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Review Article

The upstream and downstream processes of extracting oil from microalgae to produce biodiesel: A review

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Abstract: In recent years, the depletion of fossil fuel reserves and the rise in air pollution have drawn the attention of scientists towards seeking solutions and identifying suitable alternatives to fossil fuels. Biofuels derived from terrestrial plant lipids were once proposed, but due to competition with agricultural demands, this solution lost its viability. However, microalgae have emerged as a promising alternative to fossil fuels, as they neither contribute to air pollution nor compete with food production. Their rapid growth rate and substantial oil production make them a favorable option. Despite these advantages, the economics of microalgae-based biofuels remain challenged by the high costs associated with drying and current extraction methods. This work provides an overview of both upstream processes, including cultivation, harvesting, and drying, and downstream processes such as cell wall disruption, oil extraction, and transesterification, as well as the oil production potential of microalgae. The article suggests that future research should concentrate on microalgae cultivation in wastewater, novel extraction methods, and the simultaneous extraction of multiple compounds from microalgae.

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Introduction

Microalgae have garnered attention in the past decade for their potential in biodiesel production. In recent years, the focus has shifted towards their utilization in the food, chemical, and pharmaceutical sectors. Microalgae contain substantial amounts of lipids, proteins, and carbohydrates, all of which have diverse applications (Wijffels and Barbosa, 2010; Tahergorabi et al., 2017). In comparison to terrestrial crops, microalgae can thrive in a wide range of aquatic environments, including oceans, freshwater bodies, and wetlands. They can even be cultivated in wastewater and brackish water, avoiding competition for agricultural land, soil depletion, and habitat destruction. Due to their high photosynthetic rates, oil productivity, and manageable culture conditions, microalgae show promise for industrial production (Singh and Gu, 2010).

Microalgae also have the potential to mitigate the greenhouse effect and water pollution (Hosseini et al., 2008). Through photosynthesis, they can capture carbon dioxide emissions from industrial plants. Some microalgae species also fix nitrogen and absorb pollutants, such as heavy metals and phosphorus (Harun et al., 2010). Given their ability to yield biomass rich in lipids, microalgae can serve as feedstock for biodiesel production. In the face of dwindling fossil fuel reserves and fluctuating fuel prices, renewable alternatives such as biofuels have garnered global interest (Sundus et al., 2017). The advantages of biodiesel over fossil fuels, including its renewability, non-toxicity, absence of sulfur, and improved lubricity, have been extensively documented (Aransiola et al., 2014). Microalgae, due to their ability to accumulate lipids, stand out as viable candidates for biodiesel production (Hosseini et al., 2024).

Furthermore, the lipid productivity of many

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oleaginous microalgae surpasses that of traditional oil-producing crops (Chisti, 2008). The typical lipid content of microalgae ranges from 20 to 50% of dry weight, and under certain conditions, it can reach up to 90% (Mata et al., 2010). Environmental stressors, such as temperature, light, nitrogen, and phosphorus, can increase lipid content (Chokshi et al., 2016). However, without effective lipid extraction methods, large-scale production of biodiesel from microalgae would remain impractical. Current research indicates that lipid extraction from microalgae is primarily solvent-based, either directly or through cell wall disruption methods (Chisti, 2008). In this review article, we explore the methods of extracting oil from microalgae for biodiesel production. We cover both upstream processes, including cultivation, harvesting, and drying, as well as downstream processes such as cell disruption, lipid extraction, and biodiesel production.

Up stream

***Cultivation*:** For biodiesel production, several microalgae species have been identified with a high lipid percentage, such as *Nannochloropsis oculata* and *Chlorella vulgaris* (Cornejo-Corona et al., 2016). In addition to selecting specific microalgae, cultivation conditions such as temperature, salinity, pH, light intensity, and nutrient availability are crucial for achieving the desired accumulation of target substances. The choice of cultivation system also plays a pivotal role and depends on the microalgae strain being considered (Chew et al., 2018). Microalgae can be cultivated in various systems, including transparent tubes with added light and nutrients, as well as fiberglass or raceway pond tanks. Table 1 outlines the advantages and disadvantages of both open and closed cultivation systems. Also, microalgae can be cultivated using urban sewage, where the high phosphorus and nitrogen content create favorable conditions for their growth. Additionally, the abundant acetate present in urban wastewater serves as an ideal carbon source for microalgae cultivation (Lowrey et al., 2015). Overall, successful microalgae cultivation relies on several key factors, including water availability, carbon dioxide concentration, microalgae strain, nutrient supply, and light availability. There are three primary techniques for microalgae growth: autotrophic, phototrophic, and mixotrophic.

