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A Comprehensive Review of Sustainable Biodiesel Production Processes from Microalgae as Third-Generation Biofuels (2026)

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Abstract:
The escalating global demand for energy, coupled with the environmental consequences of fossil fuel dependence, has intensified the pursuit of renewable and sustainable energy alternatives. Among these, biofuels derived from renewable biomass have emerged as a pivotal solution for reducing greenhouse gas emissions, enhancing energy security, and supporting economic growth. Microalgae-based biodiesel, categorized as a third-generation biofuel, represents one of the most promising alternatives due to algae's high lipid productivity, rapid growth rates, and versatility in cultivation on non-arable land with minimal freshwater use. This comprehensive review evaluates the current state of algal biodiesel production, encompassing cultivation strategies, harvesting and dewatering techniques, lipid extraction, and conversion processes. Advances in algal biology, metabolic engineering, and biorefinery concepts have significantly improved process efficiencies while also highlighting the potential for co-production of high-value bioproducts, thereby strengthening the economic feasibility of algal biofuels. However, large-scale commercialization remains constrained by technical and economic challenges, notably high production costs, contamination risks, and difficulties in maintaining stable productivity. To overcome these barriers, continued research into advanced cultivation systems, cost-effective harvesting technologies, and sustainable extraction methods is essential. Additionally, supportive policy frameworks, targeted economic incentives, and international collaborations will be critical in facilitating the transition from pilot-scale projects to full industrial deployment. Ultimately, microalgae-derived biodiesel holds substantial potential to decarbonize the transportation sector and contribute to a sustainable global bioeconomy, provided that scientific, technical, and policy-driven innovations continue to advance in synergy.

Keywords: Microalgae, biodiesel, renewable energy, photobioreactors, lipid extraction, transesterification, commercialization

1. Introduction to Biofuels and Microalgal Biofuel:

1.1. Introduction to Biofuels:

As the global population continues to expand, so does the demand for energy—posing a significant challenge given the limited reserves and adverse environmental impacts associated with fossil fuels. This scenario has intensified the global pursuit of renewable and sustainable energy solutions. According to projections by the U.S. Energy Information Administration, global energy consumption is expected to rise considerably in the coming decades, primarily fueled by economic growth in developing nations [1]. Despite this, the current energy landscape remains dominated by fossil fuels—namely coal, oil, and natural gas—which fulfill the majority of global energy needs. Unfortunately, their combustion is a major contributor to greenhouse gas emissions, exacerbating climate change and degrading environmental health. Moreover, reliance on these finite resources brings into question the long-term stability and security of the world's energy supply. [1]

This critical juncture necessitates an urgent pivot towards sustainable and renewable energy solutions. Biofuels, derived from renewable biomass, emerge as a vital part of this transition. They offer a promising pathway to reduce reliance on fossil fuels, curb greenhouse gas emissions, and foster economic development, particularly within agricultural sectors. [1] Evidencing their growing importance, global demand for biofuels in the transportation sector has consistently risen, now accounting for a considerable share of worldwide transport energy. [2] [3]

1.2. Biofuels: Generations and Evolution:

The landscape of biofuels has evolved through distinct generations, each aiming to improve upon the sustainability and efficiency of its predecessors. First-generation biofuels are produced from conventional food crops, utilizing resources such as sugars, starches, and vegetable oils. [1] Examples include ethanol from corn and biodiesel from soybeans. However, these biofuels have raised concerns regarding their impact on food security and land use. Second-generation biofuels utilize non-food lignocellulosic biomass, such as agricultural residues, forestry waste, and dedicated energy crops. [4] While they address the food vs. fuel issue, challenges remain in the economic and efficient conversion of lignocellulosic materials. Third-generation biofuels utilize microalgae as their primary feedstock. [4] These microscopic organisms offer several advantages over land-based crops and non-food biomass. Fourth-generation biofuels are an emerging concept, focusing on advanced technologies like genetically engineered algae and carbon capture during production to achieve even greater sustainability. [1] [5] Algal biodiesel, as a third-generation biofuel, represents a significant advancement by aiming to overcome the limitations of its forerunners, particularly regarding land use competition and the ethical concerns associated with using food crops for fuel. [6]

1.3. Algal Biodiesel: A Promising Sustainable Alternative:

Microalgae are a diverse group of photosynthetic microorganisms that exhibit remarkable potential for biofuel production. [6] Their ability to grow rapidly and accumulate substantial amounts of lipids, in some cases exceeding 70% of their dry weight, makes them highly attractive as a feedstock for biodiesel production. Furthermore, microalgae are efficient in utilizing carbon resources and can play a significant role in mitigating carbon dioxide emissions through photosynthesis. [6] Compared to traditional agricultural crops, microalgae offer several key advantages for biodiesel production. They exhibit high photosynthetic efficiency and can achieve high yields of both biomass and lipids with fewer environmental constraints. Notably, microalgae can be cultivated on non-arable land, including saline and alkaline soils and deserts, thus avoiding direct competition with food crops for valuable agricultural resources. [6] Many algal species can also thrive in wastewater and seawater, reducing the reliance on freshwater, a precious resource in many parts of the world. The capacity of microalgae to capture and utilize carbon dioxide during their growth contributes to a closed-loop carbon cycle, potentially leading to a significant reduction in greenhouse gas emissions from the transportation sector. Beyond biodiesel, algae can be utilized to produce a wide array of other biofuels, including biohydrogen, biogas, bioethanol, and bio-oil, as well as various high-value bioproducts such as biofertilizers, pigments, pharmaceuticals, and nutritional supplements, highlighting their potential within a comprehensive biorefinery framework. [6]

