaments or flakes, simple structure, the whole organism can carry out

photosynthesis, so photosynthetic efficiency, short growth cycle, fast.

Algae by size can be divided into algae (such as kelp and seaweed) and microalgae (for single cells or filaments, diameter less than 1mm)<sup>[9]</sup>. Oil-producing microalgae, in order to under certain conditions can carbon dioxide, carbohydrates, hydrocarbons and ordinary oil and other carbon sources into large amounts of oil stored in the algae, and the oil content of more than 20% of the total amount of microalgae<sup>[10]</sup>. Correspondingly, the oil extracted from the oil-producing microalgae is called microalgae oil.

Since microalgae are more efficient than other plants that use light to produce oil, most microalgae produce far more oil than the best. Its photosynthetic conversion rate of up to 10%, oil content up to 30%, its yield is the best oil crops 8-24 times<sup>[11]</sup>.

Microalgae are single-celled organisms, so all biomass can be used to prepare biodiesel, and its genetic transformation is relatively easy. According to the current total of biodiesel produced by animals and plants, it can only meet 0.3% of the transport oil, so the production of biodiesel through microalgae is the real solution to the problem of insufficient fuel oil resources, the fundamental way<sup>[12]</sup>.

The United States Renewable Energy Laboratory has done a lot of work in engineering microalgae. In the 1990s, the acetyl-CoA carboxylase gene and nitrate reductase gene, which affect the photosynthesis of microalgae, were discovered and the introduction of foreign gene *Chlorella* transformation system, and built 'engineering microalgae', that is, a kind of algae 'engineering small algae'<sup>[13]</sup>. The algae in the laboratory conditions can make the lipid mass fraction of up to 60%, outdoor growth can reach more than 40%, is expected per square meter 'engineering microalgae' can produce about 116L diesel oil per year.

Microalgae as a bio-diesel raw materials, have a high biomass and fertilizer quality to have a competitive advantage. According to the current study shows that many conditions on the accumulation of lipid content will have a great impact, microalgae lipid content due to different types and individuals vary widely, accounting for dry weight ratio of 1% -70% Under certain conditions up to 80% - 90% (such as missing N, Si case)<sup>[14]</sup>. At the same time, the salinity, CO<sub>2</sub> concentration, Fe ion concentration<sup>[15]</sup> and IAA<sup>[16]</sup> in the culture medium all had an effect on the growth and lipid accumulation of microalgae.

At present, China's Tsinghua University, Miao Xiaoling<sup>[17]</sup> and so on through heterotrophic transformation of cell engineering technology to obtain high-fat content of heterotrophic *Chlorella* cells, lipid content of 55% of cell dry weight, autotrophic algae cells 4 Times Representatives of the Chinese Academy of Sciences as the representative of the relevant research institutions have been screened out of hydrocarbon-rich microalgae. US Department of Energy from 1978 to 1996 through the 'aquatic species plan', has screened more than 300 kinds of fast growing, lipid content of 40% -60% of the microalgae, belonging to green algae, diatoms, cyanobacteria<sup>[14]</sup>. 1990 - 2000, the Ministry of International Trade and Industry funded a project called 'Earth Research Update Technology Program' project, cost more than 300 million US dollars, screening out a variety of high CO<sub>2</sub> concentration and high temperature, fast growth, can open into high cell density of algae species.

Microalgae Biodiesel is the most popular form of microbial biomass utilization, and a lot of research has been carried out at home and abroad. With the development of molecular biotechnology, microalgae engineering transformation has entered a considerable scale of the study, mainly from the following aspects:

(1) Foreign DNA into the current foreign DNA can be introduced into the microalgae technology in the gene marksmanship, glass beads, emery and electric shock method, but the gene gun method is the most common. In order to increase the high expression rate of exogenous genes, nuclear matrix binding domain was added to the vector, which has been successfully used in microalgae<sup>[18]</sup>.