1- Autotrophic culture: In autotrophic cultivation, photosynthesis plays a pivotal role in the survival of microalgae cells. In this process, microalgae cells absorb light and carbon dioxide, converting them into biomass and oxygen (Falkowski and Raven, 2013). Currently, the predominant method of microalgae culture is autotrophic. In this approach, cells utilize light as an energy source and carbon dioxide as a source of carbon. The primary objective and constraint of this culture are to provide sufficient natural or artificial light to support mass growth and population increase (Mandalam and Palsson, 1998).

2- Heterotrophic culture: This system does not rely on light, and the biomass is nourished with an organic carbon source. As a result, microalgae are cultivated in stirred tanks or fermenter reactors, where higher growth and lower harvesting costs are achieved due to elevated dry biomass productivity (up to 25.0 g/L/day) and substantial accumulation of various components, such as lipids (ranging from 22-54 mg/L/day). Carbon sources such as glucose, acetate, glycerol, and glutamate are utilized by *C. vulgaris*, with glucose demonstrating the greatest improvement in growth rate. However, the main drawback of these systems is the high cost associated with sugars (Ogawa and Aiba, 2012).

3- Mixotrophic cultivation: Microalgae can adopt a combination of autotrophic and heterotrophic methods by utilizing photosynthesis alongside consuming organic substances like glucose, which is well-suited for microalgae (Liang et al., 2009). This means that cells are not heavily reliant on either light or organic matter for growth. In mixotrophic culture, carbon dioxide and organic compounds are utilized simultaneously, and both respiration and photosynthesis mechanisms work in tandem (Lee, 2004). Photosynthesis metabolism harnesses light for growth, while aerobic respiration relies on an organic carbon source (Andrade and Costa, 2007). The capacity of mixotrophs to assimilate organic substrate

Table 1. Comparison of open culture and cloze culture.

Factor	Open system	Close system
Risk of contamination	High	Low
required space	High	Low
Evaporation	High	Low
process control	Impossible	Possible
Standardization	Impossible	Possible
Density	Low	High
Time	Long	Short

implies that cell growth is not solely dependent on photosynthesis, making light energy less limiting for growth. Both light and organic substrate contribute to growth promotion (Chen et al., 1996). It has been established that mixotrophic culture is an optimal method, yielding a high range of cell densities (Um and Kim, 2009).

Harvesting

When microalgae cells reach the end of the logarithmic growth phase, they require harvesting. Various methods are used for harvesting microalgae, including filtration, centrifugation, and coagulation.

1- Filtration: Filtration is an economical and straightforward method of separating suspended solids from liquids, utilizing gravity and various filters. However, this process proves challenging with *Chlorella* and *Scenedesmus* microalgae due to their small size. For *Spirulina*, this process is more feasible due to its filamentous nature (Becker, 1985).

2- Centrifugation: Centrifugation is the most effective approach for separating filamentous and non-filamentous algae. This process accounts for 20-30% of the total cost of biomass production. The most common method employed for harvesting *C. vulgaris* is centrifugation (5000 rpm, 15 min) due to its efficiency, quickness, and suitability for treating large volumes (Grima et al., 2003).

3- Coagulation: In the logarithmic growth stage, microalgae cells carry a significant negative charge, which makes their separation challenging and results in dispersed cells. Once the stationary phase is reached, the negative charge decreases, causing cells to accumulate and form clusters. Consequently, a process termed coagulation occurs spontaneously. This phenomenon is linked to an elevated pH attributed to the uptake of CO_2, nitrate, and phosphate (Vandamme et al., 2012). To expedite coagulation, a pH elevation is necessary, which can be achieved by adding a base. Sodium hydroxide is the most effective flocculating agent, achieving over 90% flocculation at a pH of 11 (Vandamme et al., 2012). On an industrial scale, lime proves to be the most cost-effective option. Chitosan, exhibiting compelling flocculation properties, demonstrates peak efficiency at a pH of 7, yielding a 90% recovery of microalgae (Divakaran et al., 2002).

Drying

Following microalgae harvesting, biomass dry solid content remains low (Christenson and Sims, 2011). Accordingly, a drying process—such as sun drying, convection drying, or freeze drying—is typically employed based on the requirements of the final product (Dissa et al., 2010).

1- Sun drying: Sun drying, the most economical technique, demands ample time and space for drying (Brennan and Owende, 2010). Nevertheless, maintaining product quality proves challenging with sun-drying methods due to slow drying rates resulting from low temperatures, which can potentially lead to biomass degradation and elevated bacterial counts (Prakash et al., 1997).

2- Convection drying: Convection drying, a widely used method for drying algae, typically employs convective hot air drying (Oliveira et al., 2009). The optimal temperature range for Spirulina sp. using oven drying within this method lies between 40 and 55°C. Notably, prolonged exposure to high temperatures can damage fatty acid structures (Desmorieux and Decaen, 2005).