1.4. Introduction to Microalgal Biofuel:

Microalgae are emerging as a highly promising third-generation biofuel feedstock, lauded for their rapid growth, remarkable capacity for lipid (fat) production, and their ability to fix greenhouse gases, resulting in a net-zero emission balance. [5] Unlike earlier biofuel generations, microalgae cultivation avoids competition with food or feed crops and can thrive on non-arable land and in saline water. [5] Biofuels, broadly defined as solid, liquid, or gaseous fuels derived from organic matter. [5] The initial wave of biofuels, first-generation biofuels, faced significant economic, environmental, and political challenges. Their large-scale production demanded extensive arable land, diminishing acreage available for food production for both humans and animals. Furthermore, the manufacturing processes themselves contributed to environmental degradation, leading to a decline in enthusiasm for this generation. [7]

Consequently, research shifted to second-generation biofuels. However, the sophisticated and expensive technologies required for their production rendered them commercially unprofitable. [7] [8] This led researchers to focus on third-generation biofuels, with microalgae at the forefront. [5] [7]. Microalgae are now considered a feasible and sustainable alternative for biofuel production, effectively overcoming the drawbacks of both first and second-generation biofuels. [5] [7] [9] [10] They offer the versatility to produce various renewable biofuels, including methane [11], biodiesel [12], and bio-hydrogen [13]. The advantages of algal biofuel production are numerous: microalgae can yield 15 to 300 times more biodiesel per unit area compared to traditional crops [7], boast a very short harvesting cycle and high growth rate [7] [14], and crucially, do not require high-quality agricultural land for biomass production. [15]

Microalgae, microscopic photosynthetic organisms, are ubiquitous in both marine and freshwater environments. They are categorized based on various characteristics, including their pigmentation, the storage products of photosynthesis, the arrangement of their photosynthetic membranes, and other distinct morphological features. Presently, microalgae are broadly classified into four primary groups: diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cyanophyceae), and golden algae (Chrysophyceae). [16]

Among the many species, several dominate commercial production, notably Isochrysis, Chaetoceros, Chlorella, Arthrospira (Spirulina), and Dunaliella. [17] Chlorella species are particularly interesting due to their ability to switch between phototrophic (light-dependent) and heterotrophic (relying on external carbon sources like glucose) modes of nutrition. [18] [19] Some even exhibit mixotrophic growth, combining both methods. Regardless of their nutritional mode, microalgae universally comprise essential biomolecules such as carbohydrates, proteins, lipids, and nucleic acids. [20]

1.5. Why Microalgal Biomass is the Next Generation of Biofuels?

Over the past few years, there's been a surge of interest in the research and commercial applications of microalgae. Their remarkable growth rate—up to 100 times faster than land plants, capable of doubling their biomass in under a day—makes them an incredibly attractive renewable energy source. [21] This efficiency stems from their simple cellular structure and large surface-to-volume ratio, allowing them to absorb more nutrients from water sources and accelerate their growth. [16] Many microalgae strains are known for their high lipid production, which can be readily converted into biodiesel. Utilizing microalgae for biofuel production offers increased efficiency and potentially lower costs. The expenses associated with harvesting and transporting microalgae are relatively low compared to other plant biomass resources. [22]

Furthermore, microalgae cultivation avoids direct competition with the human food chain, mitigating the contentious "food versus fuel" debate. [22] They don't compete with land-based crops used for food, fodder, or other value-added products. [23] Microalgae are incredibly versatile, thriving in various environments including fresh, brackish, or saltwater, and even on non-arable lands unsuitable for conventional agriculture. [24] They can also be grown in controlled photobioreactors. [25] This adaptability and non-selective growth enable microalgae to achieve significantly higher yields per hectare with superior environmental benefits. Microalgae commonly boast an oil content ranging from 20% to 50% of their dry biomass, with potential for even higher yields. [26] These remarkable organisms typically double their biomass within 24 hours, and under optimal conditions, this exponential growth can lead to a doubling in as little as 3.5 hours. [9]

