Microalgae-Derived Biodiesel as a Scalable and Sustainable Pathway for Next-Generation Biofuels

Neeraj Choudhary,[a] Vinod Kumar Gauttam,[b] Vidyavati Hiremath,[c] Sukhpreet Singh,[a] Mukul Machhindra Barwant,[d] Emad M. Abdallah [e] Samiah Hamad Al-Mijalli [f,]* and Dinesh Kumar [a,]*

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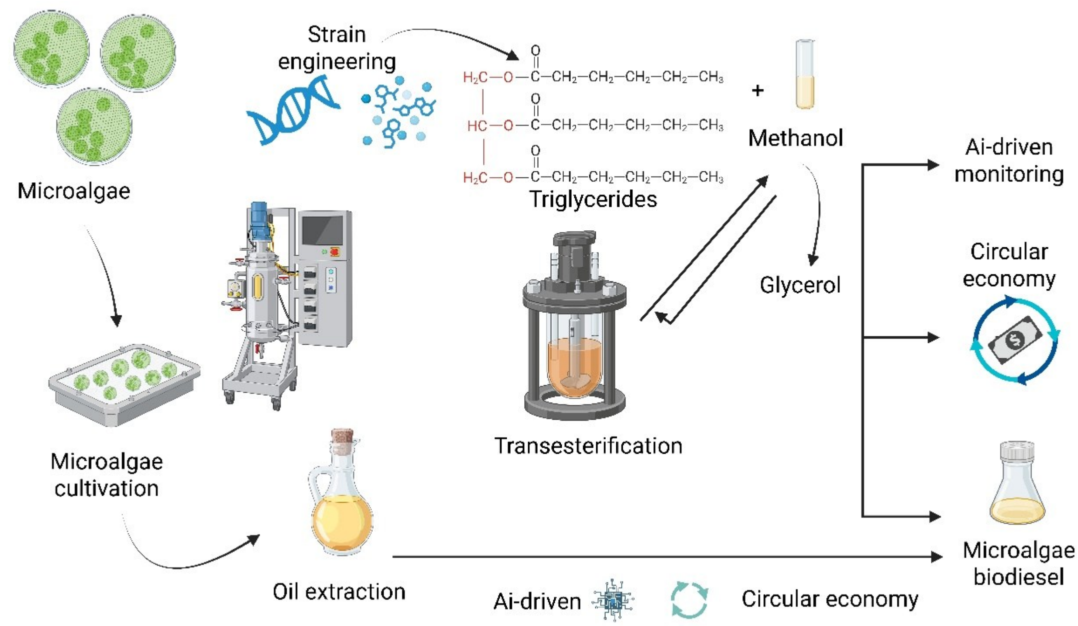
GRAPHICAL ABSTRACT



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Microalgae-derived biodiesel is increasingly regarded as a next-generation renewable fuel because microalgae combine rapid biomass growth, high lipid productivity, carbon dioxide capture, and cultivation on non-arable land or nutrient-rich wastewater. This review critically integrates recent progress in strain improvement, cultivation engineering, harvesting, lipid extraction, and conversion technologies for scalable biodiesel production. Advances in genome editing, two-stage cultivation, hybrid photobioreactor-open pond systems, and microwave- or ultrasound-assisted extraction have improved lipid yield and process efficiency. Digital tools, including artificial intelligence, predictive monitoring, digital twins, and machine-learning-based contaminant control, are also emerging as important enablers of automation and scale-up. Embedding microalgae production within circular biorefineries can support flue-gas utilization, nutrient recycling, and co-production of high-value compounds such as astaxanthin, omega-3 fatty acids, phycobiliproteins, proteins, and biodegradable biopolymers. Nevertheless, commercialization remains constrained by high cultivation costs, energy-intensive dewatering, variable outdoor productivity, and uncertain policy incentives. Integrated techno-economic optimization, carbon-credit mechanisms, and multi-product biorefinery models are therefore essential for advancing microalgae biodiesel toward industrial deployment.

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Keywords: Microalgae biodiesel; Strain engineering; Microorganisms; AI-assisted cultivation; Circular bioeconomy; Biorefinery; CO_2 sequestration; Lipid extraction; Renewable fuels

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INTRODUCTION

The global energy landscape is changing rapidly due to the depletion of fossil fuel reserves, the rise in energy demand, and the growing impact of climate change. The continuous use of coal, oil, and natural gas releases large amounts of greenhouse gases (GHGs), with the energy sector responsible for about 73% of global CO_2 emissions, leading to global warming, ocean acidification, and biodiversity loss (Zhang 2024). According to the International Energy Agency, if current trends continue, global CO_2 emissions may exceed 37 gigatonnes per year by 2030, threatening international climate targets. This situation has already caused severe effects such as rising sea levels, heat waves, and extreme weather events, resulting in global economic losses exceeding US$300 billion annually (Shirizadeh *et al.* 2023). These trends highlight the urgent need for sustainable, low-carbon, and renewable energy solutions to ensure energy security and environmental stability.

Among various renewable options, microalgae-based biodiesel has gained attention as a promising third-generation biofuel. It offers several advantages over conventional crops, such as soybean or palm oil, because of its high photosynthetic efficiency, rapid growth, and ability to produce large amounts of lipids even in saline environments (Rial 2024). Under optimized cultivation conditions, selected microalgal strains have been reported to achieve lipid contents of up to 70%, although values vary considerably among species, cultivation systems, and environmental conditions (Alishah Aratboni *et al.* 2019). They can also be cultivated using wastewater and industrial flue gases, allowing for nutrient recycling and carbon capture without competing with food or freshwater resources.

Microalgae are not only a renewable source of biofuel but also play an essential role in environmental sustainability. They can fix up to 1.8 kg of CO_2/kg of biomass, making them valuable for carbon sequestration and climate mitigation (Zhang 2024). The microalgal growth in wastewater helps recover nitrogen and phosphorus, reducing nutrient pollution. Additionally, microalgae produce valuable co-products such as pigments (β-carotene, astaxanthin), proteins, polyunsaturated fatty acids (DHA, EPA), and antioxidants that enhance the overall economics of biorefinery operations. These combined benefits make microalgae an essential component of the bio-circular-green (BCG) economy, which focuses on resource efficiency and closed-loop sustainability (Yadav *et al*. 2022)

Although many recent reviews have discussed algal biofuels, most focus on specific aspects such as cultivation, extraction, or cost analysis (Slade and Bauen 2013; Chia *et al.* 2018; Yadav *et al.* 2022). The present review provides a broader and more integrated perspective. It combines biotechnological advancements, AI-based process optimization, and circular bioeconomy frameworks to form a unified approach. This integration links metabolic and genetic engineering innovations with digital automation, techno-economic modelling, and policy mechanisms, thereby creating a complete roadmap for scaling up microalgae-based biodiesel production. This comprehensive approach distinguishes this review from earlier studies by connecting laboratory-level innovations to large-scale industrial and policy frameworks, supporting the transition to sustainable bioenergy systems (Kamarel 2020). In this context, the review discusses the technological, economic, and policy factors that influence large-scale implementation of microalgal biodiesel. It highlights new developments in AI-assisted cultivation systems that optimize nutrient and light conditions, integrated biorefinery models that use all biomass components to achieve zero waste, and carbon market integration to increase economic feasibility. The review also addresses significant challenges such as the high cost of photobioreactors, energy-intensive harvesting methods, and limited strain stability. It proposes strategies including machine learning–based process control, genetic modification for higher lipid yield, and sustainable business models supported by carbon credit incentives. Altogether, these insights position microalgae-derived biodiesel as a significant solution for achieving low-carbon, circular, and climate-resilient energy systems, aligning with global net-zero and sustainable development goals.