3- Freeze drying: Lyophilization, also known as

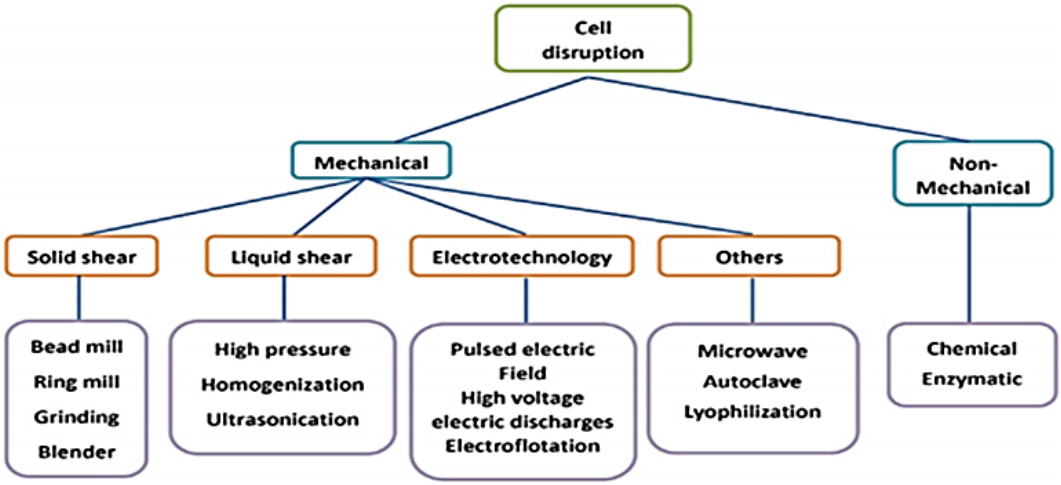

Figure 1. Types of cell wall destruction methods (Li-Beisson et al., 2013).

freeze drying, is a process in which water is removed from a product after freezing, while under vacuum, causing ice to transition directly from a solid to a vapor, bypassing the liquid phase. When compared to convection drying, infrared drying, and spray drying, freeze drying preserves the highest protein content in dried microalgal biomass (Desmorieux and Decaen, 2005).

Downstream

Pre-treatment (cell wall disruption): The cell wall of microalgae serves various functions, including disease prevention, resistance to dehydration, and regulation of cellular homeostasis. Disrupting the cell wall is a crucial step in maximizing product recovery in downstream processes of algal biorefineries. Furthermore, for the direct consumption of algae in feed or food, breaking down the cell wall is essential to enhance the absorption of algae compounds. Today, various methods are available to disrupt the cell wall. As downstream processes constitute a significant portion of production costs, cell disruption technologies should be cost-effective, energy-efficient, and ideally maintain high product quality (D'Hondt et al., 2017). In this section, we review physical-mechanical and biochemical cell disruption technologies (Fig. 1).

1- Physical methods for cell wall disruption: Physical pretreatment is categorized based on the forces disrupting the cell wall, and it can be divided into thermal and mechanical methods. Thermal pretreatment involves the use of heat and freezes techniques. High-temperature methods (X > 100°C) and freezing methods are two approaches within heat pretreatment. Freeze fracture involves subjecting microalgae cells to a series of freeze-thaw cycles, utilizing the formation of ice crystals to achieve cell disruption. Freeze-drying (lyophilization) employs low pressure (approximately 1 kPa) and temperatures below -40℃ on slowly frozen algae samples. The slow freezing process leads to porous cell walls due to the formation of large ice crystals. Thermal pretreatments offer advantages such as cost-effectiveness, availability, and the absence of chemical usage (Patel et al., 2016).

Mechanical pretreatment involves physically breaking cell wall components. Bead milling, high-pressure homogenization, and ultrasonication are standard mechanical methods for disrupting microalgae cells. Bead milling, which utilizes kinetic energy, causes small beads (made of glass, ceramic, plastic, or steel) to collide with each other and algae cells, thereby resulting in cell wall destruction. High-pressure homogenization, one of the earliest techniques for cell disruption, involves pumping microalgae concentrate through a narrow orifice (200-80 µm) into a high-pressure valve (400-138 MPa), followed by releasing the suspension into a low-pressure chamber. Microwaves have been widely utilized for thermal pretreatment of biomass feedstock, including lignocellulosic, microalgae, and

macroalgae biomass (Yun et al., 2016). Frequencies ranging from 3.0 to 300 GHz, particularly those at 2450 MHz, are commonly used to disrupt the cell walls of microalgae. This process relies on the interaction of electromagnetic waves with dielectric and polar molecules, generating heat and increasing internal pressure (Gunerken et al., 2015).

