

Sustainable Biodiesel Production by Using Microalgae: A Review

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Abstract

The study will cover literature reviews on the extraction of algae oil, algae methyl ester (AME) biodiesel production, and the effect of microalgae biodiesel on performance, combustion, and exhaust emissions in diesel engines. It is based on research published by various researchers from 2006 to 2020. At present, research on renewable energy shows that a renewable energy source is biodiesel. Biodiesel is a diesel-like fuel with good features such as quality, renewable energy, and lower exhaust emissions, along with good lubricity. Besides being a source of fuel from microalgae, they tend to lower CO₂ from the environment, resulting in improved air quality to breathe in as well as a cleaner environment. There has been significant interest in the production of biodiesel from microalgae, being regarded as one of the oldest life forms on our planet. However, in comparison with diesel, it has some drawbacks: lower heating value, higher density and viscosity, and higher NO_x emissions. Optimization strategies are still a root of concern to counter the side effects of biodiesel when blended with diesel.

Keywords: Spirulina, Microalgae, Biodiesel production

INTRODUCTION:

Algae are organisms that perform photosynthesis, transforming sunlight into chemical energy. They have simple structures for reproduction. The biomass of algae is made up of various compounds with different structures and functions. Algal biotechnology is categorized into microalgae, macroalgae, and cyanobacteria, each having its own distinct characteristic. At times, cyanobacteria are classified within microalgae. The classification of microalgae includes both prokaryotic and eukaryotic forms, which can be unicellular or multicellular. The algae that is presently farmed for its highest protein levels is the cyanobacterium species *Athrospira*, widely recognized as *Spirulina*. [8] Biodiesel is basically the main substitute of fossil fuels, and producing biodiesel has generated considerable interest round the world and was the first alternative fuel to gain public attention. Biodiesel is defined as a biofuel that is diesel-equivalent and, therefore, produced from renewable biological raw materials that need a specific process to be turned into fuel. More specifically, biodiesel is often characterized as monoalkyl esters of long-chain fatty acids that have been synthesized through the chemical reaction of transesterification, using renewable feedstocks like vegetable oils or animal fats, with alcohol, with or without a catalyst. Each biodiesel source should be evaluated concerning its overall social benefits using a life-cycle assessment

in which criteria include its impact on net energy supply, the global food system, greenhouse gas emissions, soil carbon and fertility, and water, air quality, as well as biodiversity[2].

1. SPIRULINA

In 1519, the Spanish scientist Hernando Cortez and his Conquistadors were the first to identify it. Cortez observed that the Aztecs consumed Spirulina during his travels in Lake Texcoco, Valley of Mexico. Pierr dangeard hyped Spirulina after seeing the flamingos thrived on blue-green algae. Botanist Jean Leonard endorsed dangeard's findings and spurred commercialization of Spirulina to harness its benefits. The French founded the first processing plant for Spirulina, Sosa Texcoco, in 1969[8]. Spirulina (Arthrospira) is a type of oxygenic photosynthetic bacteria that belongs to the groups Cyanobacteria and Prochlorales. These filamentous bacteria are non-heterocystous and are typically found in tropical and subtropical regions, thriving in warm bodies of water with high carbonate and bicarbonate content, elevated pH, and salinity. Their large, gas-vacuolate filaments range from 3 to 12 micrometers in diameter, making them easy to collect through filtration and other physical separation techniques.

Spirulina is one of the blue-green photolithoautotrophs that is capable of trapping light energy and exploiting carbon dioxide as the primary source of carbon. It absorbs essential minerals from inorganic substrates in the environment. Spirulina belongs to the gram-negative group of cyanobacteria and consists of a cellular membrane, a cell wall and an outer membrane. Common species of Spirulina-Arthrospira and Spirulina-are placed under the same order Oscillatoriale[7].

