Review Article

Algal Biofuels: A Comprehensive Review and Analysis

Abstract

The depletion of fossil fuels and increased atmospheric CO_2 levels have precipitated a global energy and environmental crisis. The cultivation of microalgae offers a viable remedy by utilizing solar energy and assimilating CO_2 for biofuel production via photosynthesis. Microalgae demonstrate rapid growth in diverse environments, rendering them a dependable biomass source for large-scale biofuel generation. Furthermore, their biofuel production can be tailored through the manipulation of growth conditions or genetic engineering. This review aims to highlight the variety of biofuels produced from microalgae and the methodologies to optimize their yield. This review also explores the economic implications of algal biofuel production in both open ponds and closed photobioreactors. Various strategies for the photo-production of biohydrogen utilizing the hydrogenase enzyme from green algae are investigated. Additionally, certain microalgae species are recognized for their potential as biodiesel sources due to their high lipid content. The lipid profiles of leading oil-producing algal strains under optimal conditions are assessed. This review further examines the potential of microalgae in synthesizing petroleum-based chemicals and in generating bioethanol and biogas from algal biomass.

Keywords: Microalgae, Algal biofuels, Renewable energy, Biofuel conversion, Sustainability

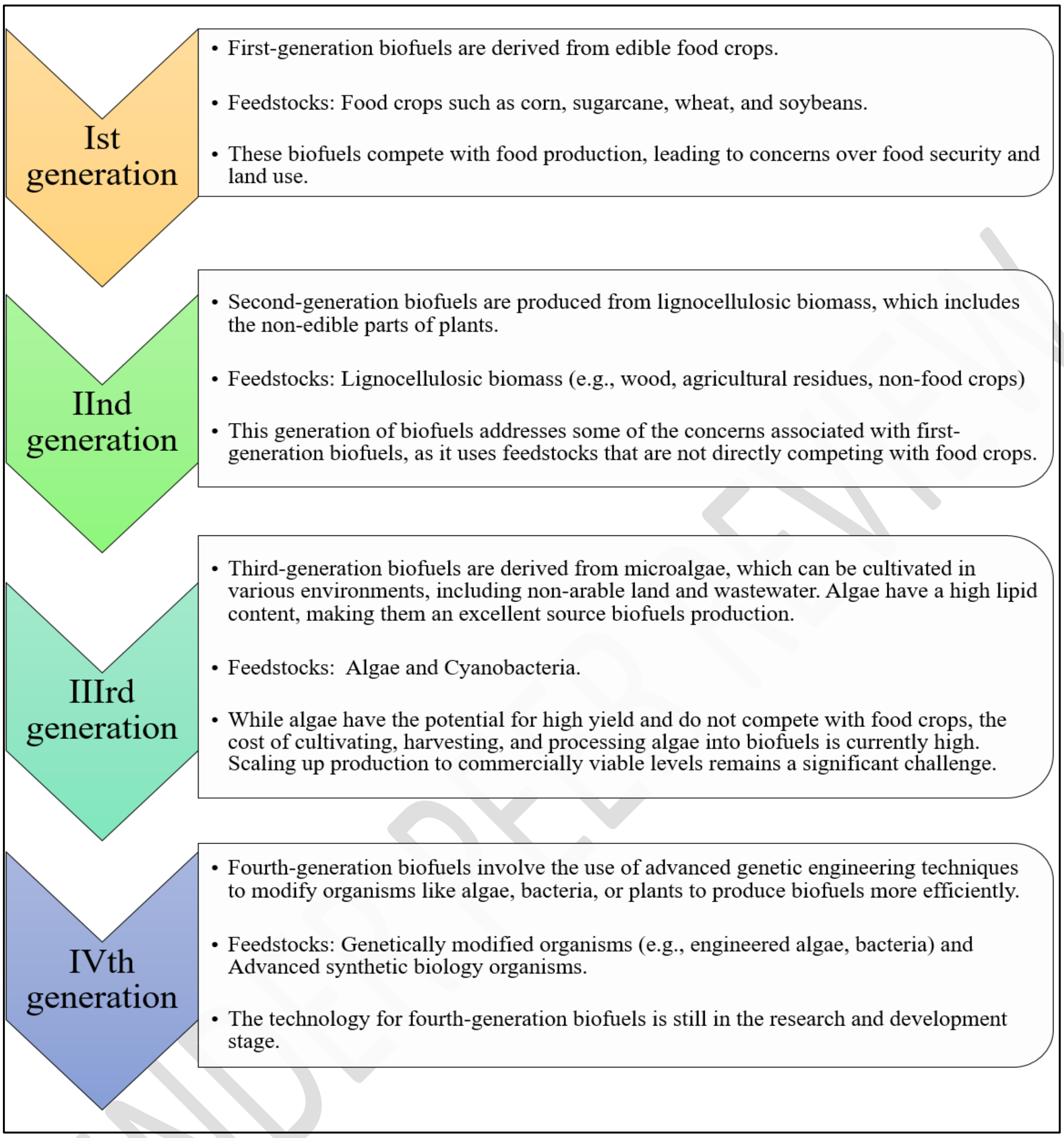
1. Introduction

Algae biofuels are advanced renewable fuels produced from algae raw materials through various conversion processes (Saad et al., 2019). This is because the raw material is rich in oil, which is related to its abundant photosynthetic capacity (Angelidaki et al., 2018). With over 3,000 different species and the fastest reproductive rate, algae are an aquatic species that is more diverse than land plants (Collet et al., 2011). Their main advantage is their ability to convert virtually all of the energy in the feedstock into various types of valuable biofuels (Passos and Ferrer, 2014). Because they produce biomass quickly and have a relatively high oil content, microalgae have long been considered promising sources of biofuels (Watson et al., 2021). Mass algae culture can be carried out on non-agricultural land using non-potable salt water and wastewater because microalgae grow much faster than terrestrial plants (Posten and Schaub, 2009). Therefore, scientists, entrepreneurs and the general public are becoming increasingly interested in the use of microalgae as a substitute feedstock for biodiesel.

This review thoroughly examines recent advancements and developments in third-generation microalgae-based biofuels, highlighting their ability as a sustainable energy source. The goal is to understand the unique properties of microalgae, evaluate different algae biofuels and their production methods, and determine the financial and environmental benefits of each. In addition to outlining the research and development requirements, the assessment also aims to identify the current barriers to large-scale commercialization.

2. Types of Biofuels

Biofuels are classified into four generations based on the raw materials used in their production. This classification depends on the origins of feedstocks and the technological processes involved in biofuel conversion (Liu et al. 2021). Figure 1 illustrates a comparative analysis of the four biofuel generations.



- First-generation biofuels are derived from edible food crops.
- Feedstocks: Food crops such as corn, sugarcane, wheat, and soybeans.
- These biofuels compete with food production, leading to concerns over food security and land use.