Beyond their oil-producing capabilities, microalgae offer a treasure trove of valuable co-products, including carbohydrates, proteins, biopolymers, and residual biomass, all of which can be repurposed as animal feed or fertilizer. What's more, their cultivation requires no herbicides or pesticides. [27] Microalgae are increasingly recognized as an efficient biological system for harnessing solar energy to produce a variety of organic compounds. [28] They play a crucial role in fixing atmospheric carbon dioxide, thereby helping to mitigate this pressing global crisis. The production of microalgal biomass can effectively bio fix waste carbon dioxide, significantly reducing the release of this major greenhouse gas (with approximately 1.8 kg of $CO2$ required for every 1 kg of dry microalgal biomass). [9] [27] The lipids produced by microalgae are typically neutral lipids. Their high degree of saturation and rapid accumulation within the cellular system throughout various growth stages make them a promising alternative to conventional diesel fuel. [29] Beyond lipid extraction, certain microalgae, such as blue-green algae (which produce glycogen instead of starch), can generate biohydrogen under anaerobic conditions. [30] [31] Their fermentation can also be leveraged for methane production. By extracting multiple types of biofuels and other value-added products from microalgae, the overall value of the biomass increases, while also contributing to additional offsets against ecological impacts. As noted earlier, the biorefinery concept can be applied to enhance ethanol production from algae. [29] This integrated model can also be combined with biohydrogen and biogas production, either by yielding valuable products before fermentation techniques or by utilizing the gaseous fermentation products to power the creation of high-value entities like methane, biodiesel, and bio-hydrogen. The full exploitation of microalgae for these combined biofuel applications remains an active area of research. [9] [32]

1.6. Scope and Objectives of the Review:

This review paper aims to provide a comprehensive analysis of the current state of development and production technologies for algal biodiesel. It will delve into the various stages of algal biodiesel production, starting from the fundamental biology and selection of suitable algal species, followed by an examination of different cultivation methods for biomass production. The subsequent sections will explore the critical steps of harvesting and dewatering algal biomass, extracting lipids from algal cells, and converting these lipids into biodiesel using various technologies. Furthermore, the review will discuss the essential properties and quality standards of algal biodiesel, as well as the economic and environmental considerations associated with its production. Finally, the paper will address the significant challenges and bottlenecks that currently hinder the widespread commercialization of algal biodiesel and highlight recent advancements and potential future directions in this rapidly evolving field.

2. Algal Biology and Selection of Microalgae Strain for Biodiesel Production:

2.1. Diversity and Characteristics of Algae:

Microalgae are single-cell microscopic organisms which are naturally found in fresh water and marine environment. There are more than 300,000 species of micro algae, diversity of which is much greater than plants. [15]. Microalgae are generally more efficient converters of solar energy comparing to higher plants. In addition, because the cells grow in aqueous suspension, they have more efficient access to water, $CO2$, and other nutrients. [7] [9] The current biofuel yields from various biomasses are shown in Table 1. The table clearly shows huge potential of microalgae compared to other biomasses. Some microalgae can double their biomasses within 24 hours and the shortest doubling time during their growth is around 3.5 hours which makes microalgae an ideal renewable source for biofuel production. [9]

Algae represent a remarkably diverse group of photosynthetic organisms that inhabit a wide range of aquatic and terrestrial environments. This group encompasses both macroscopic, multicellular forms, commonly known as seaweeds, and microscopic, primarily unicellular organisms referred to as microalgae. [34] While macroalgae find applications in various industries, microalgae have garnered significant attention as a promising feedstock for biodiesel production due to their unique characteristics. [35] Microalgae are microscopic organisms, predominantly single-celled, that share a photosynthetic mechanism akin to that of higher plants. They possess the ability to efficiently utilize carbon resources present in both water and soil. These microorganisms exhibit a broad geographical distribution, thriving in diverse aquatic habitats, including freshwater ecosystems such as rivers, lakes, and ponds, as well as marine environments like oceans and estuaries. Notably, certain species of microalgae have demonstrated the capacity to grow in more extreme conditions, such as saline and alkaline soils and even deserts, highlighting their adaptability. A key characteristic that distinguishes many microalgae species is their rapid growth rate and relatively short life cycle. This rapid biomass accumulation is a crucial factor contributing to their potential as a sustainable source for biofuel production. [36]

2.2. Key Algal Species for Biodiesel Production:

A multitude of microalgae species have been extensively investigated for their suitability in biodiesel production. Among the freshwater species, Chlorella vulgaris stands out due to its robust growth and significant lipid accumulation potential. Scenedesmus obliquus is another well-studied freshwater alga known for its rapid growth and considerable lipid yield under certain conditions. Botryococcus braunii produces hydrocarbons with lipid content reaching up to 75%, making it ideal for high-energy biofuel applications. [37] In marine environments, Nannochloropsis oculata is a commonly studied species recognized for its high lipid content and relatively fast growth. [38] Other marine species like Dunaliella tertiolecta and Tetraselmis chuii also show promise for biodiesel production. [37] Certain algal species are known to accumulate exceptionally high levels of lipids. For instance, Schizochytrium sp. has been reported to contain lipids up to 77% of its dry weight. Nannochloropsis sp. can accumulate lipids ranging from 31% to 68%. [39] Chlorella sp. can have lipid contents ranging from 28% to 53%. Rapid growth rates are also essential for achieving high biomass productivity. Picochlorum renovo exhibits a very fast doubling time of approximately 2.2 hours. [40] These species are at the forefront of algal biodiesel research and development due to their potential to provide sustainable, renewable alternatives to fossil fuels. The oil contents of various microalgae in relation to their dry weight are shown in Table 2. It is clear that several species of microalgae can have oil contents up to 80% of their dry body weight.