POLICY SUPPORT AND MARKET OPPORTUNITIES FOR MICROALGAE-BASED BIODIESEL

Microalgae-derived biodiesel occupies a strategic position within global decarbonization policies as a third-generation biofuel offering high yield, non-food competition, and substantial GHG-mitigation potential. Governments worldwide are integrating algae biofuels into advanced bioenergy frameworks to accelerate the transition toward low-carbon transport (Kang *et al.* 2021).

Policy Landscape: Europe, USA, and India

The current legislative framework for the deployment of renewables in transport within the European Union is set by the Renewable Energy Directive III (RED III; Directive (EU) 2023/2413), which amends the Renewable Energy Directive II (RED II; Directive (EU) 2018/2001). RED III sets a higher goal for Member States: to reach 29% renewable energy in transport by 2030, or 14.5% to reduce the greenhouse gas (GHG) intensity of transport, which will boost the EU's decarbonization efforts. The Directive also sets a minimum combined target of 5.5% advanced biofuels and renewable fuels of non-biological origin (RFNBOs), with at least 1% to be sourced from RFNBOs. Biofuels from algae are still considered advanced biofuels under the Directive and continue to receive policy support measures to reduce dependence on food and feed crops due to indirect land-use change (ILUC) effects. Furthermore, national scale-up and commercialization measures, such as blending requirements, investment support, carbon pricing, and tax measures, are anticipated to promote the commercialization and scale-up of microalgae-based biodiesel production throughout the European Union (European Parliament and Council. 2023. Directive (EU) 2023/2413 of the European Parliament and of the Council of 18 October 2023 amending Directive (EU) 2018/2001, Regulation (EU) 2018/1999 and Directive 98/70/EC as regards the promotion of energy from renewable sources, and repealing Council Directive (EU) 2015/652. Official Journal of the European Union, L 2023/2413).

In the United States, the Renewable Fuel Standard (RFS) in the biomass-based diesel and advanced biofuel categories offers a multi-tiered platform for algae-derived biodiesel. Producers generate Renewable Identification Numbers (RINs) and can be sold or traded, generating valuable revenue streams that are driven by compliance. The RFS structure, which recent policy updates have fortified, bolsters the argument for the expansion of algae biodiesel initiatives and cultivates a favorable investment environment for third-generation biofuels (Dusser 2019).

India's National Policy on Biofuels (2018) sets ambitious blending targets, currently 5%, with a roadmap to 20% by 2030, in the pursuit of energy security, import substitution, and emission reductions (Das 2020). While most of the existing biodiesel production is sourced from used cooking oil and non-edible oil crops, microalgae are increasingly recognized as a future-proof feedstock due to their scalability, non-food supply chain, and compatibility with circular economy approaches (*e.g.*, wastewater integration). Government incentives, pilot programs, and public–private partnerships are accelerating research-to-market translation in this sector (Wang *et al.* 2024)

Several Asia–Pacific and Middle East nations are including algae-based biofuels in their decarbonization plans. Japan's Green Growth strategy and 2030's, 10% sustainable aviation fuel (SAF) target boost demand for lipid-derived fuels, while E10 petrol by 2028 could open domestic markets for third-generation biodiesel. China's ETS and CCER programs enable verified carbon capture and utilization from microalgal systems, particularly when production plants are near industrial CO_2 sources (Moya Joëlle Carole *et al.* 2023). The UAE National Policy on Biofuels (2024) promotes biodiesel blends (up to B100) with 75% CO_2 reductions, coinciding with Net Zero 2050 goals (Ahmed and Makkawi 2025). To accelerate commercialization, governments in these regions should implement stackable incentives, including (i) LCFS or renewable fuel credits for lifecycle-based carbon-intensity reductions, (ii) ETS/CCER or carbon-credit eligibility for CO_2-to-biomass utilization, (iii) wastewater tipping-fee credits for nutrient removal, and (iv) preferential capital grants or tax relief for facilities near industrial CO_2 sources. These policies with guaranteed offtake agreements for airline and municipal fleets would decrease market risk, improve bankability, and promote regional algae-based biofuel leadership.

Integrating Carbon Markets and Pricing

In addition to meeting renewable blending mandates, microalgae-derived biodiesel can generate significant revenue through participation in carbon markets due to its potent life-cycle GHG reduction potential. Life-cycle assessments have reported GHG reductions ranging from 60 to 80%, depending on cultivation strategy, energy inputs, and system boundaries (Chamkalani *et al.* 2020). This reduction qualifies microalgae-based fuels for carbon offset credits in voluntary markets (*e.g.*, Verra VCS, Gold Standard) and compliance markets such as the EU Emissions Trading Scheme (EU-ETS) and the California Low Carbon Fuel Standard (LCFS).

Under Low Carbon Fuel Standard (LCFS) mechanisms, algae-derived biodiesel with a low carbon intensity may qualify for LCFS credits, providing an additional revenue stream that can improve project economics. However, credit values are market-dependent and fluctuate over time. Similarly, in Canada's Output-Based Pricing System (OBPS) and New Zealand's ETS, pairing microalgae cultivation with industrial CO_2 capture can reduce operating expenses by 15% to 30% due to carbon-cost avoidance (Singh *et al.* 2025). Eligibility for green bonds, climate transition loans, and sustainable aviation fuel (SAF) credits further enhance financing access for algae biofuel ventures aligned with net-zero 2050 commitments (Jungmeier 2017).

Market Outlook and Strategic Considerations

The commercial landscape for microalgae-derived biodiesel is becoming increasingly favorable as renewable fuel quotas expand and carbon pricing frameworks intensify worldwide (El-Araby 2024). Recent market analyses show that achieving cost-competitive production relies on the strategic integration of (i) policy-based financial incentives such as RINs, LCFS credits, and carbon offsets, (ii) co-product valorization through the extraction of high-value compounds such as pigments, polyunsaturated fatty acids, nutraceutical proteins, and bioplastics, and (iii) process optimization through improved cultivation, metabolic engineering, and flue-gas CO_2 utilization. Techno-economic analyses suggest that co-product recovery can contribute approximately 40 to 60% of total revenue under specific biorefinery scenarios. Wet extraction and strain engineering have the potential to improve the overall energy efficiency and economic feasibility of microalgae-based biodiesel production.

Several microalgal fatty acids have significantly higher commercial value in the markets of nutraceuticals, pharmaceuticals, cosmetics, and specialty chemicals, while biodiesel is the major product because of its compatibility with the current diesel infrastructure and significant market demand. Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have market prices that are much higher than biodiesel and can greatly enhance the economics of an integrated microalgal biorefinery. Their commercial recovery involves more purification and fractionation stages, which make the process more complicated, energy-intensive, and costly, and demand high-quality criteria. Moreover, the market demand for these specialty fatty acids is significantly less than the demand for transportation fuels, thus restricting the ability to scale these fatty acids to become the primary commercialized product. Thus, an integrated biorefinery strategy can be considered as the most economically viable commercialization pathway, in which high-value compounds are recovered first, and the oil content is then processed into biodiesel.