- Second-generation biofuels are produced from lignocellulosic biomass, which includes the non-edible parts of plants.
- Feedstocks: Lignocellulosic biomass (e.g., wood, agricultural residues, non-food crops)
- This generation of biofuels addresses some of the concerns associated with first-generation biofuels, as it uses feedstocks that are not directly competing with food crops.

- Third-generation biofuels are derived from microalgae, which can be cultivated in various environments, including non-arable land and wastewater. Algae have a high lipid content, making them an excellent source biofuels production.
- Feedstocks: Algae and Cyanobacteria.
- While algae have the potential for high yield and do not compete with food crops, the cost of cultivating, harvesting, and processing algae into biofuels is currently high. Scaling up production to commercially viable levels remains a significant challenge.

- Fourth-generation biofuels involve the use of advanced genetic engineering techniques to modify organisms like algae, bacteria, or plants to produce biofuels more efficiently.
- Feedstocks: Genetically modified organisms (e.g., engineered algae, bacteria) and Advanced synthetic biology organisms.
- The technology for fourth-generation biofuels is still in the research and development stage.

Figure. 1 Four generations of biofuels

2.1 First-Generation Biofuels: Food crops such as corn, sugar cane and soybeans are used for direct production. Ethanol and biodiesel are the most commonly used first-generation biofuels. Generally, sugars are fermented to produce ethanol, while vegetable and animal fats are transesterified to produce biodiesel (Aron et al., 2020).

2.2 Second-Generation Biofuels: They come from non-food biomass, which includes waste products, woody plants and agricultural residues. The dispute between fuel and food, which accompanied the introduction of first-generation biofuels, is intended to be resolved with second-generation biofuels. Fischer-Tropsch diesel and cellulosic ethanol are two examples (Rostek, 2016)**.**

2.3 Third-Generation Biofuels: Third-generation biofuels such as algae-derived biodiesel are known to utilize microalgae as a sustainable raw material for renewable energy production. Microalgae are a sustainable and potential source of biofuels because they can grow in wastewater and uncultivated soil (Chisti, 2007)**.** The transesterification of lipids creates algal biodiesel, which brings a number of advantages, including a relatively small land requirement, limited competition with food crops and high biomass yields.

2.4 Fourth-Generation Biofuels: These use genetically modified organisms with the aim of improving the production of biofuels, but are currently in the experimental stage. This generation also includes advanced carbon capture and utilization technologies (Nigam & Singh, 2011).

3. Types of Algal Biofuels

Algal biofuels can be categorized into various categories depending on the methods used for their production and the final products obtained, as illustrated in Figure 2. These classifications help in better understanding the diversity and potential applications of algal biofuels in the renewable energy sector.

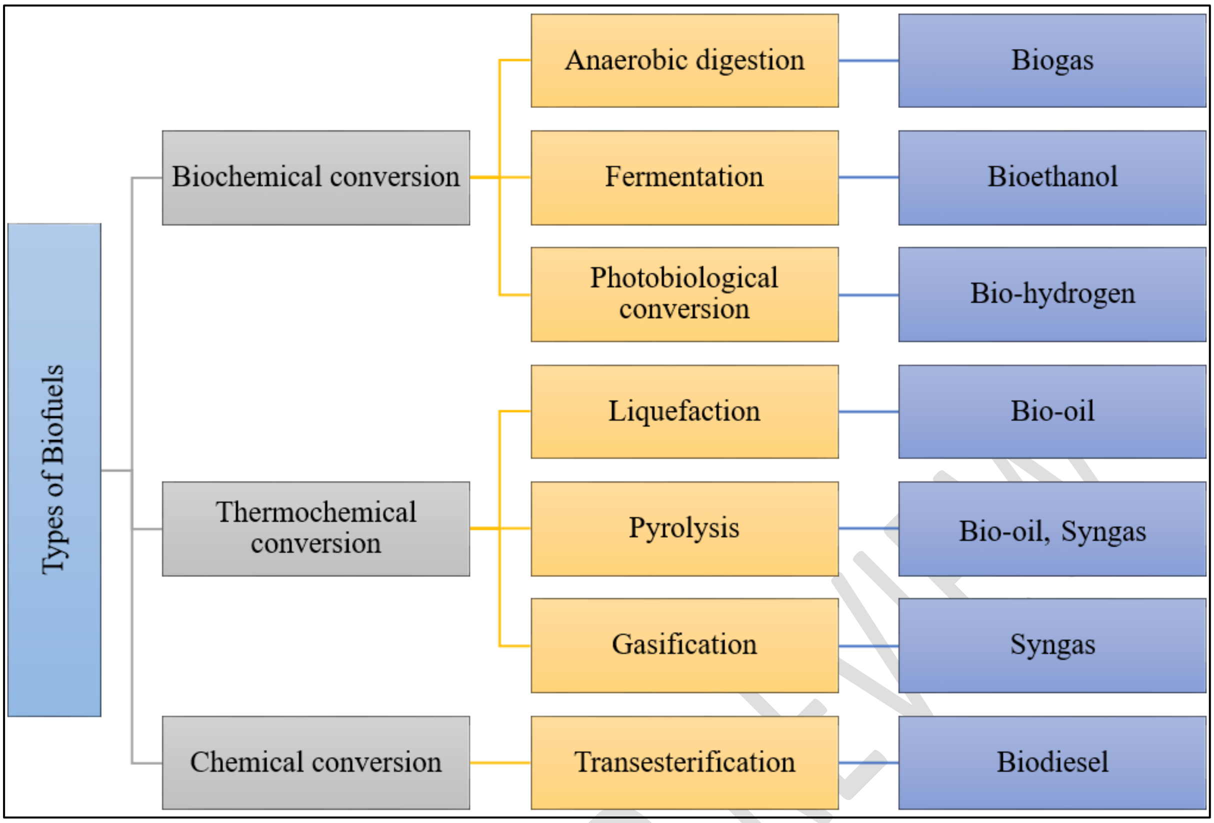


Figure. 2 Types of algal biofuels

3.1 Biogas: Biogas, produced from algal biomass through anaerobic digestion, involves bacterial breakdown of organic matter in the absence of oxygen. Methane, the primary output of this process, represents a sustainable renewable energy source. Additionally, this process produces secondary gases like carbon dioxide and various by-products (Zabed et al., 2020). The generated biogas can be effectively employed as a fuel for transportation, heating in buildings, or electricity generation, presenting a sustainable substitute for current energy sources (Abusweireh et al., 2023).

3.2 Bioethanol: Algal bioethanol is produced via the fermentation of algal carbohydrates. This process converts sugars and starches from algal biomass into ethanol, offering a renewable alternative to gasoline (Melendez et al., 2022). Algal bioethanol is a sustainable energy source capable of reducing greenhouse gas emissions, making it an appealing eco-friendly fuel option

(Chandrasekhar et al., 2023). The advancement of algal bioethanol signifies progress towards a greener energy paradigm, underscoring its role in the pursuit of sustainable energy sources.