Revised Biological Classification of *Spirulina platensis*:

- Kingdom: Eubacteria
- Subkingdom: Negibacteria
- Phylum: Cyanobacteria
- Class: Cyanophyceae
- Subclass: Oscillatoriophycidae
- Order: Spirulinales
- Family: Spirulinaceae
- Genus: Spirulina
- Species: Spirulina platensis

2.USE OF SPIRULINA:

1. Health and Medicinal Applications of Spirulina.
2. Spirulina for Consumption by Humans and Livestock.
3. Production of bioenergyetc.,

3. ALGAL CULTIVATION:

3.1 Species Selection:

The species of green algae that have been particularly recommended for consideration are Chlamydomonas reinhardtii, Dunaliella salina, Chlorella vulgaris, and Botryococcus braunii; some diatoms such as Phaeodactylum tricornutum and Thalassiosira pseudonana are also gaining favor in light of their remarkable productivity. Heterokonts such as Nannochloropsis and Isochrysis are also advancing

to receive this status. With the possible exception of angiogenesis, these species can be expected to contain over 60 weight % lipid content, according to certain conjectures. A broad spectrum of considerations is accounted for in establishing which algal species is best fitted for biofuel production[9].

3.2 Growth Pattern:

A whole lot of oil production may be heterotrophic, but these types of systems are also highly contaminated-open systems are much more at risk. Also, large-scale feed stock of organic carbon is scarcely added for further disadvantage. On the other hand, phototrophic systems have a lower rate of oil productivity; however, they capture a CO₂ feed from a nearby industrial source and are therefore more economical to large-scale production. Mixotrophic and photoheterotrophic modes of cultivation are limited by light availability and need to be mixed with specially designed photobioreactors (PBRs) [9].

FACTORS OF GROWTH OF SPIRULINA

3.2.1 Climatic Factor:

Temperature-the principal factor for the growth of Spirulina. Below 17 °C, there is almost no growth. Spirulina existed even below this limit. Optimal growth occurs at temperatures around 35 °C, and at temperatures above 38 °C, growth is somewhat restricted. Light had a strong effect on growth. Yet full sunlight is not advisable; rather, about 30% of full light intensity should be given, except more light in the early morning is required to quickly warm the culture. The light is also a very important factor for growth. However, continuous 24-hour lighting is not recommended. Important processes of Spirulina include protein synthesis and respiration, which occur during this phase [8].

3.2.2 Media:

Cultural Media involves various water sources based upon the medium used. Clean or filtered water is very important for the purpose of avoiding pollution with undesirable algae. Most waters have usually an adequate supply of calcium; however, very high hardness may induce sedimentation. Drinking water can be used but RO-treated water has proved to be the best for Spirulina culture. The main elements of the culture medium include urea, and instead of carbonate, bicarbonate is used. Both urea and ions such as sulfate, chloride, nitrate, and sodium are good sources of nitrogen, though they can become quite toxic in high concentrations. Spirulina can grow with nitrate or urea as the only source of nitrogen; however, the mixture is preferable. Excessive concentrations of phosphates, magnesium, and calcium should be avoided. In case of potash addition, levels shall not exceed five times that of sodium. Fertilizer-grade chemicals should better be used in soluble or crystalline form rather than in slow-release granulated form, wherever applicable. These data represent a few ways of media preparations employed depending upon the local growing conditions, with Zarrouk's media being the most widely used[8].

3.2.3 Temperature and pH:

Spirulina can grow between 20-37°C and optimum growth occurs between 29-35°C. At night, the growth rate of Spirulina becomes greatly hampered to almost nil. The pH of the culture medium plays a critical role in microalgae growth because it essentially rules the activity of enzymes, availability of phosphorus, toxicity of ammonia, and access to inorganic carbon. [13] Temperature variations alter the

metabolic process of microalgae, from high-temperature to low-temperature stress, promoting the accumulation of lipids within high-stress organisms. Growth may majorly take place in two forms[8].