Generally, additional separation and purification steps are used to obtain EPA, as well as DHA-rich fractions, but these operations are only applied to part of the extracted oil that will be used in high-value markets and not to the entire lipid stream. The neutral lipid fraction can still be processed into biodiesel without significant changes to conventional transesterification processes, with the remaining fraction enriched in saturated and monounsaturated fatty acids. This means that the upstream processing costs can increase with the recovery of PUFAs, but the much higher commercial value of these compounds may compensate for the extra cost, making the production of biodiesel competitive in an integrated biorefinery scheme.

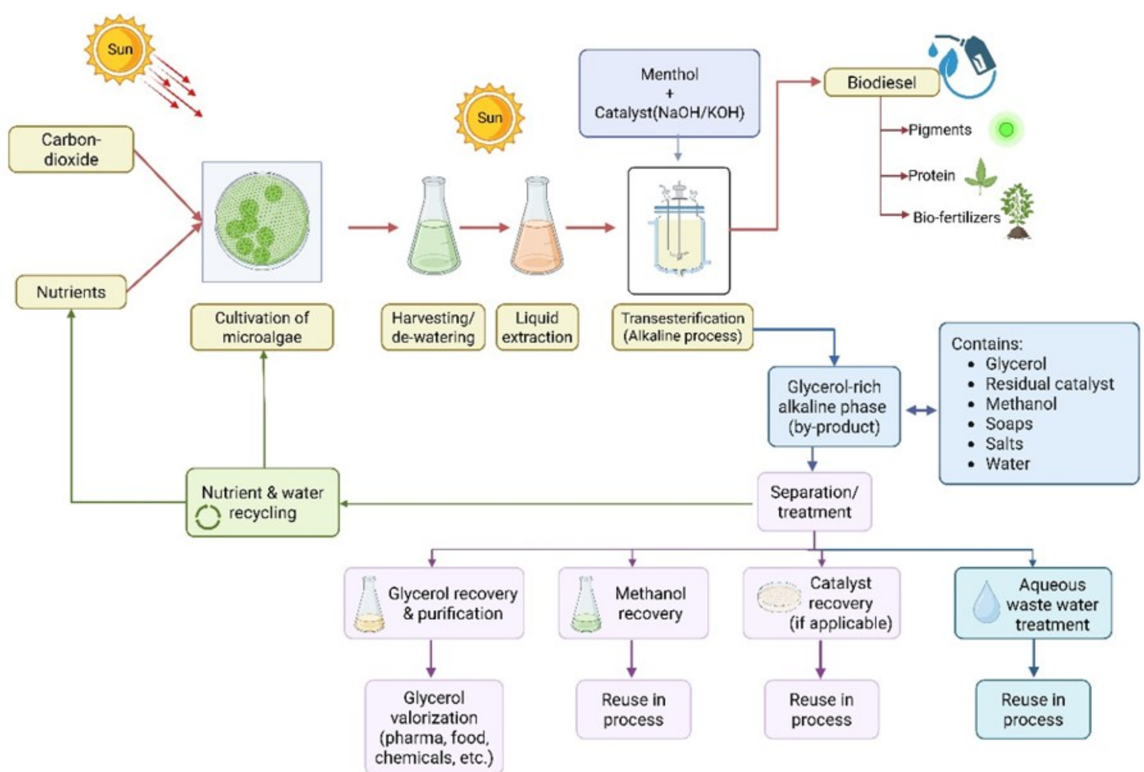

Fig. 1. Schematic representation of an integrated and sustainable microalgae-based biodiesel production process. Microalgae are cultivated using sunlight, carbon dioxide, and nutrients, followed by harvesting/dewatering, lipid extraction, and alkaline-catalyzed transesterification to produce biodiesel. The process also generates valuable co-products, including pigments, proteins, and biofertilizers. The glycerol-rich by-product stream undergoes separation and treatment for glycerol purification and valorization, methanol and catalyst recovery, and wastewater treatment. Recycling of recovered methanol, catalyst, water, and nutrients back into the production cycle highlights the circular and resource-efficient nature of the biorefinery system.

The future success of microalgae biodiesel deployment will depend on long-term regulatory stability, industry and government collaboration, and a clear demonstration of life-cycle sustainability performance (Mitra *et al*. 2024). As countries pursue more ambitious carbon neutrality commitments, sustainable aviation fuel mandates, and circular bioeconomy strategies, microalgae-derived biodiesel is poised to become a central component of next-generation low-carbon energy systems.

Figure 1 illustrates the overall process flow for biodiesel production from microalgae, beginning with microalgal cultivation and ending with fuel conversion. Microalgae are grown in the presence of sunlight, carbon dioxide, and essential nutrients, which drive photosynthesis and cellular biomass formation. Once sufficient biomass is achieved, the culture undergoes harvesting and dewatering to separate and concentrate algal cells from the growth medium. The concentrated biomass is then subjected to liquid extraction, where the intracellular lipids and oils are isolated. These extracted lipids are subsequently processed and converted into biodiesel, which can be directly utilized as a renewable and low-carbon transportation fuel. The schematic highlights the solar-driven nature of both growth and lipid accumulation, emphasizing microalgae's role in CO_2 capture, sustainable biomass production, and environmentally friendly fuel generation.

Microalgae as a Multifunctional Biodiesel Feedstock and Biorefinery Platform

Microalgae have gained prominence as a third-generation biodiesel feedstock owing to their exceptionally high areal productivity, rapid growth rates, and capacity to fix up to 1.8 kg CO_2 per kg of biomass, enabling integration with industrial CO_2-emitting sectors (Zhang 2024). Compared to soybean (~ 500- to 1,200-L oil ha^{-1} year^{-1}) and palm (~ 4,000- to 6,000-L ha^{-1} year^{-1}), several microalgal strains demonstrate potential oil yields of 20,000- to 80,000-L ha^{-1} year^{-1}, establishing them as the highest-yielding lipid biomass source (Razzak *et al.* 2022). Biomass productivities of 40- to 60-g m^{-2} day^{-1} in closed photobioreactors and 15- to 30-g m^{-2} day^{-1} in open raceway ponds are now routinely reported in pilot operations under nutrient-replete conditions (Matos *et al.* 2018).

Strain engineering and omics-driven metabolic optimization have further improved lipid yields. For instance, CRISPR/Cas9-mediated diacylglycerol acyltransferase-1 (DGAT1) gene over-expression in *Nannochloropsis oculata* increased triacylglycerols (TAG) accumulation by 43% to 58%, while acetyl-CoA carboxylase (ACCase) amplification in *Chlorella sorokiniana* enhanced lipid synthesis by 32% to 49% under nitrogen limitation (Muñoz *et al*. 2021). Similarly, transcriptome-guided suppression of AGPase and G6PDH in *Chlamydomonas reinhardtii* redirected carbon flux from starch into TAGs, increasing lipid yield from 18% to 41% of its dry weight (DW) (Leu and Boussiba 2014). These outcomes confirm the feasibility of precision metabolic rewiring for scalable lipid enhancement.