3.3 Biohydrogen: Algae utilize photolysis to convert water into hydrogen and oxygen, facilitating biohydrogen production. Biohydrogen generation can occur through both anaerobic pathways and alternative processes. This renewable biohydrogen represents a viable sustainable option for fuel cells, providing an eco-friendly alternative to fossil fuels (Goria et al., 2024). Fuel cells using biohydrogen convert water combustion byproducts into electricity, as demonstrated in the 2022 study by Karishma et al.

3.4 Bio-oil: Algal bio-oil, derived from algal lipids through processes like pyrolysis or hydrothermal liquefaction, has the potential to transform the energy sector. This bio-oil can be converted into various fuel forms, including biodiesel, aviation fuel, and biocrude, which can be refined similarly to petroleum products (Koyande et al., 2019). The adoption of algal bio-oil offers a viable response to the issues associated with fossil fuels, due to its mass production capabilities and its potential to reduce greenhouse gas emissions (Sekar et al., 2021).

3.5 Syngas: Algal syngas is produced from algal biomass through gasification or pyrolysis. This gas mixture primarily comprises carbon dioxide, hydrogen, and carbon monoxide. Algal syngas can be utilized for electricity generation, synthetic fuel production, or chemical manufacturing (Paniagua et al., 2022). The extraction of this gas from algae represents a sustainable alternative to fossil fuels, with potential benefits for reducing greenhouse gas emissions (Raheem et al., 2021).

3.6 Biodiesel: Lipids from algae biomass are extracted via transesterification to produce biodiesel. This process involves reacting lipids with methanol and a catalyst, yielding Fatty Acid Methyl Ester (FAME) and glycerol (Bošnjaković & Sinaga, 2020). The biodiesel produced is a sustainable and environmentally friendly substitute for conventional diesel fuel, offering a cleaner combustion alternative. Research by Mathew et al. (2021) and Jeyakumar et al. (2022) highlights the effectiveness and advantages of algae-derived lipids in biodiesel production through transesterification.

4. Algae Species Used for Biofuels Production

A variety of microorganisms, called microalgae, are capable of synthesizing significant amounts of lipids, which can then be converted into biofuels. The selection of algae species for biodiesel production depends on various aspects, including lipid composition, growth rate and cultivation parameters (Yaashikaa et al., 2022). A comparison of important microalgae species and their productivity is discussed in Table. 1.

Table.1 A Comparison of important microalgae species and their productivity

Microalgae species	**Yield (g/l/d)**		**Culture conditions**	**Reference**
	Biomass[a]	**Lipid**[b]		
Botryococcus braunni	0.17	0.030–0.065	Prate media, Cultivated in a conical flask with 1% CO_2, Concentration, temperature -25°C.	(Kalacheva et al., 2002)
Chlorella minutissima	0.17	0.0913	Cultivate Guillards Marine medium in a 2L stirred tank bioreactor, aerated with 1 L/min of air containing 5% CO_2, at a temperature of -25°C.	(Faried et al., 2017)
Chlorella emersonii	0.38	0.123	Cultivate in low nitrogen medium within a 230L photobioreactor, illuminated with a light intensity of 130 μmol/m^2/s, at a temperature of 25°C.	(Singh et al., 2017)
Chlorella emersonii	0.26	0.158	Cultivate in low nitrogen medium within a 2L stirred tank bioreactor, aerated with 1 L/min of air containing 5% CO_2, at a temperature of -25°C.	(Faried et al., 2017)
Chlorella vulgaris	0.25	0.16	Cultivate in low nitrogen medium within a 230L photobioreactor, illuminated with a light intensity of 130 μmol/m^2/s, at a temperature of 25°C.	(Singh et al., 2017)

Chlorella vulgaris	0.38	0.149	Cultivate in low nitrogen medium within a 2L stirred tank bioreactor, aerated with 1 L/min of air containing 5% CO_2, at a temperature of -25℃.	(Huesemann & Benemann, 2019)
Chlorella protothecoides	3.7–4.2	1.7–1.8	Heterotrophically cultivate in 1L Erlenmeyer flask using liquid basal medium supplemented with glucose (30 g/L), yeast extract (4 g/L), and reducing sugar from JA (30 g/L). Maintain the temperature at 28℃.	(Mamilla et al., 2024)
Chlorella protothecoides	2.5–7.4	1.25–4.17	Cultivate in a basal medium within a 5L bioreactor, aerated with 3 L/min of air, at a temperature of 28℃.	(Mamilla et al., 2024)
Chlorella protothecoides	0.94	0.58	N/A	(Kavindi & Rathnayake, 2024)
Chlorella protothecoides	1.4	0.655	Cultivate in a modified basal medium within an Erlenmeyer flask at a temperature of 28℃.	(Kavindi & Rathnayake, 2024)
Chlorella protothecoides	1.94	0.28–1.07	The glucose solution was batch-fed during growth in autotrophic batch cultures, cultivated in a 5L fermenter aerated with 0.5 L/min of air at a temperature of 28℃.	(Kavindi & Rathnayake, 2024)
***Nannochloropsis* sp.**	0.10	0.026	Cultivated in an airlift bioreactor.	(Kavindi & Rathnayake, 2024)
***Nannochloropsis* sp.**	0.4	0.206	Cultivated in F media with nitrogen depletion within a 20L flat panel alveor, aerated with 0.6 L/min of air containing 3% CO_2, at a temperature of -25℃.	(Kavindi & Rathnayake, 2024)
***Nannochloropsis* sp.**	0.50	0.143	Using f/2 media in artificial seawater, cultivate in a cylindrical glass photobioreactor, aerated with 2 L/min of air containing 2% CO_2. Maintain semicontinuous culture with daily replacements, at a temperature of -26℃.	(Huesemann & Benemann, 2019)
Spirulina sp.	0.22	0.4–4.3	N/A	(Rajak et al., 2020)

[a] Biomass yield is measured by the amount of dry weight produced per liter of volume per day.

[b] Lipid yield is measured by the amount of lipid produced per gram of dry weight per liter per day.

4.1 *Chlorella*

The green microalgae of the genus Chlorella are rapidly growing with a high-fat composition. Due to their robustness and high productivity, species such as *Chlorella vulgaris* are being actively researched for biodiesel production. Chlorella is a great candidate for biodiesel because in some situations it can accumulate up to 50% of its dry weight in lipids (Gouveia & Oliveira, 2009). Its adaptability to a variety of conditions, including wastewater, increases its attractiveness as a feedstock for biofuels (Sukenik, 2004).