Ultrasound waves possess a frequency higher than 15-20 kHz, which is beyond the range of human hearing. Initially, these waves can inactivate cells, but at higher power levels, they are capable of destroying cells. The mechanism involves creating numerous tiny holes in the liquid that collectively disintegrate, converting a substantial amount of sound energy into mechanical energy (elastic waves) and ultimately causing cell wall destruction (Chisti and Moo-Young, 1986).

2- Chemical and biological methods for disrupting cell walls: Chemical disruption of cells using a wide range of compounds, including antibiotics, chaotropes, detergents, solvents, oxidizing agents, acids, and alkalis, has been widely studied (Gunerken et al., 2015). In chemical methods, energy consumption is generally lower, and cell destruction efficiency is higher. However, the cost of the chemicals and the quality of the products may reduce their benefits. Among chemical methods, H_2SO_4 is the most commonly used acid, while NaOH is the most effective among bases. These materials have been proven effective as pretreatments for the fermentation and extraction of intracellular compounds, such as lipids and pigments (Mendes-Pinto et al., 2001; Nguyen et al., 2009; Miranda et al., 2012). Methods based on acids and bases are faster, but they have drawbacks, including the production of inhibitors, equipment corrosion, difficult chemical recovery, and high operating and maintenance costs. Denaturation of proteins can occur in alkaline environments, while degradation of pigments typically occurs in acidic environments (Gunerken et al., 2015).

Extraction using ionic liquids is another relatively advanced method. This method is highly effective for lipid extraction and ethanol production processes. The advantages of this method include low volatility, high solubility, a short reaction time, and the recovery and reuse of ionic liquids. Depending on the type of ionic liquid, it can also serve as a solvent for lipid extraction and a catalyst for transesterification (Mohan et al., 2014).

Enzymatic hydrolysis is a biochemical method that breaks down carbohydrates into glucose and proteins into amino acids, leading to the disruption of the cell wall. Enzymes often used for breaking down the cell wall are cellulases, glycosidases, amylases, proteases, peptidases, and lipases (Lam and Lee, 2015). The enzymatic method has several advantages over the chemical method, including biological specificity, high selectivity, high conversion efficiency, mild operating conditions, low energy requirements, and lower investment costs. However, there are drawbacks, such as inhibitor production and the high cost of enzymes, as well as difficulties in enzyme recovery (Lam and Lee, 2015).

Oxidizing agents, such as H_2O_2 or ozone, can also react with the constituents of the cell wall, leading to its destruction and disruption (Concas et al., 2015). This pretreatment can enhance extraction efficiency; however, the reaction time should be kept short to prevent oxidation of the target compounds. Ozonolysis is a promising method with advantages over traditional methods, including the low production of inhibitory compounds, minimal impact on carbohydrates, no liquid phase, no need for chemicals, mild conditions, direct in-situ ozone production, and direct ozonolysis. Disadvantages include high operating costs, toxicity, flammability, corrosion, reactivity, and the use of special equipment materials (Travaini et al., 2016). Careful consideration of performance parameters is necessary to minimize ozone consumption and the generation of by-products that could act as inhibitory compounds or impurities in downstream processes. Key parameters in the reactor design process include moisture content, ozone concentration, ozone-to-air flow ratio, and pretreatment time (Travaini et al., 2016).

Lipid extraction: To extract lipids from oilseeds, a large-scale industrial mechanical press is applied to biomass products such as peanuts, soybeans, and

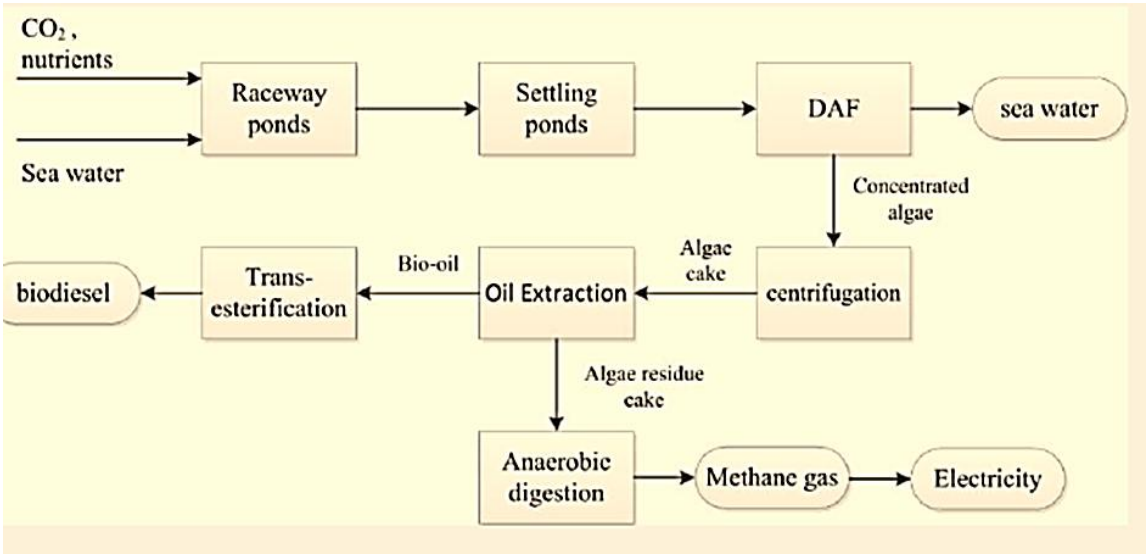

Figure 2. Schematic figure of biodiesel production from microalgae (Gunerken et al., 2015).