Microalgae also offer substantial biorefinery value beyond fuels. As illustrated in Fig. 2, microalgal biomass comprises five primary biochemical domains: proteins, carbohydrates, lipids, metabolites, and vitamins, each with distinct revenue-generating product streams. For example, *Haematococcus pluvialis* can accumulate 1.5% to 5.2% astaxanthin on its DW basis, valued at USD 2,500 to 7,000 kg^{-1}, which can subsidize biodiesel production costs (Sarker and Kaparaju 2024). Brown algae-derived fucoidans and carrageenans are used in wound dressings, antiviral therapies, and hydrogel matrices, expanding pharmaceutical co-market opportunities (Wang *et al.* 2024). Meanwhile, polyunsaturated fatty acids (PUFA)-rich lipids (EPA, DHA) extracted from

Nannochloropsis oculata and *Phaeodactylum tricornutum* offer nutraceutical revenue streams valued at USD 30 to 120 g^{-1}, dramatically improving biorefinery economic performance (Lu *et al.* 2021). Recent techno-economic assessments show that when biodiesel production is integrated with co-product recovery, the minimum fuel selling price (MFSP) can be reduced from USD 3.5–6.0 L^{-1} (fuel-only route) to USD 1.8–2.4 L^{-1}, placing microalgal biodiesel within parity of petroleum diesel in markets with carbon credits or low-carbon fuel standards (Lee *et al.* 2019). When powered by renewable resources and wastewater integration, life-cycle GHG reductions exceed 70% to 85%, confirming microalgae as a high-value, climate-positive energy system (Meitern 2024).

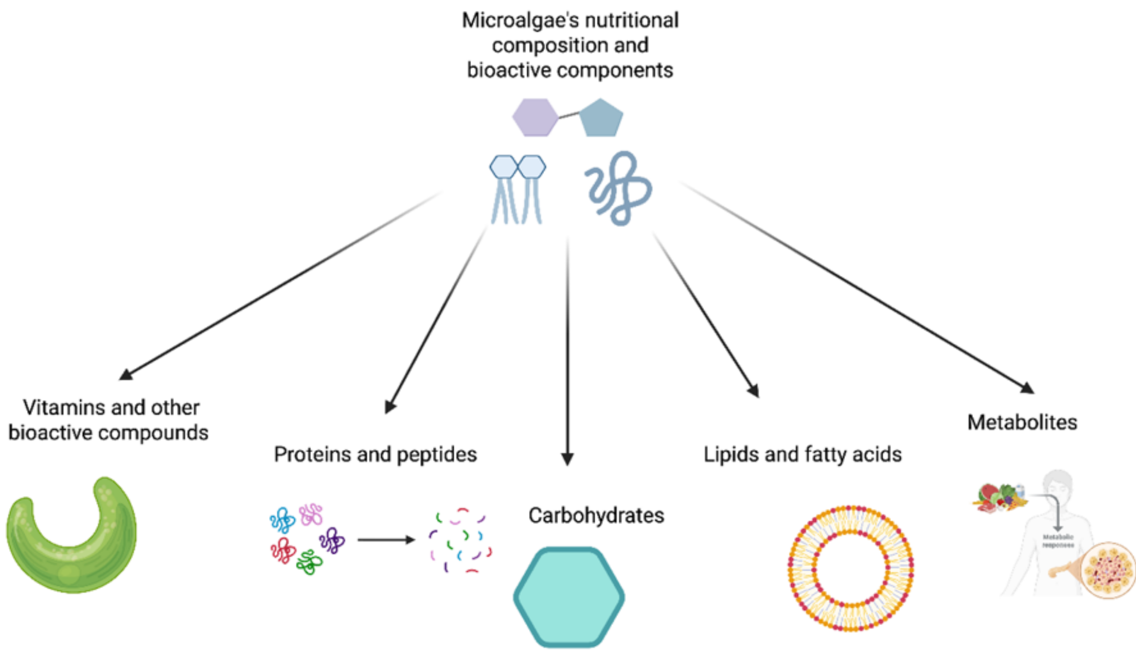

Fig. 2. Nutritional composition of microalgae, highlighting key bioactive components across major biochemical categories: Proteins (including peptides and phycobiliproteins), carbohydrates (such as polysaccharides and glucans), lipids (including sterols, carotenoids, and fatty acids), and vitamins

The quantitative data presented in this report are general ranges and are intended as general guidelines, not absolute figures, and should be interpreted as such unless otherwise noted. The ranges of reported values for lipid content, biomass productivity, CO_2 fixation, greenhouse gas mitigation, production costs, and techno-economic performance are wide depending on the microalgal species and/or strain, cultivation conditions, processing technologies, system boundaries, and production scale.

Table 1 highlights that microalgae differ widely in their biochemical composition, environmental tolerance, and co-product potential, which directly influences biodiesel feasibility. *Anabaena* sp. produces both lipids and extracellular polysaccharides, making it useful for biodiesel–biopolymer biorefineries (Mata *et al.* 2010). *Botryococcus braunii* is notable for synthesizing long-chain hydrocarbons structurally similar to those in petroleum and for accumulating exceptionally high lipid contents (up to 75% dry weight). However, its relatively low biomass productivity (0.05 to 0.2 g L^{-1} day^{-1}) and slow growth rate limit overall lipid productivity and large-scale commercial cultivation. This illustrates that high intracellular lipid content does not necessarily translate into high biodiesel productivity, which depends on both lipid accumulation and biomass production.

Chlamydomonas reinhardtii is the primary model organism for metabolic engineering and for optimizing lipid biosynthesis using CRISPR/Cas9 (Nievergelt 2025). Extremophilic species such as *Dunaliella salina* and *Picochlorum sp*. withstand high temperatures and saline environments, thereby reducing the risk of contamination and enabling their economical outdoor cultivation (Tampieri *et al.* 2023; Raja *et al.* 2022).

There are also several microalgae that offer valuable co-products that drive the economics for integrated biorefineries. *Haematococcus pluvialis* accumulates astaxanthin, and *Isochrysis galbana* and *Phaeodactylum tricornutum* are rich in DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) for nutraceutical use (Sztubecka *et al.* 2020; Celi *et al.* 2022). *Euglena gracilis* produces wax esters that can be used for sustainable aviation fuel pathways, and *Spirulina platensis* is used for its protein and pigment content that can help reduce the costs of biodiesel production.

The species *Anabaena* sp. and *Nostoc* sp. have the ability to produce extracellular polysaccharides that can be used in the production of biopolymers and bioplastics, while the green macroalga *Ulva lactuca* produces polysaccharides called ulvan, which can be included in hybrid bioethanol–biodiesel production systems (Bin Wang *et al.* 2014; Polikovsky *et al.* 2020). These examples highlight the benefits for a biorefinery concept that recovers high-value co-products alongside biodiesel to maximize the sustainability and profitability of the process.