Certain strains of Chlorella sorokiniana are also known for their rapid growth. [41] Scenedesmus bijugatus and Nitzschia recta have also shown fast growth in some studies. [37] The selection of the most suitable algal species for biodiesel production involves a careful consideration of various factors beyond just high lipid content and rapid growth. These factors include the specific composition of the lipids (fatty acid profile), the ability of the species to tolerate different environmental conditions (such as temperature, salinity, and pH), the ease with which it can be cultivated and harvested using available technologies, and its overall compatibility with the chosen biodiesel production process. For example, the capacity of certain algal species to grow effectively in wastewater can significantly reduce the costs associated with nutrient supply. [40]

2.3. Lipid Composition and Fatty Acid Profile:

Within the cells of microalgae, lipids are present in two primary forms: structural lipids, which are polar lipids that form the essential components of cellular membranes, and storage lipids, which are non-polar lipids that accumulate as intracellular energy reserves, primarily in the form of triacylglycerols (TAGs). TAGs are the primary target for biodiesel production because they can be readily converted into fatty acid methyl esters (FAMEs), the main constituents of biodiesel, through a process called transesterification. The fatty acid composition of the lipids produced by microalgae is a critical factor that determines the quality and suitability of the resulting biodiesel as a fuel. Microalgae typically synthesize a diverse array of fatty acids, with varying chain lengths ranging from 12 to 24 carbon atoms. Some of the common fatty acids found in most microalgae species include myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). For biodiesel production, saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are generally preferred over polyunsaturated fatty acids (PUFAs). This preference stems from the fact that PUFAs can negatively impact the oxidative stability of the biodiesel fuel, making it more prone to degradation during storage. Additionally, a high content of PUFAs can lower the cetane number of the biodiesel, which is a measure of its ignition quality. Conversely, a higher proportion of SFAs and MUFAs in the algal lipids can enhance the energy yield, improve the cetane number, and increase the oxidative and thermal stability of the resulting biodiesel. Oleic acid (C18:1), a monounsaturated fatty acid, has been particularly noted for its excellent fuel characteristics, including good ignition quality, high oxidative stability, high heat of combustion, low cold filter plugging point, suitable viscosity, and good lubricity. The lipid content and the fatty acid profile of microalgae are not fixed and can be significantly influenced by various environmental conditions during cultivation. For instance, subjecting microalgae to nutrient stress, particularly nitrogen starvation, is a well-established strategy to enhance the accumulation of lipids within their cells. Other factors such as the salinity of the growth medium, the intensity and spectrum of light provided, the cultivation temperature, and the concentration of carbon dioxide available can also have a substantial impact on both the total quantity of lipids produced and the specific types of fatty acids that are synthesized by the microalgae. [42]

3. Cultivation, Harvesting and Dewatering Techniques for Microalgae:

Producing biodiesel from microalgae begins with cultivating the algae to generate sufficient biomass, a foundational and critical phase in the process. Multiple cultivation methods have been developed over time, each offering unique characteristics tailored to specific operational needs and environmental contexts. [43] These systems are broadly classified into two main types: open pond systems and closed photobioreactors (PBRs). Furthermore, ongoing research is investigating hybrid systems that integrate the strengths of both traditional open ponds and advanced closed reactors. [44]

Choosing the most suitable cultivation system involves a complex interplay of factors. Key considerations include the intended production scale, the particular strain of microalgae being used, the targeted biomass quality—such as lipid concentration and overall purity—and the environmental conditions of the site, such as sunlight exposure and temperature variability. Economic viability remains a central determinant in this decision-making process. [45] Each system type carries its own set of advantages and limitations, necessitating a thorough assessment to identify the most effective approach for specific production goals and contextual parameters.

3.1. Closed Photobioreactors (PBRs):

Closed photobioreactors (PBRs) represent a cultivated approach to algal cultivation where microalgae are grown within enclosed, transparent vessels, typically constructed from materials such as glass or plastic. These systems afford a significantly enhanced degree of control over the cultivation environment when compared to traditional open pond systems. A paramount advantage of PBRs lies in their capacity for precise regulation of diverse growth parameters, encompassing temperature, light intensity and spectrum (frequently managed through artificial illumination), pH, nutrient supply, and carbon dioxide concentration, thereby enabling the optimization of conditions for specific algal strains. The inherent enclosed nature of PBRs confers substantial protection against contamination by undesirable microorganisms and grazers, consequently facilitating the cultivation of target algal strains with heightened reliability and consistency. [43] This augmented control and protection collectively contribute to PBRs typically achieving superior biomass productivity and supporting higher cell densities in contrast to open pond systems. [46] Furthermore, the closed configuration of these systems mitigates water losses attributable to evaporation. [47]