wheat germ. However, this method is not suitable for microalgae biomass due to differences in morphological and biochemical characteristics, as well as their cultivation in an aquatic environment. Therefore, the presence of water greatly affects the extraction of oil from wet microalgae. Consequently, for lipid extraction from microalgae, methods involving organic solvents and supercritical carbon dioxide (known as the green extraction method) are recommended.

1- Lipid extraction by organic solvents: The lipid extraction procedure employing organic solvents is based on the principle of chemical affinity. Non-polar solvents, such as hexane, are used to extract non-polar lipids, while polar solvents, like methanol, are utilized to extract polar lipids like phospholipids (Pragya et al., 2013). A common solvent mixture of chloroform and methanol is frequently used for lipid extraction from microalgae in the literature (Chatsungnoen and Chisti, 2016). Bligh and Dyer's method, Folch et al.'s method, and Soxhlet's method are widely recognized as effective techniques for lipid extraction from microalgae. In industrial production, hexane stands as the most commonly employed solvent for lipid extraction from microalgae (Khattab and Zeitoun, 2013). While hexane offers relatively lower efficiency compared to chloroform and methanol, its advantages include reduced toxicity, low latent heat of vaporization, and high selectivity for extracting neutral lipids. Hence, hexane is widely used in this context (Halim et al., 2011).

2- Lipid extraction by supercritical carbon dioxide: The supercritical carbon dioxide method is commonly used for extracting lipids from microalgae. Carbon dioxide, serving as a suitable alternative to organic solvents, is safe and non-toxic, while also possessing a favorable solubility for non-polar lipids (Letisse et al., 2006). An advantageous aspect of the supercritical carbon dioxide method is its adjustable solvent power, which can be achieved by adjusting the pressure and temperature during the process. Supercritical carbon dioxide (SC-CO_2) extraction is typically conducted on a laboratory scale. The efficacy of SC-CO_2 extraction is influenced by a combination of factors, including extraction duration, particle size, and the temperature and pressure settings (Del Valle et al., 2005) (Table 2).

3- Transesterification: Biodiesel is produced from extracted lipids (Fig. 2). However, lipid extraction is often costly due to the processes involved in cell wall disruption and dehydration (Cheng et al., 2017). The transesterification process, developed several years ago, has proven to be highly successful (Islam et al., 2017). This reaction involves the interaction of methanol or ethanol with oil in the presence of a catalyst, resulting in the conversion of the oil into biodiesel and glycerol (Islam et al., 2017). In recent years, extensive research has been conducted on the direct production of biodiesel from both wet and dry

Table 1. Comparison of supercritical CO_2 and solvent extractions.

Solvent extraction	Supercritical CO_2 extraction
The presence of solvent is inevitable; residual solvent concentration (usually in the order of ppm) depends on the solvent used.	The procedure is completely free of solvents, and thus, the extracts are very pure.
Heavy metal contamination is also unavoidable, and depends on the solvent, solvent recycling procedure, source of raw material, and what the machinery parts are made from	Free of heavy metals; not extracted, even if they're present in the raw material. There are no heavy metals present in CO2 or the equipment.
Inorganic salt content is also difficult to avoid (same reasons as above)	Free of inorganic salts (same reasons as above)
Solvents have poor selectivity; during solvent removal, polar substances form polymers, which lead to discoloration of the extract and poor flow characteristics	CO_2 is highly selective, so there is no chance of polar substances forming polymers
Both polar and non-polar colors are extracted	Only non-polar colors get extracted
Solvent removal requires extra unit operations, which results in higher cost and lower recoveries	No extra unit operations required, and the yield is very high