Table 1. Microalgae Species for Biodiesel Production

Microalgae Species	Lipid Content (% DW)	Biomass Productivity (g L^{-1} day^{-1})	Notable Features	References
Anabaena sp.	12 to 25	0.15 to 0.4	Cyanobacterium; co-produces polysaccharides suitable for bioplastics	Singh *et al.* 2016
Botryococcus braunii	25 to 75	0.05 to 0.2	Produces petroleum-like long-chain hydrocarbons; high-value bio-crude	Gouveia *et al.* 2017
Chlamydomonas reinhardtii	18 to 28	0.2 to 0.5	Genetically tractable; CRISPR-engineered strains show enhanced lipid flux	Shin *et al.* 2024
Chlorella protothecoides	30 to 65	0.5 to 1.5	Heterotrophic fermentation dramatically increases lipid yield	O'Grady and Morgan 2011
Chlorella vulgaris	20 to 50	0.5 to 1.2	Fast growth; adaptable to wastewater; widely used in pilot-scale biodiesel units	Mohamed *et al.* 2025
Dunaliella salina	10 to 40	0.1 to 0.4	Extreme halotolerant; major commercial β-carotene producer	Pourkarimi *et al.* 2020
Euglena gracilis	20 to 35	0.3 to 0.9	Produces wax esters ideal for sustainable aviation fuels	Iwasaki *et al.* 2019
Fistulifera solaris	25 to 40	0.2 to 0.5	Marine strain; substantial lipid turnover; tolerant to salinity variation	Matsumoto *et al.* 2017
Haematococcus pluvialis	25 to 40	0.1 to 0.3	Produces high-value astaxanthin under stress; improves biorefinery economics	Matsumoto *et al.* 2017
Isochrysis galbana	22 to 40	0.2 to 0.8	High DHA & sterols; widely used in aquaculture + nutraceutical sectors	Señoráns *et al.* 2020
Monoraphidium sp.	20 to 35	0.3 to 0.6	High lipid accumulation under nitrogen stress; suitable for open ponds	Dhup and Dhawan 2014
Nannochloropsis oculata	31 to 68	0.3 to 0.8	Exceptional EPA and lipid content, leading to a marine biodiesel strain	Navalho *et al.* 2025

Microalgae Species	Lipid Content (% DW)	Biomass Productivity (g L⁻¹ day⁻¹)	Notable Features	References
Neochloris oleoabundans	30 to 54	0.3 to 0.7	Grows in fresh & saline water; strong nitrogen-stress lipid induction	de Jaeger *et al.* 2018
Nostoc sp.	9 to 22	0.15 to 0.35	Cyanobacterium producing extracellular polysaccharides for hydrogel polymers	Quan *et al.* 2015
Phaeodactylum tricornutum	20 to 30	0.15 to 0.5	Marine diatom; major EPA producer; strong candidate for nutraceutical co-products	Ebbing *et al.* 2025
Picochlorum sp.	18 to 36	0.6 to 1.4	Extremophile; thrives in high-temperature & hypersaline outdoor conditions	Chin-On *et al.* 2024
Scenedesmus obliquus	18 to 55	0.4 to 0.9	Robust strain; handles pH and salinity stress; ideal for wastewater systems	Ferreira *et al.* 2021
Spirulina platensis (Arthrospira)	6 to 18	0.5 to 1.3	High protein biomass; co-product revenue reduces biodiesel cost	Kumari *et al.* 2023
Tetraselmis suecica	15 to 45	0.2 to 0.7	Marine green alga; high PUFA; ideal for saline cultivation	Rida *et al.* 2024
Ulva lactuca (Sea Lettuce)	5 to 20	0.4 to 1.0	Macroalgae with valuable ulvan polysaccharides, suited for integrated biorefineries	Rodríguez-Iglesias *et al.* 2024

CULTIVATION SYSTEM SELECTION AND GROWTH OPTIMIZATION FOR MICROALGAE-BASED BIODIESEL PRODUCTION

Microalgae cultivation for biodiesel production is primarily achieved through open raceway ponds or closed photobioreactor (PBR) systems, each offering distinct techno-economic trade-offs (Table 2). Open raceway ponds are widely adopted for large-scale biomass generation due to their low capital cost, simple construction, and reliance on natural sunlight, making them suitable for cost-effective operations and for integration with wastewater and flue-gas CO_2 utilization systems (Sitorus and Brito-Parada 2020). However, open ponds have limitations, including uncontrolled environmental conditions, contamination risks, low light penetration, and relatively lower biomass productivity, which can reduce lipid yields and overall system efficiency (Paul *et al*. 2021). In contrast, closed PBR systems enable precise regulation of CO_2 delivery, temperature, pH, light intensity, and nutrient supply, supporting high cell densities and enhanced lipid accumulation while minimizing contamination. The PBR systems are particularly advantageous for high-value strains or genetically engineered microalgae requiring sterile or controlled conditions. However, the high capital and energy costs, particularly for mixing, gas exchange, and illumination, currently limit their scalability for low-margin biofuel applications (Abdur Razzak *et al*. 2024). Thus, while open ponds enable cost-effective extensive cultivation, PBRs are preferred where high productivity, purity, or strain stability is required (Qin *et al*. 2023).

Optimal biodiesel-oriented cultivation also depends on nutrient availability, particularly nitrogen, phosphorus, trace metals, and inorganic carbon. Nitrogen limitation is widely used to trigger lipid accumulation *via* carbon flux redirection from protein synthesis toward TAG formation, which is a key strategy in industrial lipid induction (Khoo *et al.* 2023). Similarly, CO_2 supplementation directly influences photosynthetic carbon assimilation and biomass productivity, making flue gas integration an effective cost-reduction strategy (Cuellar-Bermudez *et al*. 2015). Environmental factors such as light intensity, temperature, salinity, and pH also play critical roles in determining biomass productivity. Excess light causes photo-inhibition, while insufficient light reduces growth rates (Gani *et al*. 2021). Temperature influences enzyme activity and metabolic kinetics, with most industrial strains exhibiting optimal performance in a 20 to 30°C window (Sriphochanart and Skolpap 2022). Salinity tolerance varies greatly among taxa, and pH imbalances can inhibit nutrient uptake and lipid biosynthesis (Atta *et al*. 2023). Therefore, nutrient management and environmental optimization are essential for achieving high biomass yields and biodiesel suitability.