4.2 *Nannochloropsis*

Marine microalgae of the genus *Nannochloropsis* are known for their high content of lipids and eicosapentaenoic acid (EPA). The lipid-rich biomass and adaptability to culture conditions of species such as *Nannochloropsis oculata* and *Nannochloropsis gaditana* make them particularly valuable for biodiesel production (Spolaore et al., 2006). These algae can produce a significant amount of the polyunsaturated fatty acids desired in biodiesel, accounting for up to 30–50% of their dry weight of lipids (Hu et al., 2008).

4.3 *Botryococcus braunii*

The high lipid content of *Botryococcus braunii* is well known, especially when it comes to long-chain hydrocarbons. Under optimal conditions, this green microalga can produce up to 70% of its dry weight in lipids (McCauley et al., 2013). Due to its distinct lipid profile, it is a viable option for producing biodiesel with exceptional properties. However, its economic viability needs to be improved by addressing problems such as slow growth rates and high cultivation costs (Becker, 2007).

4.4 *Spirulina*

Spirulina is a blue-green algae with high protein and fat content and is also being studied for the production of biodiesel. Because Spirulina platensis grows in a variety of conditions and has high nutritional content, it has potential, although it has not been studied as intensively as *Chlorella* and *Nannochloropsis* (Belay, 2002). Although lower than other species, its normal lipid content of 6-8% of dry weight is promising for the production of biodiesel (Renaud et al., 1996).

5. Algae cultivation

The process of bioethanol production begins with the growth of microalgae. To increase biomass productivity, algae must be grown in regulated environments. There are two types of cultivation systems as shown in Figure 3:

5.1 Open Ponds: These are naturally occurring ponds or shallow watercourses in which algae thrive. They are affordable and easy to maintain but can become contaminated and change with the environment (Pulz & Gross, 2004).

5.2 Photobioreactors (PBRs): These closed systems provide a regulated framework for algae cultivation. PBRs are more expensive to build and operate but offer higher production and better protection from pollution (Richmond, 2004).

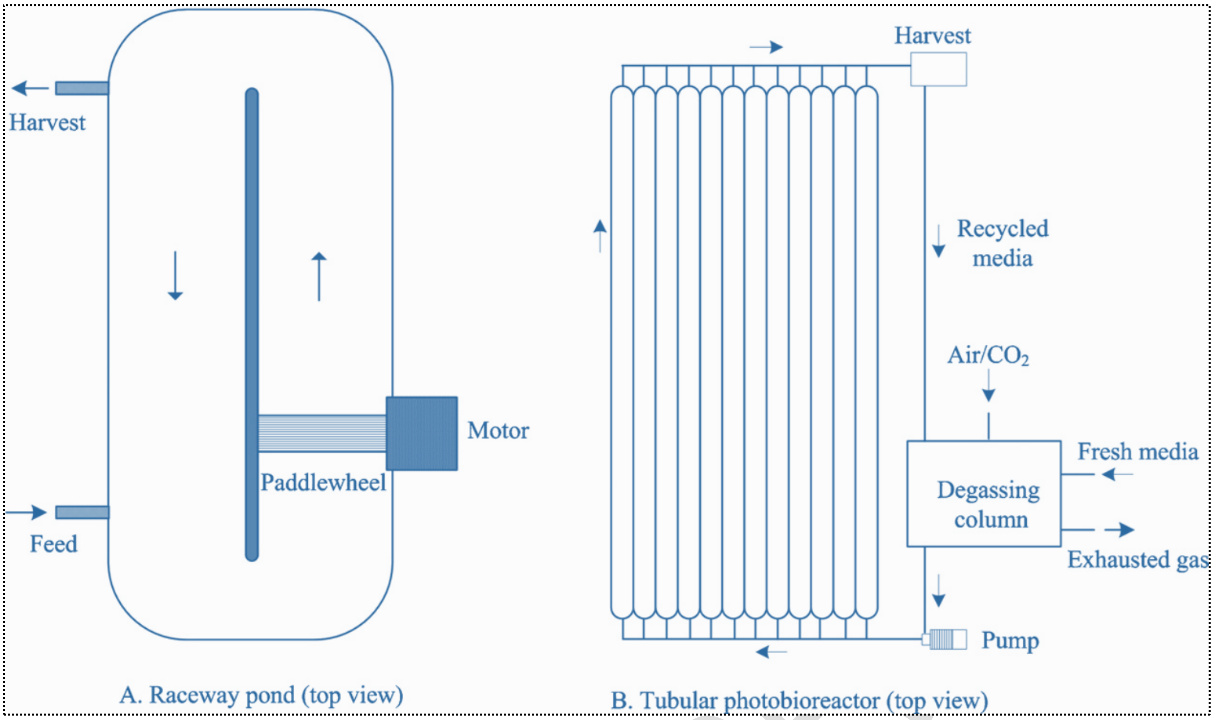


Figure. 3 An example showing the differences between an open and closed system.

6. Comparative analysis of open and closed system

Table 2. A comparative study of closed vs open systems.

Issue	Closed system[b]	Open system[a]	References
Control of mass and gas transfer	Easy	Difficult	(Krishnaiah et al., 2009)
Surface-to-volume ratio	High	Moderate	
Evaporation rate	Low	High	
Irradiance supplied (MJ)	29	13	
Preheating	High	Low	
Biomass productivity (t/ha/yr)	20–33	20	
Total energy consumption (GJ/yr)	730	450	
Volumetric productivity (kg/l/d)	0.28–0.57	0.036	
Energy recovered as biomass (MJ)	2.8	1.3	
Total energy content in 100 MT (GJ/yr)	3156.30	3156.30	
Energy produced as oil (GJ/yr)	1156.49	1156.49	
NER of biomass production	4.35	7.0	
NER of oil production	1.60	2.57	

[a] The open system was based on a raceway pond.

[b] The tubular photobioreactor served as the primary basis for the closed system study

7. Harvesting and Drying/Dewatering

Upon reaching the necessary algal density, harvesting is conducted using methods such as centrifugation, flocculation, or filtration, selected according to algal species and operational efficiency (Latif et al., 2019). The central aim of this phase is to isolate algal biomass from the aqueous medium while reducing energy use and minimizing biomass loss during separation (Xiu & Shahbazi, 2012). Following harvesting, algae undergo dewatering to reduce moisture content, which improves oil extraction suitability for biofuel production. This dewatering process is vital for transitioning to oil extraction and enhancing biomass utilization efficiency in renewable energy contexts (Baskar et al., 2019). The Process of harvesting and drying is illustrated in the figure 4.