Despite these considerable benefits, PBRs are not without their limitations. The requisite initial capital investment for the design and construction of PBRs, alongside their ongoing operational expenditures, are notably higher than those associated with open pond systems. [46] Overheating of the algal culture can pose a significant challenge, particularly in outdoor PBRs exposed to direct sunlight, often necessitating the implementation of robust temperature control mechanisms. Biofouling, characterized by the proliferation of algae and other microorganisms on the internal surfaces of the reactor, can diminish light penetration into the culture and mandates periodic cleaning. The accumulation of dissolved oxygen, a byproduct of photosynthesis, within the confined environment of a PBR can occasionally reach inhibitory concentrations for algal growth. Moreover, scaling up PBRs to attain very large production volumes while concurrently sustaining efficient light distribution and adequate temperature control presents considerable engineering complexities. [48] Ensuring uniform light distribution throughout the culture volume, especially in larger PBRs, can also prove challenging. [49]

A diverse array of PBR designs has been conceptualized and developed to cater to varying requirements and scales of operation. These encompass tubular PBRs, which can be configured horizontally or vertically; flat plate PBRs, distinguished by their high surface area-to-volume ratio conducive to efficient light capture; bag PBRs, recognized for their relative cost-effectiveness and ease of setup; stirred tank reactors, providing effective mixing; bubble column reactors, where air serves as the medium for mixing and gas exchange; airlift reactors, which leverage buoyancy for culture circulation; and, more recently, a variety of hybrid designs that endeavor to capitalize on the strengths of different fundamental configurations. [43] Each of these PBR typologies presents a unique set of advantages and disadvantages concerning factors such as light utilization efficiency, ease of temperature regulation, mixing characteristics, and overall cost-effectiveness. [50]

3.2. Cultivation of Microalgae: Overview

Producing microalgal biomass generally entails higher costs and greater technological complexity compared to conventional crop cultivation. Photosynthetic growth of microalgae necessitates essential inputs including light, CO2, water, and inorganic salts. Maintaining a stringent temperature regime is crucial, with optimal growth for most microalgae typically occurring between 20°C and 30°C. To mitigate production expenses, biofuel generation often relies on ambient sunlight, despite the inherent daily and seasonal fluctuations in natural light intensities. [51] [52] [53] [54] The growth medium must supply the inorganic elements vital for algal cell composition. Key essential elements include nitrogen (N), phosphorus (P), and iron (Fe), with silicon (Si) also being critical for certain species. Microalgae can be cultivated in diverse aquatic environments, such as freshwater, marine water, and various wastewaters (e.g., municipal, industrial, and animal), provided there are adequate concentrations of carbon (organic or inorganic), nitrogen (urea, ammonium, or nitrate), phosphorus, and other trace elements. [55]

For marine microalgae, seawater supplemented with commercial nitrate and phosphate fertilizers, along with a few other micronutrients, is a common practice. [56] Wastewaters possess distinct chemical profiles and physical properties when compared to fresh and marine waters. Recent research highlights the significant potential of utilizing wastewaters for the mass production of algal biomass for biofuel and other applications. However, wastewater-based algal cultivation presents numerous uncertainties and challenges. These include variability in wastewater composition due to source, infrastructure, weather conditions, and pre-treatment methods; suboptimal nutrient ratios (e.g., C/N and N/P); elevated turbidity from pigments and suspended solid particles, which impedes light transmission; the presence of competing microflora and toxic compounds; and the accumulation of growth-inhibiting compounds, a problem exacerbated by water recycling and reuse [55]. Table 3 gives comparison of various systems for algal growth.

Microalgae can be cultivated using various methods, broadly categorized into two widely employed systems: suspended cultures (encompassing open ponds and closed reactors) and immobilized cultures (including matrix-immobilized systems and biofilms). The most prevalent large-scale production systems in current practice are high-rate algal ponds or raceway ponds. Raceway ponds are open, shallow systems equipped with paddle wheels to facilitate the circulation of algae and nutrients. While relatively inexpensive to construct and operate, raceway ponds frequently exhibit low productivity for various reasons. Among closed systems utilized for large-scale algae production, tubular photobioreactors are the only type employed. Photobioreactors systems can be further sub-classified into vertical, flat or horizontal, and helical photobioreactors, with the helical photobioreactors generally considered the easiest to scale up for production. Compared to open ponds, tubular photobioreactors offer superior control over pH and temperature, enhanced protection against culture contamination, improved mixing, reduced evaporative losses, and the ability to achieve higher cell densities. The comparison of various types of photobioreactors yields the most optimal design parameters for closed photobioreactors. [57] [58] [59]

3.3. Harvesting Techniques:

Various methods are currently employed for algal harvesting, encompassing chemical, mechanical, biological, and to a lesser extent, electrical operations. Frequently, combinations or sequential applications of these methods are utilized to optimize efficiency. Given the typically small cell size of algae, chemical flocculation is often implemented as a pre-treatment step. This process increases the effective particle size of the algae, facilitating subsequent harvesting by methods such as flotation. Among mechanical approaches, centrifugation stands out as the most reliable and rapid method for recovering suspended algae. In the realm of electrical-based methods, the inherent negative charge of algal cells is leveraged for separation. These charged cells can then be concentrated through movement within an applied electric field. [60]