(2) Screening markers and promoter markers and promoters are the key to the expression of exogenous genes. At present, the main screening factors are kanamycin resistance gene nptII, antibiotic Zeocin resistance gene sh-ble, N-acetylation in the gene nat1, sulfur ion transport protein gene sat-1 and so on. The promoter of the acetyl-CoA carboxylase ACC1 and the chloroplast a / c-binding protein gene fcp promoter are widely used<sup>[19]</sup>.

(3) Related gene research fatty acid biosynthetic pathway with pyruvate synthesis of acetyl COA as the substrate, acetylated coenzyme A carboxylase (ACCase) catalyzed into the fatty acid synthesis pathway, which is the enzyme reaction to the whole process of the speed limit Key steps, so ACCase is the key rate limiting enzyme. At present in the 'engineering microalgae' research, mainly to improve

the ACC gene expression efficiency, to control the level of lipid accumulation<sup>[20-22]</sup>.

## Culture of microalgae

Microalgae culture technology is another key factor affecting the rate of biomass synthesis of microalgae. In the nature of microalgae are classified as autotrophic microorganisms, because of their intracellular presence of photosynthetic system PSII is also known as microalgae plants. In the process of artificial culture, the microalgae are divided into autotrophic microalgae and heterotrophic microalgae and polyculture microbes<sup>[23]</sup>, depending on whether the outside world is required to provide organic matter. Microalgae autotrophic with less energy consumption, easy to grow. But there are still more growth rate

Low, high cost of harvesting, low cell density, low yield, large area, long growth cycle and other issues. Microalgae heterotrophic culture is not affected by light, faster growth than self-support, so you can achieve higher yields, while shortening the culture cycle. Microalgae heterotrophic is generally fermented, heterotrophic medium are generally in the autologous medium based on the appropriate amount of organic matter after the improvement made. However, heterotrophic microalgae has high energy consumption and high cultivation cost. It is widely used to produce high value-added fatty acids. If used to produce biodiesel, the cost is too high and the CO<sub>2</sub> emission will increase<sup>[24]</sup>.

## Microalgae photobioreactor

At present, the method of large-scale autotrophic culture of microalgae is: the open cell (the open type) and the closed type according to its structure.

### Open photobioreactor

The reactor structure is simple, easy to operate, low cost, the most typical is the runway reactor, which is actually covers an area of 1000 - 5000 square meters; culture medium depth of 15CM circular shallow pool, with natural light for the light, Heat source, the impeller rotation of the way the culture medium in the pool mixing, circulation, to prevent algae precipitation, and improve light utilization, but also access to air or CO<sub>2</sub> gas for bubbling or air stirring up<sup>[23]</sup>. To prevent moisture evaporation and contamination usually cover the transparent membrane above the cell body, make it a closed cell. Although the use of open photobioreactor has been early, but for the mild culture conditions and weak competition in the micro-algae it has some

technical defects: easy pollution, a large amount of water, CO<sub>2</sub> supply is insufficient, CO<sub>2</sub> utilization is low, Due to environmental factors, so under normal circumstances for the engineering microalgae are closed reactor<sup>[25]</sup>.

### Closed photobioreactor

Closed photobioreactors can extend the culture time of purebred microalgae and have been successfully used for mass production of microalgae. (3) free from external environmental factors, the annual growth period is longer; (4) can maintain a high level of growth; (2) the cultivation of the conditions, easy to control; Cultivate density and easy to harvest. However, compared to open-type photosynthetic bioreactor its cost is too high, however, because of its advantages have been more and more people's attention. Currently used in closed bioreactors are: plate photobioreactors, columnar photobioreactors, tubular photobioreactors, in which plate photobioreactors, in particular flat plate photobioreactors, have a relatively high light energy Utilization and the most promising large-scale application<sup>[27]</sup>.