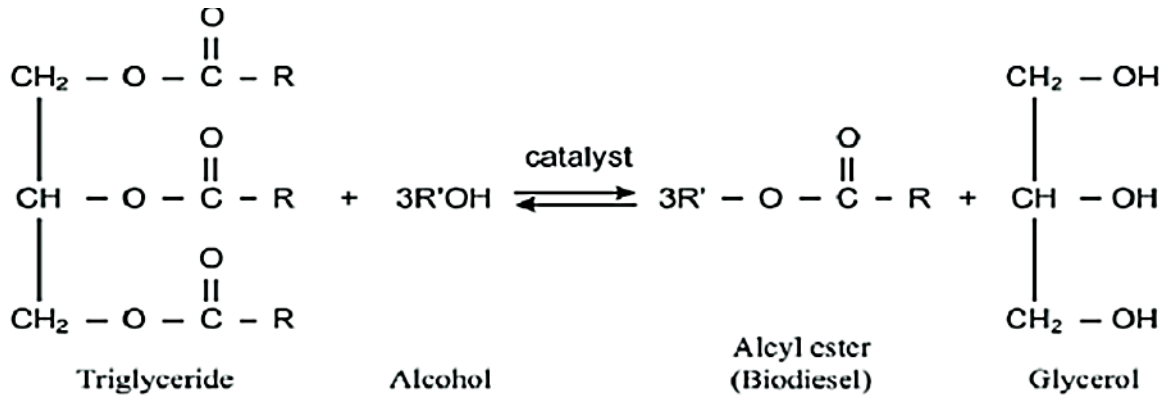

Figure 3. Schematic of transesterification (Yun et al., 2016).

microalgae biomass. Studies have shown that higher humidity results in a decreased biodiesel yield (Im et al., 2014). The addition of a co-solvent has been explored to enhance process efficiency by functioning as an extraction agent and forming a homogenous system among microalgae oil, alcohol, and catalyst (Martínez et al., 2017). However, Chen (2015) noted that direct transesterification from biomass necessitates a higher catalyst loading compared to the transesterification of extracted oils. Several parameters influence the biodiesel yield from a single-step transesterification process, including catalyst application, the use of ultrasonic or microwave technologies, and the utilization of supercritical alcohols (Fig. 3).

Cultivation strategies for optimised TAG production

Numerous studies have confirmed that nitrogen limitation conditions (nitrogen stress) in the microalgae culture environment alter the carbon skeleton utilization pathway, directing it towards triacylglycerol production. Two nitrogen limitation systems have been tested (Fig. 4). In the sudden nitrogen limitation system (N starvation), optimal cultivation conditions are initially provided to achieve maximum biomass production, followed by the onset of the starvation phase (nitrogen deficiency). During this phase, biomass production halts, and triacylglycerols accumulate. This stage continues until cells begin to break down the accumulated triacylglycerols. Another nitrogen stress application system involves gradual limitation. In this approach, the amount of nitrogen supplied in the culture medium is limited to the extent that biomass production necessary for cell growth and development is restricted by nitrogen availability. However, the received light energy exceeds the required amount, resulting in an energy imbalance, and as a result, triacylglycerols continue to accumulate (Rodolfi et al., 2009). Dortch and Conway (1984) observed a decline

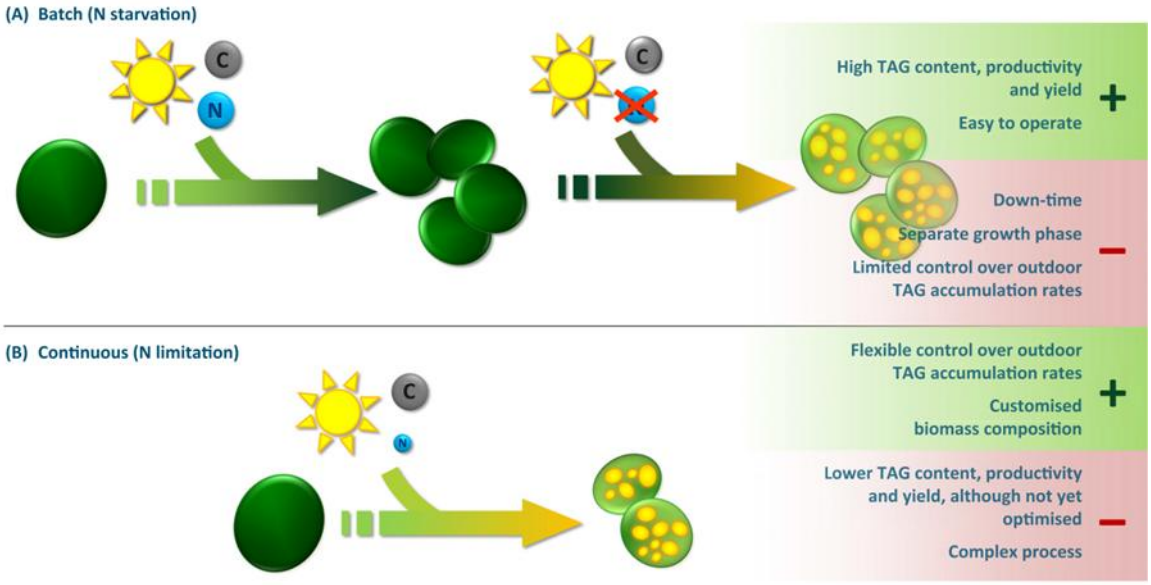

Figure 4. A. Nitrogen starvation, and B. Nitrogen limitation (Um and Kim, 2009).