A variety of techniques have been developed to separate and collect microalgal biomass from the culture medium. [42] These methods can be broadly categorized based on their underlying principles. Flocculation is a common initial step, which involves aggregating individual algal cells into larger clumps or flocs, thereby facilitating their subsequent separation. Flocculation can be achieved through the addition of chemical flocculants, such as inorganic salts like aluminum sulfate (alum) and ferric chloride, or synthetic polymers. Auto-flocculation can occur under specific conditions, such as by adjusting the pH of the medium or by adding carbon dioxide. Bio-flocculants, produced by microorganisms or derived from natural sources, are also being explored as more environmentally friendly alternatives. Electro-flocculation, which utilizes electrodes to induce cell aggregation, represents another emerging technique. The efficiency of flocculation is highly dependent on the specific algal species and the characteristics of the culture medium. [61]

Sedimentation, or gravity settling, relies on allowing the denser algal cells to settle to the bottom of the cultivation vessel. This is a relatively low-cost method but can be slow and necessitates large settling areas. Flotation involves introducing air bubbles into the culture, which attach to the algal cells and cause them to float to the surface, where they can be skimmed off. Flotation can be a faster method compared to sedimentation. Centrifugation utilizes centrifugal force to rapidly separate the algal cells from the liquid medium. While centrifugation is highly efficient and fast, it is also energy-intensive and can be costly, particularly when processing large volumes of culture. [35]

Filtration involves passing the algal culture through membranes or screens with pore sizes smaller than the algal cells, effectively trapping the biomass. This method can be effective but is prone to clogging, particularly with high cell densities or filamentous algae. Microstrainers and vibrating screens are often employed for an initial coarse filtration step. [35] Magnetic separation, a noveler approach, involves attaching magnetic nanoparticles to the algal cells, which can then be separated from the medium using a magnetic field. [42] The selection of the most appropriate harvesting technique, or a combination thereof, depends on a variety of factors, including the specific algal species being cultivated, the scale of operation, the energy consumption associated with the method, and the overall cost-effectiveness of the process. [35]

3.4. Dewatering Techniques:

Following the initial harvesting step, the resulting algal slurry typically has a relatively low solids content (ranging from 5% to 15% TSS) and requires further dewatering to concentrate the biomass before subsequent downstream processing, such as lipid extraction. Reducing the moisture content of the algal biomass is essential as high moisture levels can significantly increase the cost and energy requirements of subsequent steps, particularly drying. Several dewatering techniques are commonly employed. Mechanical dewatering methods, such as centrifugation and filtration (using equipment like filter presses or belt filters), are often used to further concentrate the algal slurry. Thermal drying methods involve using heat to evaporate the remaining water from the biomass. Common thermal drying techniques include sun drying, air drying, freeze-drying, spray drying, and drum drying. However, thermal drying is generally an energy-intensive process and can represent a significant economic burden in the overall algal biodiesel production process. Osmotic dehydration, a less commonly used method for algae, involves using high concentrations of salts or sugars to draw water out of the algal cells. The selection of the most suitable dewatering technique is influenced by factors such as the initial solids content of the harvested biomass, the desired final moisture content for the next processing step, the energy consumption and cost associated with the method, and the potential impact on the quality of the algal biomass. [35] Table 4 gives overview of advantages and disadvantages of various harvesting and dewatering techniques for algal growth.

4. Lipid Extraction Methods for Microalgal Biomass:

Microalgae are emerging as a strong contender for sustainable biodiesel production, with current research highlighting their potential to meet global energy demands. Notably, their oil content can be remarkably high, sometimes surpassing 80% of their dry weight. [27] While such extreme levels are possible, it's more common to find oil content ranging from 20% to 50% (Table 5). [9] The amount of oil produced from microalgae, known as oil production yield, is determined by the mass of oil generated per unit volume of microalgal culture broth daily. This takes into account both the microalgae's growth rate and the oil content within their biomass. Many algal species naturally accumulate a high concentration of lipids, serving as storage materials, often reaching 50-60% of their dry weight. These lipids are chemically similar to oils found in traditional oilseed crops, making algae a promising and sustainable resource for biodiesel production. [62]

4.1. Lipid Extraction Overview and Challenges in Lipid Extraction:

To maximize oil yield, various techniques are employed for efficiently extracting lipids from microalgae. Common approaches include the expeller/oil press, which mechanically squeezes oil from the biomass; ultrasound techniques, which use high-frequency sound waves to break down cell walls and release lipids; solvent extraction, which utilizes chemical solvents to dissolve and separate lipids; and supercritical fluid extraction, which employs supercritical fluids like CO2 as solvents, offering tunable properties for extraction. The ideal extraction methods should be fast, non-damaging to the lipids, and easily scalable. [63] A widely used method for lipid extraction is the modified Bligh and Dyer method. [64] [65]

Efficient extraction of lipids from algal biomass presents several technical challenges. The rigid cell walls of many algal species act as a significant barrier, hindering the release of intracellular lipids. Therefore, a crucial step in the process often involves cell disruption to break open the algal cells and make the lipids accessible for extraction. [68] However, some algal species, such as Botryococcus braunii, have the unique ability to store significant amounts of hydrocarbons extracellularly, outside the cell wall, which potentially allows for the use of milder extraction methods that do not necessarily require complete cell lysis. [69] Another challenge lies in the fact that the lipid content within algal cells can vary considerably depending on the species and is also significantly influenced by the growth conditions under which the algae are cultivated. This variability necessitates the optimization of extraction methods for the specific algal species and its lipid characteristics.