Pathways of lipid biosynthesis which are known or hypothesized to occur in algae

### Synthesis of Microalgae Lipid

Microalgae contains a lot of chlorophyll, has a strong ability to photosynthesis. The synthesis of microalgae grease begins with photosynthesis. Light energy can be transformed into chemical energy through PS and PS in microalgae photosynthetic system, and then pass through a series of electrons to pass into the Calvin cycle. In the Calvin cycle, ribulose-1,5-diphosphate carboxylase catalyzes ribulose-1,5-bisphosphate to immobilize carbon dioxide to produce 3-phosphoglycerate, and 3-phosphoglycerate can further produce other fats. This enzymatic reaction is a critical step in limiting the rate of lipid synthesis and oxidation, so ribulose-1,5-diphosphate carboxylase is the critical rate of lipid biosynthetic pathway<sup>[28]</sup>.

### Lipid extraction and transformation

Microalgae lipid extraction method<sup>[29]</sup> mainly chloroform - methanol method, acid hydrolysis, Soxhlet extraction method. Chloroform-methanol extraction of oil is highly toxic and difficult to recover, and the determination of high water samples is more effective; acid hydrolysis hydrolysis is likely to cause a lot of water loss; the measured sample without sufficient grinding, the combination of So

that the results can be completely free, while the results are low, while the organic solvent extraction is often easy to emulsify; Soxhlet extraction method is a classic method, the majority of the sample results are more reliable, but time-consuming, large amount of solvent, and the need for special cable The extractor. Miao Xiaoling and so on using n-hexane from the heterotrophic growth of Chlorella (lipid content up to 55% of cell dry weight is generally 4 times the number of autotrophic algae cells) to obtain a large number of fat.

The transesterification method is a commonly used method for the preparation of biodiesel<sup>[30]</sup>. The higher fatty acid glycerides or higher fatty acids extracted from microalgae are usually transesterified or esterified with alkyl monoalcohols under the action of the higher fatty acid alkyl esters, mainly methanol, mainly because the methanol is cheap and convenient Easy to get, high chemical and alkaline catalyst soluble in methanol, and the resulting methyl ester heat value is high, the density is small. Production also used ethanol, 2-propanol and butanol. According to the different catalyst, ester exchange method can be divided into acid catalysis, alkali catalysis, enzyme catalysis three. The acid catalyst<sup>[31]</sup> generally used as a catalyst for 100% concentrated sulfuric acid, so the material requirements of the device is high, it is the unsaturated fatty acids and water containing oil has obvious advantages; acid can catalyze the acid and alcohol ester Reaction, in the production of biodiesel with microalgae without pretreatment. Therefore, acid catalysis is suitable for the preparation of biodiesel from microalgae. At present, in order to reduce the pollution of the environment and the pollution and corrosion of the equipment, some scientists began to use incomplete sulfur and solid acid as a catalyst instead of concentrated sulfuric acid, the product and the catalyst easy to separate, and the catalyst can also be recycled. The catalyst used in general catalysis is sodium hydroxide and potassium hydroxide, and sometimes sodium alkoxide, usually sodium methoxide. Due to the higher basicity of sodium methoxide, it is easier to carry out the transesterification reaction with glyceride, which is more significant for those with less active glycerides. Since the transesterification reaction is a reversible reaction, both the biodiesel can be produced and the glycerol ester can be produced. Therefore, the alcohol / oil ratio is chosen to be 6: 1 in order to allow the chemical reaction to proceed in the direction of the formation of the biodiesel. The reaction temperature is generally selected in the vicinity of the boiling point of the alcohol compound, the alcohol is generally



methanol, so the choice of its boiling point of 65. The most important advantage of the biocatalytic production of biodiesel is that the reaction speed is fast, the reaction time is short and the yield of bio-oil is high. The biggest drawback is that the oil used must be anhydrous, no free fatty acid glycerides, because if there is free fatty acids, must react with the base, so that the esterification reaction cannot be carried out; if the oil contains a lot of water, prone to saponification reaction The reaction yield is reduced and introduced into by-products, which are not easily separated. Therefore, the use of alkali as a catalyst before the oil must be pretreated<sup>[33]</sup>, that is, in addition to acid removal. Another problem with base catalysis is the recovery and utilization of the catalyst base (NaOH and KOH). Under normal circumstances, NaOH and KOH cannot completely avoid water during the catalytic process, and the saponification reaction takes place more or less, and NaOH and KOH are Strong bases are also likely to react with the equipment and are therefore not readily available for purification<sup>[34]</sup>. It has been proposed to synthesize biodiesel with solid bases, such as Verziu et al.<sup>[25]</sup> catalyzed the synthesis of biodiesel with solid base MgO and achieved a yield of more than 90%. The catalyst is not active after 4-5, the rate is still above 90%.