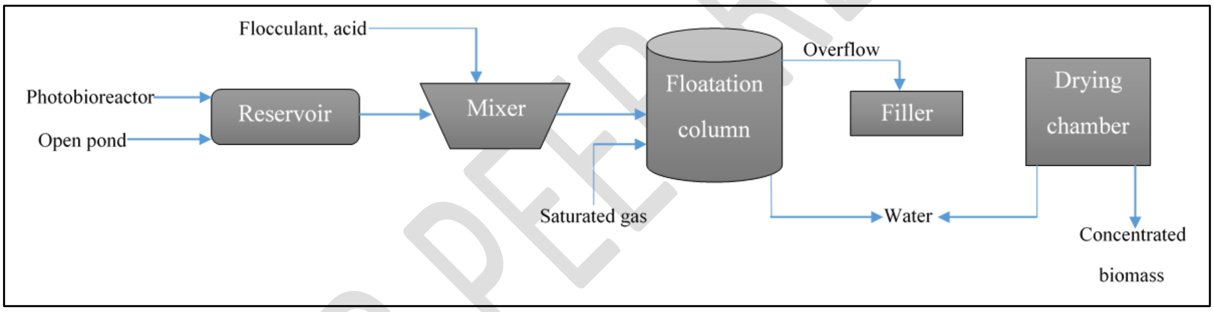


Figure. 4 The method and process of microalgae harvesting.

8. Pretreatment of algal biomass

A major challenge in biofuel production is the resilient nature of the algal cell wall. Pretreatment of algal biomass is essential to enhance biofuel extraction efficiency. The pretreatment process aims to optimize extraction and increase biomass and biofuel yields. This phase involves disrupting the cell wall using physical, chemical, and enzymatic methods. Table 3 summarizes various studies on the pretreatment of algal biomass.

Table. 3 The impact of various pretreatment methods on different types of algal biomass

Pretreatment methods	**Algae**	**Result of Pretreatments**	**References**
Mechanical Pretreatment	Algal mixture	Up to 60% of the organic matter was dissolved and made available.	(Bhatia et al., 2022)
Biological Pretreatment	Algal mixture	Up to 9 – 29 % of the organic matter was dissolved and made available.	(Bhushan et al., 2023)
Thermal Pretreatment	Algal mixture	Up to 63% of the organic matter was dissolved and made available.	(Bhatia et al., 2022)
Thermal Pretreatment	Mixed biomass from wastewater	There was a 70% increase in methane production.	(Bhatia et al., 2022)
Alkaline pretreatments were performed using three different concentrations of sodium hydroxide (NaOH): 0.5%, 2%, and 5% by weight.	*Chlorella vulgaris & Scenedesmus sp.*	Between 20 and 43% of the sugar content was dissolved.	(Yoo et al., 2015)
Autohydrolysis was conducted at a low temperature of 50℃.	*Chlorella vulgaris & Scenedesmus sp.*	Up to 6-12 % of the organic matter was dissolved and made available.	(Zieliński et al., 2014)
A crude solution containing extracellular enzymes was extracted.	*Chlorella vulgaris*	Hydrogen production was 43.1 milliliters of hydrogen gas per gram of dry cell weight.	(Prajapati et al., 2015)
Hydrolytic enzymes were obtained from Novozymes.	*C. reinhardtii & C. vulgaris*	Methane production increased by 14% for C. vulgaris, but there was no change for C. Reinhardtii.	(Prajapati et al., 2015)
Crude enzymes extracted from fungi under optimized conditions.	*Chroococcus sp.*	Up to half of the biomass was dissolved within 150 minutes at a temperature of 50℃, leading to a 28% increase in methane production.	(Prajapati et al., 2015)
Fungal crude enzymes are produced under suboptimal conditions.	*Chroococcus sp.*	After 48 hours at 30℃, 44% of the total sugar and 46% of the total organic matter (COD) from the biomass were released.	(Prajapati et al., 2015)

The biomass was subjected to high-pressure and high-temperature conditions.	*C. vulgaris*	Methane production increased by up to 64%.	(Zieliński et al., 2014)
A combined process involving acid catalysis for pretreatment followed by extraction.	*Scenedesmus & Chlorella*	Up to 90% of the sugars were dissolved. Fatty acid recovery from wet algal biomass reached a maximum of 97%.	(Laurens et al., 2015)
Alkaline pretreatment using sodium hydroxide.	*Chlorococcum infusionum*	Glucose yield was 350 milligrams of glucose per gram of biomass. Bioethanol production reached 0.26 grams of ethanol per gram of algae.	(Yoo et al., 2015)

9. Biodiesel from microalgae: Production Process

Algae oil is chemically transformed into biodiesel through a process called transesterification. This process involves combining the oil with alcohol and a catalyst to produce glycerol and a fuel called fatty acid methyl ester (FAME). FAME is essentially biodiesel and can be used as a replacement for regular diesel fuel (Kim et al., 2022).

9.1 Transesterification Process

The transesterification process involves several distinct phases, as shown in Figure 5. Each phase plays a critical role in the efficiency and yield of the biodiesel production process.

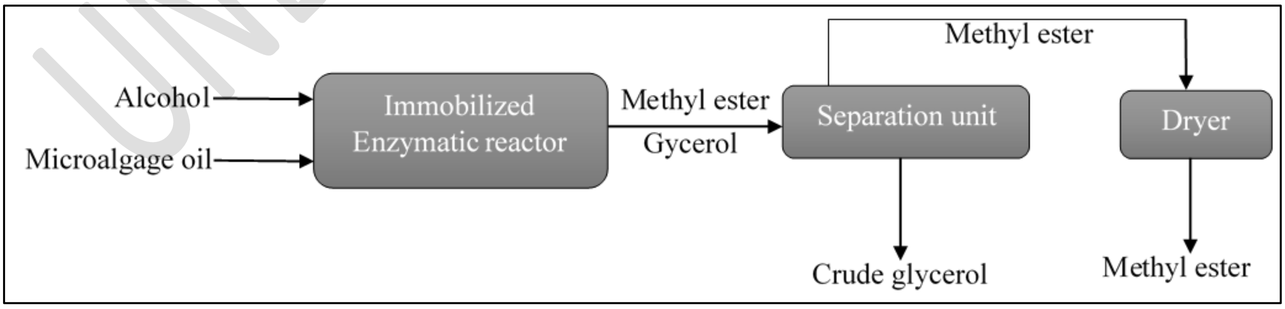


Figure 5. A depiction of the immobilized transesterification process.

9.1.1 Lipid Extraction: The process of removing lipids from algal biomass is the initial phase. Mechanical pressing, supercritical fluid extraction and solvent extraction are some techniques that can be used to achieve this. The most popular solvent extraction technique is the use of organic solvents such as hexane due to their effectiveness and affordability (Santos et al., 2010).

9.1.2 Algal oil preparation: Enzyme extraction and refining are the first steps in producing algae oil. This eliminates free fatty acids and other pollutants. Ensuring the quality of the biodiesel and increasing the efficiency of the transesterification reaction depends on this phase (Mittelbach & Remschmidt, 2005).