- **Changes in available carbon, which can disrupt photosynthesis.**

- **Interference with cellular membrane functions.**

3.2.4 Light Intensity:

This chapter deals with photo-autotrophic organisms, including photosynthetic bacteria, cyanobacteria, and higher plants. They convert light energy into chemical energy via photosynthesis. Hence, light quality, intensity, and duration are considered some of the most important factors for algal productivity. In natural outdoor cultivation, it is only natural light or solar radiation that provides light. The availability of light is greatly determined by location, climate, season, and local ambience. Spirulina operates under conditions where it grows on light for food. The required light for growth may differ for different species. The range of light intensity for Spirulina growth is already known. The first detailed work on the effect of light on Spirulina maxima was conducted by Zarrouk (Zarrouk, 1966). The optical density of cultures is directly proportional to light flux. A denser culture will require more light. Conversely, less optical density would require less light. Light intensity is one of the most important parameters in cyanobacterial cultivation. With higher light intensity arises a high specific growth rate and with low intensity, a low growth rate combined with biomass being pigmented and proteinaceous. Outdoor algal systems are subject to the cycles of light and dark running diurnally. Such diurnal cycles establish unique physiological conditions for the outdoor algal cells on making their adjustments to light. Increased cell density in cultures tends to promote self-shading and induce inhibition on the growth rate of Spirulina[8].

3.2.3 Nutrient Content:

Different nutrients that are essential for the growth of microalgae include carbon (C), oxygen (O₂), hydrogen (H₂), nitrogen (N), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), sulfur (S), and phosphorus (P). These include carbon, oxygen, and hydrogen from water and air, and nitrogen, phosphorus, and potassium from the medium. The major nutrient components responsible for microalgal growth are nitrogen and phosphorus, while diatoms require certain species of elements, such as silicon (Si) [13].

4. BIODIESEL INTRODUCTION ORGANISMS FROM WHICH BIODIESEL IS PRODUCED

The major merit of biodiesel is that being renewable and biodegradable, it has far less impact on the environment than fossil fuels. However, biodiesel can be produced from natural and sustainable materials derived from vegetable oils (such as soybean, palm oil, and canola), animal fats, and recycled cooking oils. There are also other emerging sources, such as microalgae species including Spirulina (Arthrospira)[4].

4.1 SELECTION OF MICROALGAE:

Spirulina (Arthrospira) is a filamentous, blue-green microalga that exists in a symbiotic relationship and absorbs nitrogen from the ambient atmosphere. Its shape is mostly that of a spiral rod, although in some cases, it may take a disk form. The main photosynthetic pigment in Spirulina giving it a blue hue is phycocyanin. Spirulina also contains two other pigments: chlorophyll-a and carotenoids. Some strains

contain the pigment phycoerythrin, which provides a red or pink color for the microalgae. The spirulina is autotrophic and carries out photosynthesis; it reproduces by binary fission. The most promising group of microalgae includes Cyanophyceae (blue-green algae), Chlorophyceae (green algae), Bacillariophyceae (which covers diatoms), and Chrysophyceae (which comprise the golden algae).

Tablet:1

SPECIES\ GROUP	PRODUCT
<i>Arthrospira (Spirulina) platensis</i>	Phycocyanin, biomass
Arthrospira (Spirulina)	Protein, Vitamin B12
Chlorella spp.	Biomass, Carbohydrate extract
Chlorella vulgaris	Biomass, Carbohydrate extract
Dunaliella salina	Carotenoids, β -carotene
Odontella aurita	Fatty acids, EPA
Cryptocodinium cohnii	DHA

SOME MAJOR MICROALGAL SPECIES AND PRODUCTS

In the last two decades, the application of biotechnology has centered on four primary microalgae. These are: a) Spirulina (*Arthrospira*); b) Chlorella vulgaris; c) *Dunaliella salina*; and d) *Haematococcus*. Key characteristics, production methods, and chemical composition of each microalgal strain will be highlighted in the next sections.