The use of acid catalysis or alkali catalysis, but also in combination with acid-base catalysis<sup>[36]</sup>, the use of two-step treatment of oil, that is, first with acid catalytic treatment of free fatty acids, and then use the alkali exchange reaction to produce biodiesel can use the characteristics of alkali-catalyzed reaction time to shorten the reaction time, reduce production costs. Zhang et al<sup>[29]</sup> on the use of two-step synthesis of biodiesel.

Enzyme catalytic production of biodiesel is generally used in lipase, the reaction conditions are mild, the reaction temperature is generally room temperature (about 30) enzyme production of biodiesel biggest advantage is: mild conditions, specificity, product recovery process is simple, Emissions, for a wide range of applications. However, the enzyme catalysis has high production cost and low reaction efficiency compared with other methods, and the enzyme is prone to inactivation in excess methanol<sup>[37]</sup>.

However, for the third generation of biodiesel, due to its structure and the particularity of the process, the traditional collection, pressing, fermentation esterification; hexane or supercritical CO<sub>2</sub> extraction, acid / base catalyzed transesterification, fractionation, has become Improve the

yield, reduce costs and promote bottleneck of the industrialization of technology, from the full use of resources, environmental and cost comprehensive evaluation point of view, its sustainable development capacity is very limited. Yang Zhizhong<sup>[37]</sup> and so on using ultrasonic chemical technology, make full use of its physical, physical and chemical and chemical effects, design and manufacture of suitable focusing, mixing, continuous combination of kettle or countercurrent, ring ultrasonic chemical reactor, The separation of inorganic components and oil components and the extraction of C14-C18 fatty acid esters were carried out in the low temperature, normal pressure and water suspension system, and then the decomposition of the algae toughness cells, cellulose, carbohydrate glial, (Such as specific structure and morphology of nano-ZnO, TiO<sub>2</sub>) catalyzed by alcoholysis and ester exchange reaction, to complete the use of microporous / mesoporous molecular sieve or glass fiber mat, ferroalloy wire mesh semiconducting nano-oxide (such as the specific structure and morphology of nano-ZnO, TiO<sub>2</sub>) Low energy consumption, high efficiency, high purity, high raw material utilization of biodiesel preparation.

## Conclusion

The use of microalgae to produce biodiesel has its unparalleled advantages, and the current production of microalgae biodiesel is the main problem is that the cost is too high. However, in the case of prenatal studies, microalgae can be used not only to produce biodiesel, but also to produce bio-pyrolysis oil, bio-hydrogen, and extract chemicals<sup>[38-39]</sup>, if both The production process can greatly reduce the cost of production, while the remaining residues in the reaction can be used as animal feed, most of the residue can also be produced by anaerobic digestion to generate electricity, anaerobic digestion of waste water can be used as fertilizer and Irrigation water<sup>[40]</sup>. As the carbon element accounts for more than 5% of the dry weight of microalgae, and these Care from the CO<sub>2</sub>, the study found that the biodiesel production process can use the power plant generated by CO<sub>2</sub>, and so this series of measures can greatly reduce the production process the cost of<sup>[41]</sup>. The autotrophic culture of microalgae can be used to improve the eutrophication of water bodies and to increase the cost of N-source (0.3) for the growth of microalgae. -0.4 million / ton of spirulina), P source cost (about 0.3 million / ton spirulina) is almost reduced to zero. It was found that the N, P content of the sweat treated by microalgae was reduced significantly<sup>[42-44]</sup>. At the same time, with the rapid progress of science and technology, the genetic

engineering of microflora will surely make a major breakthrough, and the microalgae with high fat and high yield will become an important direction in the future development of microalgae.

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