4.2. Cell Disruption Methods:

To overcome the barrier posed by the algal cell wall and facilitate the release of intracellular lipids, various cell disruption methods are employed. [42] These methods can be broadly classified into mechanical, chemical, and enzymatic approaches. Mechanical methods involve the physical breakdown of the cell structure. Techniques such as bead beating, which uses small abrasive beads to grind the cells; high-pressure homogenization, which forces the algal suspension through a narrow valve at high pressure; ultrasonication, which uses high-frequency sound waves to disrupt cells; and microwave-assisted disruption, which utilizes microwave energy to heat and rupture cells, are commonly used. Chemical methods involve the use of chemical agents to degrade the cell wall. These can include the use of strong acids, such as sulfuric acid, or alkalis, such as sodium hydroxide, as well as various organic solvents that can dissolve or permeabilize the cell membrane. [68] Enzymatic methods offer a more biological approach, utilizing specific enzymes, such as cellulase, hemicellulase, and protease, to selectively break down the polysaccharide and protein components of the algal cell wall. Enzymatic methods can be milder and more environmentally friendly compared to chemical methods but may have lower efficiency or require the use of specific enzyme cocktails tailored to the cell wall composition of the target algal species. The choice of the most appropriate cell disruption method depends on several factors, including the specific algal species being processed, the scale of operation, the energy consumption and cost associated with the method, and the potential impact on the quality of the extracted lipids. [42]

4.3. Lipid Extraction Methods:

Once the algal cells have been disrupted, the lipids need to be separated and extracted from the resulting biomass slurry. Several methods are commonly used for this purpose. Solvent extraction is a traditional and widely used technique that involves using organic solvents, such as hexane, chloroform, and methanol, to dissolve and separate the lipids from the remaining cellular material. While effective, this method raises environmental concerns related to the use, recovery, and disposal of organic solvents. Supercritical fluid extraction (SFE) utilizes supercritical carbon dioxide (CO2) as a solvent. Supercritical CO2 extraction is often considered a more environmentally friendly alternative to traditional organic solvents, although it may require high operating pressures and specialized equipment. Enzymatic extraction involves the use of lipases, which are enzymes that catalyze the hydrolysis of lipids into free fatty acids and glycerol. These products can then be more easily separated from the remaining biomass. Enzymatic extraction can be a milder and more specific method but may result in lower lipid yields compared to solvent-based techniques. Microwave-assisted extraction utilizes microwave energy to enhance the efficiency of lipid extraction and can reduce the amount of solvent required and the extraction time. For greater versatility in biofuel production, microalgal fatty acids can be extracted through a multi-step process that combines direct esterification, simultaneous extraction, and transesterification. This comprehensive method is adaptable to various types of biomasses and typically involves a sequence of steps: solvent extraction, ultrasonication, high-pressure heating, filtration, density-based separation of solvents and liquids, and finally, oil recovery through evaporation to dryness. [66] For a detailed comparison of the advantages and limitations of different lipid extraction methods for microalgae oil, refer to Table 6.

Aqueous enzymatic extraction (AEE) is a promising technology that uses enzymes in an aqueous environment to extract lipids. This method is considered environmentally safe, reduces the consumption of volatile organic solvents, and can potentially allow for the simultaneous extraction of both lipids and proteins from the algal biomass. In-situ transesterification, also known as extractive transesterification, is a process that combines the lipid extraction and biodiesel production steps into a single operation. In this method, the wet algal biomass is directly treated with an alcohol and a catalyst, leading to the simultaneous extraction of lipids and their conversion into biodiesel, potentially reducing the overall processing steps and costs. The ongoing development of more sustainable and efficient lipid extraction methods is a critical area of research aimed at reducing the environmental footprint and the overall cost of algal biodiesel production. In-situ transesterification, in particular, represents a promising avenue for simplifying the process and improving its economic viability. [42]