4.1.1 CHLORELLA:

Chlorella are microalgae that are tiny, spherical, and range from about two to ten micrometers in diameter; they are capable of living exclusively through photosynthesis. These green creatures possess chlorophyll-a and -b pigments within their chloroplasts and reproduce rapidly when carbon dioxide, water, adequate sunlight, and certain minerals are provided. Commercial operations may grow Chlorella in photobioreactors, large circular tanks, paddle-wheel mixed open ponds, or circular open ponds. The cultivation process usually starts indoors in small culture flasks, which then serve as starters for larger outdoor tanks and ponds. The most-used outdoor systems are circular tanks and ponds. The most considerable cultivation pond measures approximately 500 m² with a depth of about 200 mm. Most microalgal production for aquaculture occurs, however, on smaller scales: usually indoors in carboys of 20-40 liters-or more often in larger plastic bags totalling about 1000 liters [5].

4.1.2 Dunaliella:

Microalgae from the *Dunaliella* genus are edible and nutrient-rich, single-celled extremophiles that can occur in many types of freshwater or marine environments. *D. salina*, however, has drawn recent interest because of its high antioxidant capabilities. It is recognized as the leading source of the carotenoid β -content due to its exceptionally high β -carotene production, reaching up to 14% dry biomass, which is above any other known source of carotenoids.[5]

4.1.3 Cryptecodinium cohnii:

Cryptecodinium cohnii is a dinoflagellate microalgae used for commercial synthesis of docosahexaenoic acid (DHA). Unlike many algal types, *C. cohnii* is heterotrophic, hence it doesn't exist without photosynthesis for the supply of energy to the growth process. The ratio of DHA to polyunsaturated fatty acids in *C. cohnii* can be altered; thus, there is continuous investigation among various researchers for mutant strains to provide the chance of increased DHA production. Results indicate that dissolved oxygen could be increased with better DHA yield. In addition to that, *C. cohnii* could adapt to changes in salinity affecting growth rate. The growth of this species is largely dependent on the microbiome as well as on other abiotic environmental factors. Most of the DHA is stored in the form of the phospholipid phosphatidylcholine. *C. cohnii* culture can accumulate DHA only when an organic carbon source is made available. Under nutrient starvation, other fatty acids may also accumulate, along with starch, while the maximum lipid distribution was noted in a pH-controlled auxostat culture [5].

4.2 CULTIVATION OF MICROALGAE:

Open systems have environments such as ponds, lakes, or lagoons, while closed systems comprise large-scale industrial bioreactors. Spirulina is currently being cultivated in both closed photobioreactors (PBR) and open ponds that are commercially employed for high-value product generation [8].

4.2.1 OPEN POND SYSTEM:

Open systems can be either natural water bodies such as lakes, small ponds, or lagoons, or artificial ponds or containers. The other systems normally used are large shallow ponds, circular ponds, tanks, and raceway ponds. These open spraying systems are easier and economical to construct and operate. Another problem is that, according to their very nature, they could not make good use of sunlight because of evaporation, CO₂ loss, and huge area of land required. Low-generation rates of mass transfer also occur since mixing in open cultivation systems is inefficient. Other influencing factors are location, season, temperature, pH levels, nutrients, and availability of sources supplying carbon dioxide. Another major problem that was associated with these systems was contamination due to wildlife and rapidly multiplying heterotrophic organisms. The researchers worked towards developing closed systems in order to resolve the problems associated with open systems [8].