Table 2. Comparison of Open Pond and Photobioreactor Systems for Microalgal Biodiesel Cultivation

Parameter	Open Raceway Ponds	Closed Photobioreactors	References
Capital Cost	Low construction and setup cost; suitable for large outdoor installations	High capital investment due to specialized materials and modular designs	Maqbool *et al.* 2023
Operating Cost	Low operating cost; relies on natural sunlight; minimal energy inputs	High operating cost due to mixing, aeration, lighting, and temperature regulation	Cappiello 2024
Biomass Productivity	Moderate (typically 15 to 30 g m^{-2} day^{-1}); impacted by weather and seasonality	High (typically 40 to 60 g m^{-2} day^{-1} under optimized control conditions)	Krzemińska *et al.* 2014
Lipid Content Potential	Variable; stress-induced lipid accumulation is possible but inconsistent	More consistent lipid yields due to controlled conditions	Oh *et al.* 2019
Contamination Risk	High susceptible to invasive algal species, protozoa, and pathogens	Low closed system limits contamination and culture competition	Wang *et al.* 2013
Environmental Control	Poor control over light, pH, temperature, and salinity	Precise regulation of CO_2, light intensity, temperature, and nutrient supply	Gruda *et al.* 2025
Scalability	Highly scalable to hectares; suitable for outdoor mass production	Scalability is limited by cost and complexity	Zhang *et al.* 2025
Best Application Scenario	Low-cost, large-scale biomass generation using non-sterile robust strains	High-value or genetically engineered strains requiring sterility and control	Zhao *et al.* 2025

HARVESTING AND DEWATERING TECHNIQUES OVERVIEW

Harvesting and dewatering are recognized as significant energy and operating cost bottlenecks in the microalgal biodiesel production chain, given that microalgal cultures typically contain only 0.02% to 0.1% dry biomass, requiring concentration before lipid extraction (de Morais *et al.* 2023). Among available techniques, centrifugation is widely used owing to its rapid processing time and ability to achieve >90–95% biomass recovery, producing dense pastes suitable for downstream conversion (Menegazzo and Fonseca 2019). However, its high specific energy demand (~0.2 to kWh m^{-3}) significantly impacts operational costs, making full-scale implementation challenging without process integration (Fasaei *et al.* 2018). In contrast, flocculation offers a cost-effective alternative wherein microalgal cells are aggregated into larger flocs that settle or float rapidly. Chemical coagulants (*e.g.*, Al^{3+}, Fe^{3+}) and bio-flocculants such as chitosan and tannin-based polymers have shown 70% to 98% harvesting efficiency with considerably lower energy input (Pugazhendhi *et al.* 2019). Recent attention has focused on environmentally benign bio-flocculants to avoid introducing contaminants that may interfere with lipid extraction or fuel quality (Borges *et al.* 2011).

Filtration and membrane separation (microfiltration/ultrafiltration) are also widely applied, especially where consistent biomass quality is required, though their efficiency is often constrained by membrane fouling, necessitating periodic cleaning or replacement (Castro-Muñoz and García-Depraect 2021). Dissolved air flotation and electrocoagulation-flotation have recently gained prominence for industrial-scale integration, offering rapid clarification and reduced chemical load, primarily when operating as pre-concentration stages before centrifugation or membrane finishing (Muñoz-Alegría *et al.* 2021). Meanwhile, magnetic flocculation, using recoverable Fe_3O_4 nanoparticles, has demonstrated rapid harvesting with easy magnetic retrieval, although nanoparticle recovery efficiency and cost recyclability remain under optimization for commercial viability (Zhang *et al.* 2019).

Overall, no single harvesting method optimizes cost, recovery efficiency, and scalability across all production systems. As a result, recent techno-economic analyses recommend hybrid harvesting trains, for example, bio-flocculation or dissolved air flotation for low-energy pre-concentration, followed by polishing centrifugation, which can significantly reduce energy demand while maintaining high recovery efficiency. These integrated strategies are therefore considered essential for improving the minimum fuel selling price and achieving commercially viable, climate-positive microalgal biodiesel production.

LIPID EXTRACTION APPROACHES IN MICROALGAL BIODIESEL PRODUCTION

Efficient lipid extraction is a critical determinant of the technical feasibility and economic competitiveness of microalgae-based biodiesel production. Traditionally, solvent-based extraction has been the most widely employed method due to its high extraction efficiency, yielding 90% to 98% total lipids depending on species and pretreatment (Zhang *et al.* 2022). Solvents such as hexane, ethanol, or chloroform–methanol mixtures effectively disrupt intracellular lipid associations, allowing high recovery even in species with moderate lipid content. However, the use of large solvent volumes, environmental toxicity, stringent solvent recovery requirements, and fire/explosion risks pose significant limitations in commercial implementation (Aboagye *et al.* 2021). This has driven industry interest toward reduced-solvent or solvent-free extraction trains.

Mechanical pressing and bead milling provide a more environmentally benign alternative, particularly suitable for high-lipid microalgae, where lipids accumulate in easily rupturable lipid bodies (Patel *et al.* 2018). These methods preserve oil integrity and avoid toxic residues but typically achieve 60% to 85% lipid recovery and are less effective for species with rigid cellulosic or silicified cell walls, such as *Scenedesmus* and diatoms (Abdel Ghaly and Al-Hattab 2015). Therefore, mechanical extraction is frequently used as a first-step cell disruption technique, followed by solvent or enzymatic polishing to maximize recovery.

More recently, supercritical CO_2 extraction (SFE) has attracted considerable attention as a green, solvent-free, high-purity extraction method, producing lipids with preserved bioactive compounds suitable for nutraceutical-grade biodiesel co-products. Supercritical CO_2 possesses gas-like diffusivity and liquid-like solvation power, enabling 85% to 95% lipid recovery while eliminating solvent toxicity (Nastić *et al.* 2025). However, the necessity for high-pressure equipment (200 to 400 bar) and skilled operational control increases capital and energy requirements, limiting its commercial uptake to high-value biorefineries rather than commodity-scale biodiesel plants.

Enzymatic extraction represents another emerging strategy in which cell wall-degrading enzymes (*e.g.*, cellulases, proteases, lysozymes) selectively hydrolyze structural polymers, allowing lipid bodies to diffuse from the cell with minimal mechanical force. The method is highly selective, low-temperature, and environmentally safe, often yielding high-quality, pigment-rich oils (Wu *et al.* 2017). Its limitations include high enzyme cost, strain-specific optimization requirements, and comparatively slower reaction kinetics. Enzymatic extraction is becoming increasingly feasible in integrated biorefineries, where enzymes are recovered or co-produced using microbial expression systems.

Meanwhile, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) have emerged as hybrid physical–chemical disruption methods, enhancing mass transfer and reducing solvent requirements. MAE enables rapid intracellular heating, improving lipid release but requiring careful thermal control to avoid oxidative degradation (Bagade and Patil 2021). UAE improves solvent penetration *via* acoustic cavitation, providing 70% to 88% lipid recovery with comparatively low energy input, though prolonged exposure can oxidize unsaturated fatty acids (Shen *et al.* 2023).

Taken together, these findings indicate that no single extraction technique optimizes lipid recovery, product purity, safety, and operating cost simultaneously. Consequently, hybrid extraction configurations, for instance, UAE/MAE pre-treatment with solvent finishing, mechanical disruption with enzymatic hydrolysis, or bio-flocculation with supercritical CO_2 polishing, are increasingly favored in recent techno-economic assessments. Such integrated trains enable higher lipid yields, reduce energy consumption, and enhance sustainability, making them central to advancing commercial-scale microalgal biodiesel production, as illustrated in Table 3.

The transesterification process is essential for transforming oils derived from microalgae into biodiesel. In this chemical reaction, triglycerides, which are the primary components of these oils, interact with alcohol, most commonly methanol or ethanol, while being facilitated by a catalyst, typically sodium hydroxide or potassium hydroxide (Farouk *et al.* 2024). This process yields methyl esters (biodiesel) and glycerol as a byproduct. The reaction generally occurs under moderate temperature and pressure conditions, but achieving optimal efficiency requires precise management of the catalyst and reactant ratios. Several factors, including the quality of the extracted oil, the choice of alcohol, and the selected catalyst, influence the final purity of the biodiesel. Biodiesel conversion efficiency is all about how well algal oil turns into biodiesel during the transesterification process. Several factors such as the lipid content in the algae, the type of alcohol and catalyst used, as well as the reaction time and temperature (Gaurav *et al.* 2024). Generally, a higher lipid content in the algae means better conversion efficiency. On the flip side, impurities or leftover water in the oil can decrease biodiesel yield (Nashath *et al.* 2025).