9.1.3 Reaction of Transesterification: The refined algae oil is combined with methanol or ethanol and a catalyst, usually potassium hydroxide (KOH) or sodium hydroxide (NaOH). Temperature (60-70°C) and pressure are regulated during the reaction (Ganesan et al., 2020). The chemical reaction that occurs during the transesterification process is represented by equation (1).

$$\text{Triglyceride} + 3\ \text{Methanol} \longrightarrow 3\ \text{Fatty Acid Methyl Esters (FAME)} + \text{Glycerol} \qquad (1)$$

Triglycerides are broken down into FAMEs and glycerin by the catalyst, which speeds up the process. After glycerol and FAMEs are separated through the reaction, which typically takes one to two hours, the FAMEs are collected for the synthesis of biodiesel (Knothe, 2005).

9.1.4 Purification and separation: After the reaction, the glycerin and any remaining catalysts are removed from the biodiesel (FAME). Techniques such as centrifugation and water purification can be used for this purpose. After purification, only the highest quality fuel remains in biodiesel (García et al., 2011). In this way, any remaining pollutants are removed from the fuel.

10. Bioethanol from microalgae: Production Process

With their high carbohydrate content and fast growth rates, microalgae have become one of the most promising raw materials for the production of bioethanol. Microalgae culture, biomass harvesting, and drying, hydrolysis of carbohydrates to fermentable sugars, fermentation and ethanol recovery are the main processes in the production of bioethanol from microalgae (Özçimen et al., 2020). The process of producing bioethanol from microalgae is covered in detail in this section.

10.1 Carbohydrate Hydrolysis

Carbohydrates in the form of cellulose and starch are found in the dried algae biomass; They must be converted into fermentable sugars (Özçimen et al., 2020). This happens through hydrolysis, which is carried out by enzyme or acid methods:

10.1.1 Acid hydrolysis: This process converts complex carbohydrates into simple sugars by heating dilute sulfuric acid in the biomass to a high temperature. Although acid hydrolysis is efficient, neutralizing steps are required and inhibitory byproducts may be formed (Himmel et al., 2007).

10.1.2 Enzymatic hydrolysis: The process of converting carbohydrates into fermentable sugars using enzymes such as cellulases and amylases. Because enzymes are more expensive, this process is generally slower and more expensive, but is also more environmentally friendly and produces fewer by-products (Zheng et al., 2009).

10.2 Fermentation

After hydrolysis, the fermentable sugars are fermented into ethanol. The microorganisms used in this process are usually yeast (Saccharomyces cerevisiae), which breaks down the sugar into carbon dioxide and ethanol as by-products (Demirbas, 2009). Anaerobic fermentation takes place in bioreactors, usually at 30-35°C. Sugar concentration, pH and nutrient availability are some of the variables that affect the course of fermentation. To maximize the amount of ethanol produced, these factors must be optimized (Bai et al., 2008).

10.3 Ethanol Recovery

The recovery and purification of ethanol is the final phase in the production of bioethanol. Typically, the process of distillation is used for this, in which the fermentation broth is heated to separate the ethanol from water and other ingredients according to their boiling temperature. To achieve fuel-grade purity, further purification of the ethanol is required using methods such as rectification or molecular sieving (Zabed et al., 2014).

11. Biogas from microalgae: Production processes

11.1 Anaerobic Digestion of Algal Biomass

The anaerobic digestion of organic materials, especially algae biomass, produces biogas, a renewable energy source. Biogas, a mixture mainly of carbon dioxide and methane, is created by breaking down organic waste without oxygen. This process, called anaerobic digestion, involves microorganisms that decompose the waste into these gases (Vargas-Estrada et al., 2021).

11.1.2 Hydrolysis: Hydrolytic enzymes convert complex organic polymers (lipids, proteins, and carbohydrates) in the algal biomass into simpler monomers (sugars, amino acids, and fatty acids) during the hydrolysis process (Batstone et al., 2002).

11.1.3 Acidogenesis: Acid-producing bacteria transform the initial breakdown products into volatile fatty acids, alcohols, hydrogen, and carbon dioxide. Since this prepares the substrates for methanogenesis, this step is critical to the overall efficiency of the process (Zamalloa et al., 2012).

11.1.4 Acetogenesis: Acetogenic bacteria convert acid-forming products into acetic acid, hydrogen and CO_2. In (Angelaidaki et al., 2011), this step is the transition between acidogenesis and methanogenesis.

11.1.5 Methanogenesis: Methanogenic archaea are able to produce methane and water from acetic acid, hydrogen and CO_2. In this step, the primary biogas production phase, a mixture of approximately 50-70% methane and 30-50% CO_2 is created (Ward et al., 2008).

12. Biohydrogen from microalgae: Production processes

The production of biohydrogen using photobiological and dark fermentation processes results in a sustainable and clean energy source. Fuel cells can use hydrogen, which is a more attractive fuel than fossil fuels because it has high energy content and only produces water as a byproduct (Goswami et al., 2021).

12.1 Photobiological Hydrogen Production

With the help of light energy, photosynthetic microorganisms such as cyanobacteria and green algae are used in photobiological hydrogen production to produce hydrogen. There are two main ways that play a role here:

12.1.1 Direct Photolysis: In direct photolysis, water molecules are split into hydrogen and oxygen using the light energy absorbed by photosystem II as illustrated in equation (2). This process occurs in green algae such as *Chlamydomonas reinhardtii under* anaerobic conditions (Melis & Happe, 2001).

$$2H_2O \longrightarrow 2H_2 + O_2 \qquad (2)$$

12.1.2 Indirect Photolysis: Indirect photolysis is the process by which photosynthetic microorganisms convert light-sensitive carbohydrates into hydrogen by fermenting them in the absence of light (Ghirardi et al., 2000).

12.2 Dark Fermentation

Dark fermentation is an anaerobic process that produces hydrogen from organic materials without the use of light. Fermentative bacteria such as *Clostridia* and *Enterobacter* are usually responsible for this process (Singh & Das, 2020).

12.2.1 Substrate Breakdown: Glycolysis is a process in which organic substrates (e.g. sugars) are broken down into pyruvate.

12.2.2 Hydrogen Production: Hydrogenase enzymes carry out further pyruvate metabolism to produce CO_2, acetate and hydrogen as illustrated in equation (3).

$$C_6H_{12}O_6 \longrightarrow 2C_2H_4O_2 + 2H_2 + 2CO_2 \qquad (3)$$

Higher hydrogen production rates and the ability to use various organic wastes as substrates are two advantages of dark fermentation (Levin et al., 2004).

13. Bio-oil from microalgae: Production process

The intricate and multifaceted process that encompasses the production of algal bio-oil is characterized by several pivotal steps, which range from the initial cultivation of algae in various environments to the subsequent extraction of the oil that these organisms produce (Guo et al., 2015).

13.1 Oil Extraction

13.1.1 Cell Disruption: The algal cellular structures undergo a process of disruption in order to facilitate the release of the valuable intracellular oil contained within them (Chaiwong et al., 2013). This release can effectively be accomplished through a variety of mechanical techniques, including but not limited to bead milling, ultrasonic disruption, or high-pressure homogenization, while also incorporating several chemical methodologies such as solvent extraction to enhance the efficiency of the process (Saber et al., 2016).