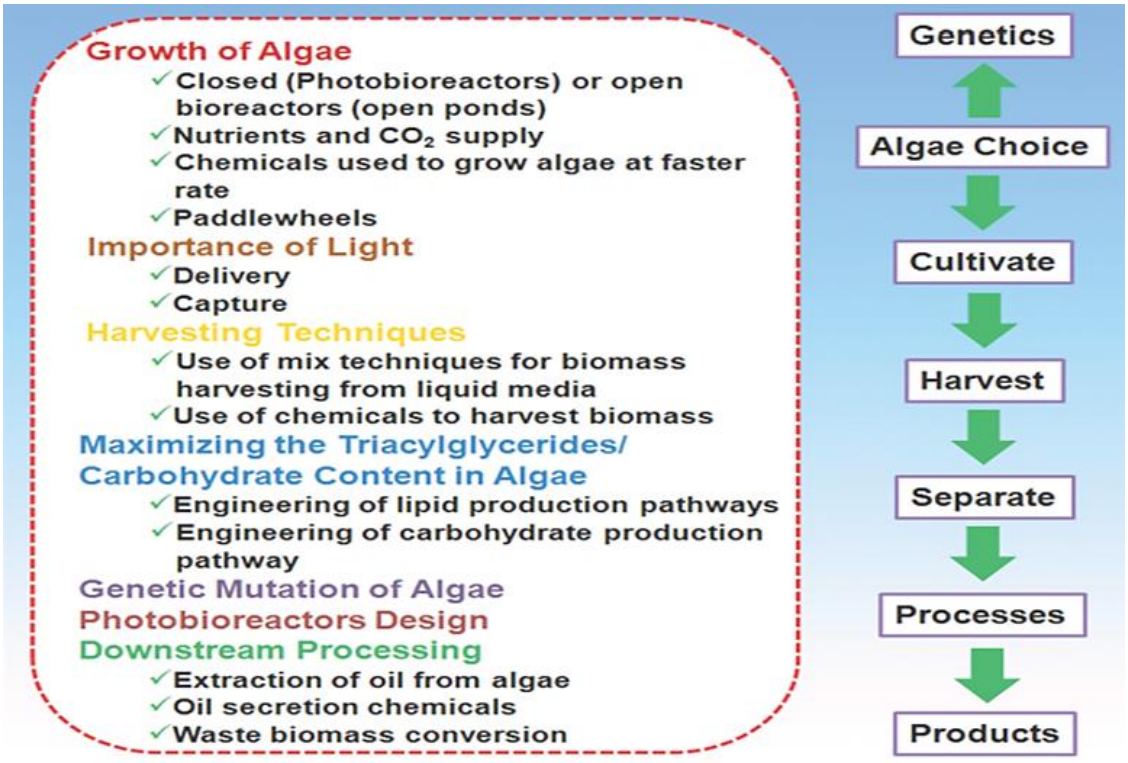

Figure 5. Schematic diagram of microalgae-based biofuel production and associated aspects including growth conditions, yield enhancement strategies and downstream process (Oliveira et al., 2009).

in the protein-chlorophyll complex during nitrate limitation. This complex is situated in the thylakoid membrane and transfers light energy to the reaction center of the photosystems. As photosynthesis continues while cell division stops due to nutrient scarcity, lipids build up within the cell. Under nutrient-deficient conditions, lipid production shifts toward storing triglycerides and neutral lipids (Fig. 5).

The mechanism of lipid production in microalgae

The first step is the synthesis of fatty acids by the fatty acid synthase (FAS) complex, which takes place in the chloroplast. During this stage, pyruvate, a precursor of fatty acids, is converted into acetyl coenzyme by the plastid pyruvate dehydrogenase complex, which is the