Table 3. Comparison of Lipid Extraction Methods for Microalgae Biodiesel Production

Method	Lipid Yield (% of Total Lipids)	Energy Consumption (kWh kg^{-1} oil)	Advantages	Limitations	References
Solvent Extraction (hexane, chloroform–methanol, ethanol)	90 to 98	1.5 to 3.0	High extraction efficiency; suitable for most species	Toxic solvents, high solvent recovery cost, and environmental concerns	Cravotto *et al.* 2022
Mechanical Pressing (expeller, bead milling)	60 to 85	0.8 to 2.5	Non-toxic solvents; preserves oil quality; simple operation	Lower yield for tough cell walls; more effective for high-lipid species	Bhuiya *et al.* 2020
Supercritical CO_2 Extraction	85 to 95	2.0 to 4.5	Non-toxic; solvent-free; preserves bioactivity; high purity	High equipment cost; high-pressure operation	López-Hortas *et al.* 2022
Enzymatic Extraction	80 to 90	1.0 to 2.0	Mild conditions; environmentally friendly; selective	High enzyme cost; process optimization required	Jiang *et al.* 2025
Microwave Assisted Extraction	75 to 90	1.2 to 2.0	Rapid heating; high cell disruption; reduced solvent use	Risk of overheating; uneven heating for large volumes	Kapoore *et al.* 2018
Ultrasound Assisted Extraction	70 to 88	0.9 to 1.8	Enhances solvent penetration; fast; low cost	Possible oil oxidation; less effective for dense biomass	Kumar *et al.* 2021

The process wastewater generated during microalgae transesterification and the subsequent purification step is also crucial to the environmental sustainability of the biodiesel. The alkaline, glycerol-rich wastewater from conventional alkali-catalyzed transesterification contains residual catalysts, soaps, methanol, salts, and organic impurities that require treatment before disposal. Direct disposal can cause an increase in chemical oxygen demand, salinity, and pH, which is a concern for the environment.

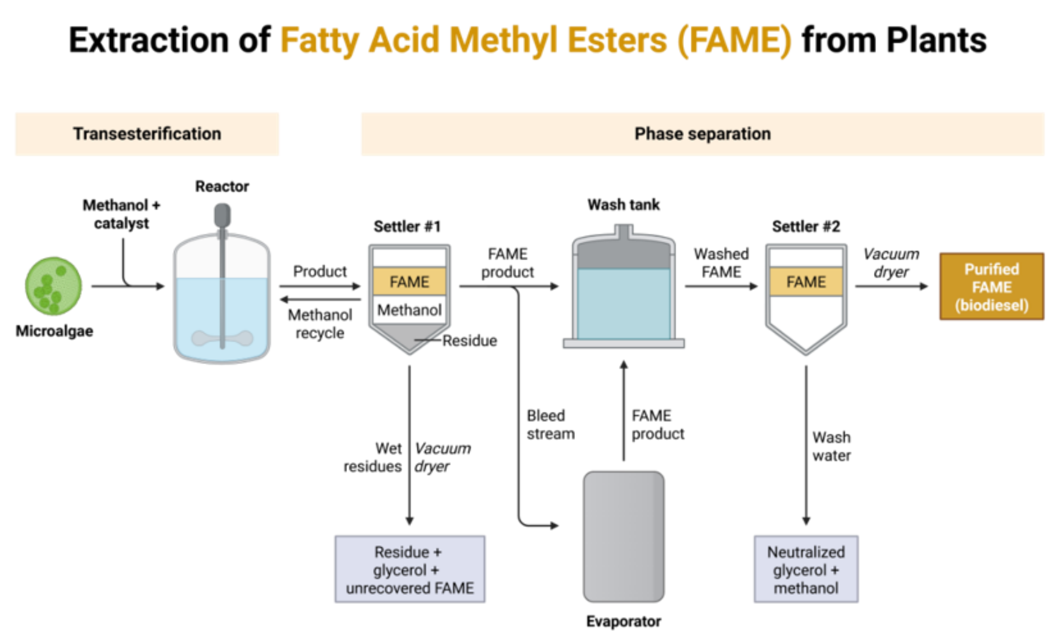

Fig. 3. Biodiesel production from the microalgae process

Catalyst recovery, methanol recycling, glycerol purification and valorization, membrane-based separation, and water recycling within the biorefinery can significantly reduce waste and enhance process sustainability. Furthermore, the use of heterogeneous catalysts and continuous processing methods helps reduce wastewater generation and improve the environmental footprint of biodiesel production.

By optimizing the transesterification process, such as by adjusting reaction conditions and utilizing advanced catalysts, the conversion efficiency can increase biodiesel yield, ultimately making large-scale biodiesel production from microalgae more feasible (Lee *et al.* 2022), as shown in Fig. 3.

SAFETY AND ENVIRONMENTAL CONTROLS FOR LIPID EXTRACTION

The extraction and recovery of lipids from microalgal biomass often involve the handling of flammable organic solvents (*e.g.*, hexane, chloroform–methanol, ethanol) and pressurized extraction systems, which present significant occupational and environmental safety challenges. These extraction media are classified as volatile organic compound emission sources. Thus, production facilities must incorporate closed-loop extraction assemblies with explosion-proof motors, grounded stainless-steel lines, solvent vapor condensers, and real-time leak detection. Industrial guidelines increasingly emphasize ≥95% solvent recovery efficiency to minimize solvent replacement costs and reduce scope-1 emissions, a key performance target confirmed in life-cycle assessments of lipid biorefineries (Prommuak *et al.* 2012). Failure to achieve high solvent recovery increases carbon intensity scores, directly impacting eligibility for low-carbon fuel standard credits in regulated markets.

Effluent streams generated from flocculation, dewatering, and solvent washing often contain surfactants, coagulants, or residual hydrocarbons, requiring treatment through anaerobic digestion, membrane polishing, advanced oxidation, or zero-liquid-discharge loops to prevent soil and aquatic toxicity. In large-scale harvesting trains, coupling nutrient-rich effluents back into cultivation ponds supports circular nutrient cycling and reduces nitrogen and phosphorus fertilizer demand (Chojnacka *et al.* 2020). To minimize chemical risk, green solvent approaches are increasingly being adopted. Supercritical CO_2 extraction provides high-purity, solvent-free lipid fractions and preserves heat-labile carotenoids, sterols, and PUFA co-products. Similarly, natural deep eutectic solvents (NADES) such as choline chloride–glycerol and lactic acid–betaine replace petroleum solvents while enabling biodegradable, low-toxicity lipid recovery, making them promising for food-grade algal oils and nutraceutical supply chains (Amador *et al.* 2025). However, both SC-CO_2 and NADES extraction require process intensification strategies (*e.g.*, elevated CO_2 density, ultrasound assistance, or microwave-coupled heating) to match the extraction kinetics of conventional hexane systems. In addition to chemical hazards, thermal and mechanical safety controls must be considered. MAE and UAE methods create localized heating and cavitation forces, and require inline thermal regulation and cavitation shielding to prevent oxidative degradation of polyunsaturated lipids (Ameer *et al.* 2017). Regular audits, flammable-area classification compliance, and operator training are therefore essential components of safe lipid extraction process design (Choi and Byeon 2020).