13.1.2 Solvent Extraction: In this context, a specific solvent, for instance, hexane, is employed to dissolve the lipids that have been extracted from the now-disrupted algal cells (Xiu & Shahbazi, 2012). Following this dissolution process, the resulting mixture of solvent and oil is subsequently subjected to a separation procedure, which involves isolating the solvent-oil combination from the residual biomass that remains (Latif et al., 2019).

13.1.3 Solvent Recovery: Ultimately, the solvent utilized in the extraction process is meticulously removed from the oil through various techniques, which may include distillation or evaporation, thereby resulting in the production of crude algal bio-oil that holds significant potential for further applications in biofuel and bioproduct development (Kumar et al., 2018).

13.2 Oil Refining

13.2.1 Purification: The initial purification phase of crude bio-oil meticulously targets impurities that may jeopardize oil integrity, such as free fatty acids and pigments (Baskar et al., 2019). This essential purification process ensures the oil adheres to rigorous standards for further processing, thus improving its applicability in biofuels (Ahmed et al., 2023).

13.2.2 Upgrading: Algal bio-oil may require additional refining to produce various biofuels, including biodiesel and jet fuel (Mondal & Soni, 2012). Advanced processes like hydrotreating and catalytic cracking are necessary to enhance biofuel properties to meet industry standards (Galadima & Muraza, 2018).

14. Syngas from microalgae: production process

The generation of synthesis gas, commonly referred to as syngas, which is a complex amalgamation of hydrogen, carbon monoxide, and various other gaseous components, necessitates the execution of a series of pivotal stages that encompass the cultivation of algal organisms, the meticulous preparation of the resultant biomass, and the subsequent process of gasification (Faraji & Saidi, 2021).

14.1 Gasification

The desiccated algal biomass is introduced into a gasification reactor, wherein it is exposed to elevated temperatures, which typically range from 700 degrees Celsius to 1000 degrees Celsius, all while being maintained in the presence of a meticulously regulated amount of either oxygen or steam to facilitate the process effectively (Voloshin et al., 2016). The primary chemical reactions that occur during this transformative phase are presented in equation (4).

$$C_xH_y + O_2 \rightarrow CO + H_2 + CO_2 + CH_4 \qquad (4)$$

14.1.1 Partial Oxidation: The algal biomass undergoes a partial oxidation process, resulting in the formation of a diverse mixture of gaseous products, including but not limited to carbon monoxide (CO), hydrogen (H_2), carbon dioxide (CO_2), methane (CH_4), along with various other gaseous compounds that may also be present in smaller quantities, thereby enriching the composition of the resultant syngas (Azadi et al., 2014).

14.2 Syngas Cleanup and Conditioning

14.2.1 Removal of Impurities: The unrefined synthesis gas contains contaminants such as tar, sulfur compounds, and nitrogen oxides that detrimentally impact gas quality (Di Ingegneria "Enzo Ferrari & Di Scienze Della Vita, 2016). To enhance syngas purity, these impurities require removal via engineering processes, including filtration, scrubbing, and catalytic cracking (Toledo-Cervantes et al., 2017).

14.2.2 Gas Conditioning: Following purification, syngas is conditioned to achieve an optimal hydrogen-to-carbon monoxide ratio essential for subsequent applications. This conditioning may utilize various methodologies, including water-gas shift reactions that convert carbon monoxide and steam into carbon dioxide and hydrogen. This process adjusts the gas mixture's composition to align with the desired specifications for efficient downstream utilization, as illustrated in equation (5).

$$CO + H_2O \rightarrow CO_2 + H_2 \qquad (5)$$

The cleaned and conditioned syngas typically consist of hydrogen (H_2) and carbon monoxide (CO), along with small amounts of carbon dioxide (CO_2) and methane (CH_4) (Kumar & Aarthi, 2020).

15. Challenges in Algal Biofuel Production

Challenges in algal biofuel production include high costs and difficulties in large-scale implementation. Optimizing growth conditions for algae is complex and resource-intensive. Additionally, there are technical challenges in extracting and converting algal biomass into biofuels. Environmental sustainability issues, particularly concerning water and nutrient use, hinder the broader acceptance of algal biofuels (Tazikeh et al., 2022). Figure 6 depicts the various challenges associated with algal biomass biofuel production.

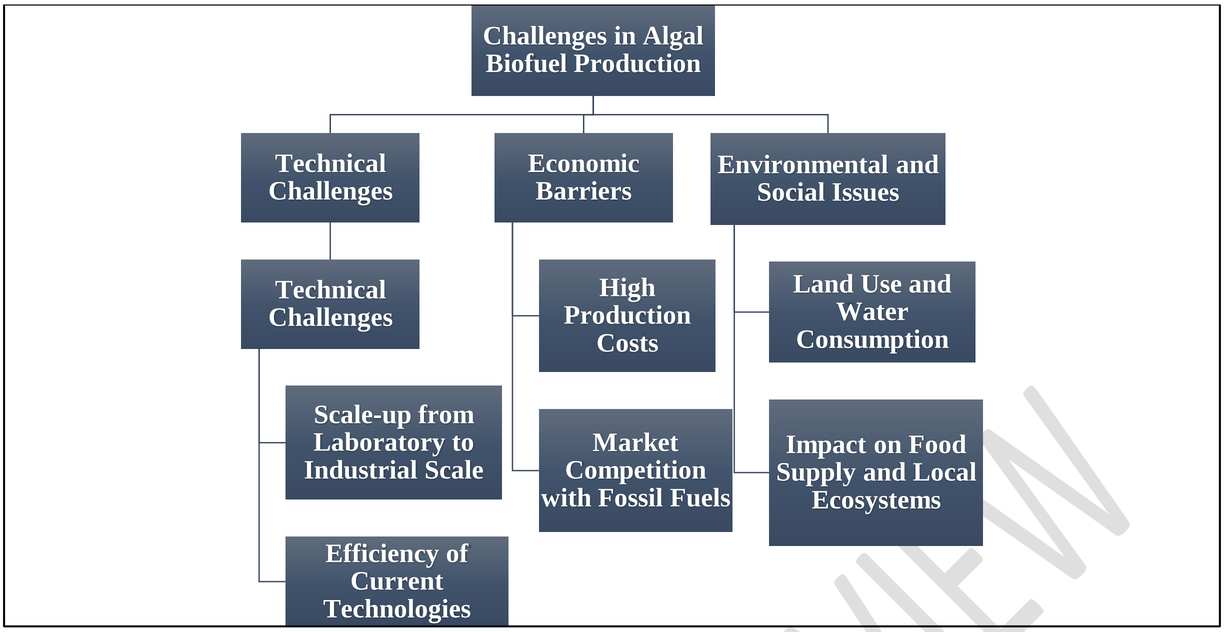


Figure 6. Various challenges in the biofuel production from algal biomass

15.1 Technical Challenges

15.1.1 Scale-up from Laboratory to Industrial Scale

There are many technical obstacles to increasing algae biofuel production from laboratory to industrial scale. Although conditions in laboratories are strictly regulated, it is difficult and expensive to reproduce these conditions in larger environments. In large-scale cultivation systems such as open ponds and photobioreactors, problems with light transmission, contamination, and maintaining ideal growing conditions often arise. Based on research by (Acién et al., 2012), significant investments in infrastructure and technology development are required for the transition from laboratory to industrial scale.