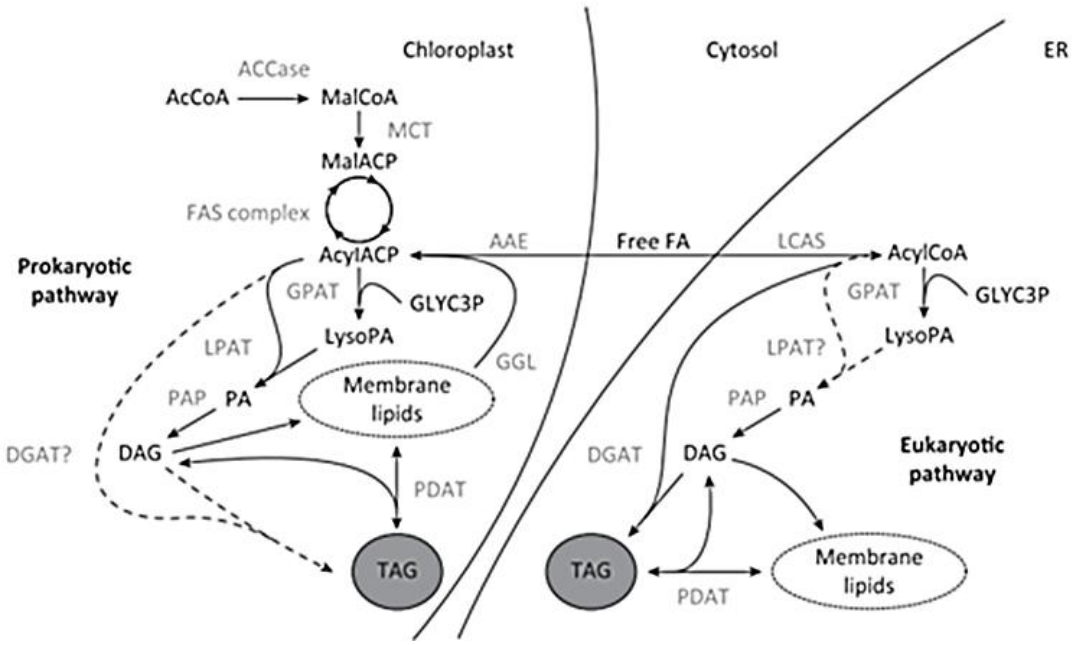

Figure 6. Biochemical pathway of TAG production in plant cells and microalgae (Mata et al., 2010).

building block for fatty acid production. Next, fatty acids are elongated by FAS complex enzymes through successive condensations of two-carbon units. In each cycle, four reactions occur: condensation, reduction, dehydration, and oxidation. Acyl carrier protein (ACP) serves as a cofactor in all of these reactions. The synthesis of a 16-carbon fatty acid requires seven repetitions of this cycle. In the first cycle, III ketoacyl-ACP synthase (KAS) catalyzes the condensation reaction. In the following six cycles, KAS II performs the condensation, and finally, KAS II is responsible for converting the 16:0 fatty acid. Most of the 18:0 fatty acids resulting from elongation are converted into 18:1 unsaturated fatty acids by the enzyme stearoyl-ACP desaturase (SAD). Additionally, fatty acids produced during this process can be hydrolyzed by the activity of fatty acyl thioesterase (FATA, FATB) enzymes, resulting in the production of free fatty acids (without ACP) (Li-Beisson et al., 2013).

The second stage involves the synthesis of triacylglycerols using fatty acids and glycerol. Four enzymatic steps are involved in attaching acyl-CoAs to the glycerol-3-phosphate (G3P) skeleton, known as the Kennedy pathway. GPAT and LPAT perform the first two acylations of G3P, followed by PAP activity, and DGAT carries out the third acylation. Additionally, TAG production from other intermediate compounds, such as membrane lipid phosphatidylcholine and diacylglycerol derived from it, occurs in this stage. Finally, in the third stage of TAG formation, nine glycerol molecules, along with proteins such as oleosin, caleosin, and steroleosin, are converted into lipids and released into the cytoplasm (Li-Beisson et al., 2013) (Fig. 6).

Future prospects and challenging aspects

Microalgae are highly promising as new renewable sources for biofuels. Despite these advantages, several obstacles still hinder the commercialization of biofuels derived from microalgae. Presently, the cost of producing algal biodiesel remains at least twice as high as that of petroleum-based biodiesel. Within the stages of algal biodiesel production, reducing the price of the dewatering stage is crucial, as it accounts for approximately 20-30% of the total production cost (Su and Ang, 2017). Furthermore, future research must emphasize mass algae cultivation using diverse wastewaters. Enhancing the survival and growth of chosen microalgal species in wastewater should involve genetic modification techniques. More efficient methods for lipid and product extraction should also be developed to lower biofuel production costs. While microalgae hold high market value as a protein source, commanding a higher selling price per weight compared to biofuels, lipid losses occur during

protein recovery. Consequently, new research should concentrate on wastewater-based microalgae cultivation, novel extraction methods, and the simultaneous extraction of multiple compounds from microalgae.

Conclusions

The commercial viability of algae biofuels hinges on microalgae species, cultivation conditions, and extraction approaches. Overcoming challenges, particularly in the upstream processes like drying, is essential. Therefore, algae dewatering and extraction techniques play pivotal roles in algal biofuel production. Urgently, the identification of lipid-rich and rapid-growing algae strains for oil production, coupled with advanced extraction processes, is imperative to enhance lipid extraction efficiency. Ultimately, coupling algae-based wastewater treatment with biofuel production could offer a promising avenue for simultaneously treating wastewater and generating crude oil for biofuel production.

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