5. Transesterification of Microalgal Oil:

5.1. Transesterification: The Primary Biodiesel Production Route:

The primary method for producing biodiesel from algal lipids is transesterification, a chemical reaction that converts triglycerides (the main components of algal oils) into fatty acid methyl esters (FAMEs) or fatty acid ethyl esters (FAEEs), collectively known as biodiesel, and glycerol as a byproduct. This reaction involves reacting the triglycerides with an alcohol, typically methanol or ethanol, in the presence of a catalyst, which can be an acid, a base, or an enzyme. [68] Base-catalyzed transesterification is the most commonly used method in commercial biodiesel production due to its high efficiency and relatively mild reaction conditions. [70] However, this method is sensitive to the presence of free fatty acids (FFAs) and water in the feedstock, which can lead to saponification and reduce the biodiesel yield. [70] Acid-catalyzed transesterification, on the other hand, is less sensitive to FFAs and water but typically requires higher reaction temperatures and longer reaction times. [68] Enzymatic transesterification, which utilizes lipases as catalysts, offers the advantages of milder reaction conditions, the production of high-purity biodiesel, and easier separation of glycerol. [71] However, the cost of enzymes and their potentially lower reaction rates compared to chemical catalysts can be limiting factors. The development of stable and reusable biocatalytic systems is an active area of research. [72] Supercritical transesterification is a non-catalytic method that utilizes alcohol under supercritical conditions (high temperature and pressure) to convert lipids into biodiesel. [73] This method can handle wet feedstock and high FFA content without the need for a catalyst but requires specialized equipment and high energy input. The use of microwave and ultrasound energy to assist the transesterification reaction has been shown to significantly reduce reaction times and improve biodiesel yields. [74] Several factors influence the efficiency of the transesterification reaction, including the molar ratio of alcohol to oil, the type and amount of catalyst used, the reaction temperature, the reaction time, and the presence of impurities such as water and FFAs in the algal oil. [75] In-situ transesterification, where the algal biomass is directly subjected to the transesterification reaction without prior extraction of the lipids, is a promising approach that can potentially simplify the overall process and reduce production costs. [42]

5.2. Other Conversion Routes for Algal Biomass:

While transesterification of algal lipids is the most direct route to biodiesel, the entire algal biomass, including the lipid-extracted residue, can be utilized to produce other types of biofuels through various conversion technologies. [42] Anaerobic digestion is a process where microorganisms break down organic matter, including algal biomass, in the absence of oxygen to produce biogas, which is primarily composed of methane. [61] This process can be applied to the whole algal biomass or the residue remaining after lipid extraction for biodiesel production, potentially improving the overall energy balance of the system. Microbial fermentation involves using microorganisms, such as yeast, bacteria, and fungi, to convert the carbohydrate content of algal biomass into bioethanol. [68] Different types of algae, including green, brown, red, and blue-green algae, can be used as feedstocks for bioethanol production. [68] Hydrothermal liquefaction (HTL) is a thermochemical process that converts wet algal biomass into a bio-oil under high temperature and pressure. The resulting bio-oil can then be further processed and refined into various types of fuels. Pyrolysis involves the thermal decomposition of algal biomass at moderate temperatures in the absence of oxygen, yielding bio-oil, biochar, and bio-syngas. Different types of pyrolysis, such as slow, fast, and flash pyrolysis, can be employed depending on the desired product distribution. Gasification is a process that converts algal biomass into syngas, a mixture primarily composed of carbon monoxide, hydrogen, and carbon dioxide, through partial oxidation at high temperatures. Syngas can be used as a fuel or as a feedstock for the production of other chemicals. Direct combustion involves simply burning the algal biomass to produce heat and electricity. Finally, some algal species have the ability to produce biohydrogen under specific conditions, such as through anaerobic pathways or photolysis. These alternative conversion routes highlight the versatility of algal biomass as a feedstock for a range of biofuels, supporting the development of integrated algal bio-refineries where the entire biomass is valorized. Table 7 gives all comparisons of different transesterification methods. [36]

6. Properties and Comparison of Algal Biodiesel with Diesel:

Algal biodiesel shows strong potential as a renewable fuel, with key properties like a high cetane number for better combustion, a higher flash point for safer handling, and a very low sulfur content reducing emissions. While it generally has higher kinematic viscosity and density than conventional diesel, and potentially higher cloud and pour points (especially in colder climates), research is ongoing to optimize its fatty acid composition and develop additives to meet international standards like ASTM D6751 and EN 14214. The overall quality of algal biodiesel is influenced by numerous factors, including the algal feedstock's fatty acid profile and impurities, the transesterification process parameters, effective purification and washing, proper storage conditions, and strategic blending with other fuels. Consistent monitoring and control across the entire production chain are crucial for maintaining high quality. [76] [77] Table 8 compares algal biodiesel to conventional diesel.

7. Conclusions:

Algal biodiesel presents a promising and sustainable alternative to conventional fossil fuels, with the potential to reduce greenhouse gas emissions and strengthen energy security. Considerable progress has been achieved in areas such as algal biology, cultivation systems, harvesting, dewatering, lipid extraction, and conversion processes. The ability of algae to generate not only biofuels but also high-value bioproducts further enhances its role in a future bioeconomy.

Nonetheless, large-scale commercialization is hindered by economic and technical barriers, including high production costs, contamination risks, and challenges in maintaining consistent productivity and fuel quality standards. Overcoming these limitations requires advances in cultivation, harvesting, and extraction technologies, as well as innovative approaches like genetic and metabolic engineering. Looking ahead, supportive policies, targeted incentives, and global collaborations will be critical to accelerate the transition from pilot projects to industrial-scale deployment, enabling algal biodiesel to play a pivotal role in decarbonizing transportation and advancing sustainable energy systems.

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