4.2.2 PHOTOBIOREACTOR:

A photobioreactor is an artificial or constructed setting equipped to cultivate biomass in controlled settings. One of the essential features of a photobioreactor is a totally sealed environment which prevents any unwanted gases or contaminants from entering. This configuration is what enhances algae productivity since better control can be achieved over a number of growth parameters. These include controlling the levels of carbon dioxide and water, temperature optimization and light intensity, as well as culture density and pH management. Depending on specific needs, a culture can be illuminated by any one or the combination of natural sunlight, artificial light, or both. Certain photobioreactors facilitate relatively easier temperature control, while larger systems, more so of the tubular type, require advanced techniques to control their conditions. The new innovation contains double-walled photobioreactors, which allow for heating and cooling systems to be integrated for improved temperature

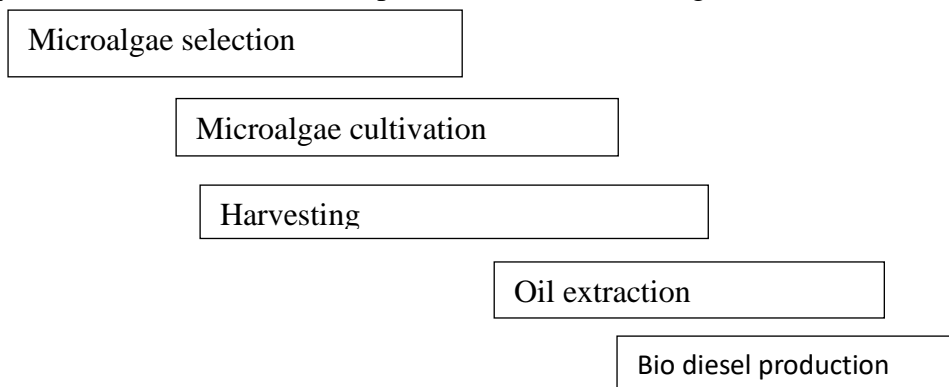
control. Although the initial cost of photobioreactors can be high, they typically offer considerable benefits compared to conventional open systems, making them ideal for biomass production [8].

5. HARVESTING:

Harvesting refers to the process of recovering or collecting biomass from the culture medium, which is primarily algal biomass contributing for about 20-30% of the total cost of biomass production. In practice, steps taken in harvesting utilize a variety of techniques and may involve combinations of physical, chemical, or biological processes whereby large volumes of water are removed, and large quantities of algal biomass are handled to achieve the target solid-liquid separation. Trials have shown this; so far, the field remains in flux with a lot of ongoing research tailored with specialization to develop cost-effective harvesting systems for specific algal species. Following are improved methods for harvesting[9].

6. OIL EXTRACTION:

The next step after harvesting and drying microalgae is lipid extraction. Lipid extraction is critical, especially in the case of microalgae with lower lipid contents, as any losses of lipids in that process can greatly affect the cost for biodiesel production from microalgae. [4]