15.1.2 Efficiency of Current Technologies

Another significant obstacle is the effectiveness of the technologies used today to produce algae biofuel. The techniques used today to collect and separate lipids from microalgae are not effective enough to be used in a commercial setting. Energy-intensive and expensive processes include flocculation, solvent extraction, and centrifugation. To increase overall efficiency, further developments in genetic engineering, cultivation techniques, and biorefinery approaches are required. The efficiency levels required for commercial production cannot be achieved with current technologies, as (Posten and Schaub, 2009) pointed out.

15.2 Economic Barriers

15.2.1 High Production Costs

The high production costs of algae biofuels compared to traditional fossil fuels are one of the biggest economic obstacles. High energy expenditure for cultivation and harvesting, expensive nutrients, and the costs of building and maintaining production facilities are, among other things, cost-driving factors. According to the U.S. Department of Energy (2016), lower costs are essential for the commercialization of algae-based biofuels.

15.2.2 Market Competition with Fossil Fuels

Fossil fuels, which are currently more affordable and have established supply chains, pose a serious threat to algal biofuels. The competitiveness of biofuels is also influenced by changes in crude oil prices. Without subsidies or legal incentives, it is difficult for algae biofuels to compete in the market. According to the International Energy Agency (2020), for the biofuel market to become competitive with fossil fuels, significant political support is required.

15.3 Environmental and Social Issues

15.3.1 Land Use and Water Consumption

The production of algae biofuels has a significant impact on the environment due to high water and land consumption. Large-scale seaweed cultivation requires large areas of land that can compete with other land uses, even if this is possible on uncultivable land. Water consumption is particularly high in open pond systems, where evaporation losses are significant. Based on (Clarens et al., 2010), solving these land and water use problems is necessary for the sustainability of algae-based biofuels.

15.3.2 Impact on Food Supply and Local Ecosystems

The resources required by algae can have an impact on local ecosystems and food availability, even if they are not in direct competition with food crops. Water availability and local agriculture can suffer if nutrients and water are diverted to algae farming. Large-scale seaweed farming can also alter the environment in the region, which can have negative impacts on biodiversity and other aspects of the environment. To reduce these impacts, (Campbell, 2011) emphasizes the need for careful planning and management.

16. Recent Advancements and Innovations in Algal Biofuel Production

16.1 Genetic Engineering approaches for enhanced Algal Biofuel generation

16.1.1 Improved Strains for Higher Yields

The yield and productivity of microalgae used to produce biofuels have been significantly increased through genetic engineering. Higher lipid content, faster growth rates, and increased resistance to environmental stressors are among the characteristics of genetically modified microalgae that researchers have created. As an example, (Radakovits et.al, 2010) showed that genetic engineering can increase the lipid content of microalgae, thereby increasing their suitability for the production of commercial biofuels. In addition, algae metabolic pathways are optimized through synthetic biology techniques, improving biofuel yields (Georgianna and Mayfield, 2012).

16.2 Process Optimization

16.2.1 Advances in Cultivation and Harvesting Technologies

To increase productivity and reduce costs, significant progress has been made in the cultivation and harvesting of microalgae. With the advent of tube and flat panel photobioreactors and other designs, the productivity of photobioreactors has increased due to improved light penetration and CO_2 utilization (Tredici, 2010). In addition, new harvesting methods have been developed to reduce the cost and energy requirements for biomass recovery, such as membrane filtration and electrocoagulation (Uduman et al., 2010). The scalability and financial viability of algae-based biofuels depend on these technological advances.

16.3 Integration with Waste Streams

16.3.1 Using Wastewater and CO_2 for Cultivation

A sustainable way to reduce production costs and environmental impact is to combine algae biofuel production with waste streams. Wastewater can be repurposed as a nutrient source for algae growth, thereby improving water quality and lessening the demand for synthetic fertilizers. Research has shown that algae can efficiently produce biomass for biofuels while removing pollutants and nutrients from wastewater (Pittman et al. 2011). In addition, algal growth can be improved and greenhouse gas emissions reduced by using CO_2 emissions from industrial sources as food for algal cultures (Singh and Olsen, 2011).

Conclusion

In conclusion, we can say that algae biofuels are a viable replacement for traditional fossil fuels thanks to several developments and innovations that have made them more viable. Genetically modified strains have been improved to achieve higher lipid yields and faster growth rates, increasing the efficiency and commercial attractiveness of algal biofuels (Radakovits et al. 2010; Mayfield & Georgianna, 2012). Advances in photobioreactor technology and effective harvesting techniques, as well as other innovations in cultivation and harvesting technologies, have increased overall productivity and reduced costs (Tredici, 2010; Uduman et al., 2010). Recycling waste and capturing greenhouse gases are two ways in which the use of wastewater and industrial CO_2 emissions for algae farming benefits the environment in addition to reducing production costs (Pittman et al., 2011; Singh & Olsen, 2011). In addition to this, scalability, integration with other renewable technologies, thorough environmental impact assessments, cost reduction tactics, and optimization of cropping systems are among the areas that require more research (Molina Grima et al., 2003; Clarens et al., 2010; Acién et al., 2012).

The Future Outlook for Algal Biofuels

The prospects for algae biofuels are promising due to technological advancements and increased environmental consciousness. Research and development are anticipated to lower production costs, enhancing the competitiveness of algae biofuels against fossil fuels. The integration of algae biofuel production with waste management and renewable energy technologies can bolster economic viability and sustainability. Demand for algae biofuels is likely to rise as regulations promote low-carbon and renewable energy alternatives. Development of genetically modified algae strains with enhanced resilience and lipid production is underway. Enhancements in open pond systems and photobioreactor designs aim to optimize light and nutrient use. The creation of cost-effective, energy-efficient harvesting methods is essential for reducing production expenses. Additionally, thorough life cycle assessments are crucial for evaluating environmental impacts and identifying necessary improvements, alongside financial incentives to foster innovation in algae biofuel production and encourage synergies between carbon capture, wastewater treatment, and algae biofuels, while fostering collaboration among governmental entities, enterprises, and research institutions to expedite the commercialization of algae-derived biofuels.

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