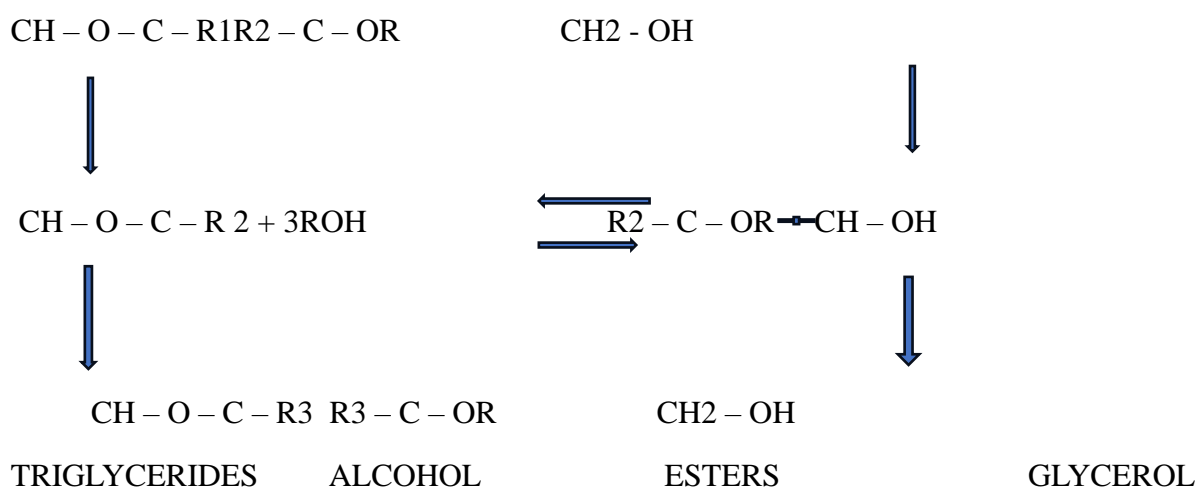


7. BIODIESEL PRODUCTION FOR MICROALGAE:

Green diesel and algal biodiesel are indeed some of the most efficient energy sources obtained from microalgae, which makes them appropriate for use in internal combustion engines. It is essential to assess and analyse the characteristics of green diesel fuel by converting it into biodiesel regarding its application in IC engines. Considerable research has been conducted on the extraction of oil from microalgae, the conversion of that oil into biodiesel, and the subsequent characterization of this biodiesel for the purpose of evaluating engine performance and emission analysis [1]. Whereas biodiesel represents a complex mix of fatty acid alkyl esters formed by means of transesterification (ester-exchange reactions), oils from plants or animals form the basis for such preparations. These sources of lipids, for example, consist of 90-98% triglycerides (mainly), whereas mono and diglycerides occupy negligible amounts and also contain as little as 1-5% free fatty acids, among other remaining traces of phospholipids, phosphatides, carotenes, tocopherols, Sulfur compounds, and small amounts of water [2]. Transesterification has many names but is in fact a multi-step process, where three sequences of reversible reactions lead to the formation of fatty acid esters (biodiesel) and glycerol, a by-product. The

transesterification overall reaction is depicted in with radicals R1, R2, and R3 representing long-chain hydrocarbons, that is, the fatty acids. A short-chain alcohol such as methanol or ethanol, together with a catalyst-typically NaOH-combine with oil or fat substrate reactants entry into the transesterification reaction [4]. A catalyst-whether homogeneous, heterogeneous, acidic, or basic-can be used to improve the rate of the transesterification reaction, although in certain processes which involve supercritical fluids (methanol or ethanol), the presence of a catalyst might not be required [2].

Base catalysts, for instance, KOH and NaOH, have been generally used to catalyze transesterification. High levels of FFA present in microalgae oil (larger than 0.5% w/w) prevent the use of homogeneous base catalysts for the transesterification reaction. The use of soap resulting from the reaction of FFAs with base catalysts lowers biodiesel yield and complicates the separation of biodiesel from the co-product, glycerol. Therefore, sulfuric acid (H₂SO₄) can be used advantageously as an acid catalyst because it is not affected by the amount of FFA present in the oil and can allow both esterification (conversion of FFA to alkyl ester) and transesterification to occur simultaneously [4]. Heterogeneous catalysts have been extensively studied in performing biodiesel transesterification either as basic or acidic. By allowing different ways to be reused, recycled, and regenerated in subsequent cycles of transesterification, heterogeneous catalysts provide for improved economics in biodiesel production compared to homogeneous catalysts [4].



CONCLUSION:

Microalgae possess the capability to utilize carbon resources available in natural water and soil environments, and they can be converted into biodiesel, presenting a more sustainable and environmentally friendly alternative to traditional fossil fuels. This review investigates the various elements that affect the production of biodiesel from microalgae, along with the advanced technological aspects involved. Essential factors that influence microalgae growth, lipid production, harvesting, and lipid extraction are thoroughly discussed, and the most recent state-of-the-art technologies for biodiesel production from microalgae, such as cultivation systems, lipid induction techniques, harvesting methods, and lipid extraction processes, are also analyzed. While producing biodiesel from microalgae is technically feasible, it is not currently economically practical. Integrating microalgae biodiesel production within a hybrid refinery, alongside conventional microalgae product manufacturing, could improve the market viability of microalgae (Zhang et al., 2022) [13].

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