Finally, energy integration significantly affects environmental performance. Heat recovery from solvent distillation, biogas generation from residual biomass, and biochar production from spent residues can improve overall EROI and move the biorefinery closer to closed-loop circular economy operation (Xiao *et al.* 2020). When combined, these measures ensure that lipid extraction not only meets industrial safety and environmental regulations but also contributes to scalable, resource-efficient, and climate-positive biodiesel production.

CHALLENGES AND LIMITATIONS

The commercial deployment of microalgae-derived biodiesel remains challenged primarily by high production costs associated with cultivation, harvesting, and lipid extraction processes. Cultivation infrastructure, whether based on open ponds or closed PBRs, demands substantial capital investment and operational inputs. PBRs offer precise control over light, temperature, nutrient supply, and contamination risk. Still, their capital and energy requirements can be two to four times higher than open pond systems, leading to significantly elevated operational expenditure (Sharma *et al.* 2025). In contrast, open ponds require lower capital input but often suffer from seasonal limitations, higher susceptibility to contamination, and 20% to 45% lower biomass productivity compared to PBR systems (Abdur Razzak *et al.* 2024). A major contributor to both cost and environmental burden is the harvesting and dewatering stage, as microalgal cultures typically contain only 0.02% to 0.1% dry biomass, necessitating energy-intensive concentration steps. Centrifugation alone can consume 0.2 to 8 kWh m^{-3}, accounting for 30% to 45% of total operating costs in several process configurations (Fasaei *et al.* 2018). Likewise, lipid extraction using solvent-based or transesterification methods increases the energy and environmental footprint due to solvent handling, recovery, and associated emissions control (Lestari 2015).

Advanced lipid extraction approaches, including supercritical CO_2 extraction and deep eutectic solvents, improve oil purity and mitigate hazardous solvent emissions, yet remain limited by equipment cost, pressure requirements, and optimization needs for different microalgal strains (de Aguiar *et al.* 2023). Parallel advances in genome editing, metabolic engineering, and adaptive laboratory evolution have demonstrated 20% to 60% increases in TAG accumulation, particularly through CRISPR/Cas9-mediated modulation of lipid biosynthesis pathways, but scaling these gains to outdoor or industrial conditions remains a developing field (de Aguiar *et al.* 2023). To objectively evaluate feasibility, integrated Techno-Economic Analysis (TEA) and Life-Cycle Assessment (LCA) frameworks are needed to identify cost bottlenecks and quantify environmental trade-offs (Mahmud *et al.* 2021). Recent TEA data indicate that pilot-scale open pond facilities typically require USD 2 to 5 million, whereas PBR installations may cost USD 5 to 12 million, depending on automation and environmental control systems (Koko *et al.* 2025). Correspondingly, operational production costs currently range between USD 1.5 to 3.0 L^{-1} for optimized open ponds and USD 3.0 to 6.0 L^{-1} for PBR-based systems. Energy return on investment values for microalgae biodiesel currently range from 0.5 to 3, indicating that specific production configurations may still require more energy than they yield. However, integrating low-energy harvesting strategies, waste heat recovery, and flue-gas CO_2 capture can elevate EROI values above 5, improving competitiveness relative to other biofuels (Vadiveloo *et al.* 2022). Life-cycle assessments further show that microalgae biodiesel can achieve 50% to 70% lower greenhouse gas emissions compared to petroleum diesel, where CO_2 capture and wastewater nutrient recycling are implemented, but these reductions may diminish when fossil-derived energy is used for drying and solvent recovery (Saranya and Ramachandra 2020). Commercial-scale payback times currently range from 8 to 12 years for optimized open-pond biorefineries and >15 years for PBR-based systems; however, integrated biorefineries co-producing biodiesel, omega-3 oils, pigments, and biofertilizers can reduce payback to 5–7 years, improving economy (Branco-Vieira *et al*. 2020). Continued innovation in AI-assisted process optimization, nutrient recycling, metabolic engineering, and circular bioeconomy integration will be central to reducing costs and advancing the commercial feasibility of microalgae biodiesel.

Microalgae biodiesel shows significant life-cycle GHG reductions compared to fossil-fuel-based diesel; the sustainability of microalgae biodiesel should also be compared with the rise of emerging battery-electric transportation powered by renewable electricity. In general, solar or wind power for electric vehicles (EVs) results in greater energy efficiency and lower operational emissions than liquid biofuels for light-duty transport. Biodiesel, however, will continue to be significant for challenging areas such as heavy-duty transportation, marine and aviation, and off-road equipment, where liquid fuels have greater energy density and compatibility with current infrastructure. Hence, future life-cycle assessments would be required to compare microalgal biodiesel with renewable electricity-based transport systems to identify the most sustainable application for each technology.

FUTURE PROSPECTS AND SUSTAINABLE SOLUTIONS

Genetic engineering has emerged as a powerful tool to enhance lipid productivity in microalgae for biodiesel applications (Muñoz *et al.* 2021). Scientists are increasingly focusing on metabolic engineering to redirect carbon flux toward TAG synthesis by modifying key enzymes in the lipid biosynthetic pathway. The acetyl-CoA carboxylase (ACCase) complex catalyzes the conversion of acetyl-CoA to malonyl-CoA, which is an essential, rate-limiting step for fatty acid biosynthesis. At the same time, diacylglycerol acyltransferase (DGAT) and phospholipid: diacylglycerol acyltransferase (PDAT) governs the final assembly of TAG molecules. Overexpression of ACCase and DGAT, coupled with suppression of carbohydrate synthesis genes such as AGPase and G6PDH, has been shown to increase lipid yield significantly (Ku *et al*. 2020).

Recent advances in CRISPR/Cas9 and RNA interference (RNAi) technologies allow precise editing and regulation of metabolic pathways to enhance fatty acid accumulation. Through gene knockouts, promoter modifications, and pathway rebalancing, these approaches reduce carbon loss toward carbohydrates and improve overall lipid flux. Multi-omics studies, encompassing transcriptomics, proteomics, and metabolomics, have further identified key transcription factors (bZIP, DOF, WRINKLED1-like) that regulate lipid metabolism under nutrient stress, enabling predictive flux balance modelling and data-driven strain design (Rajput *et al*. 2021). Synthetic biology approaches are now being applied to engineer next-generation strains with superior lipid accumulation, rapid growth kinetics, and enhanced tolerance to salinity, light, and temperature stress. Collectively, these molecular innovations have the potential to make microalgae-derived biodiesel more commercially viable, scalable, and sustainable than conventional biofuels.

An integrated biorefinery approach further strengthens the economic potential of microalgae-based biodiesel by enabling simultaneous recovery of multiple value-added products such as proteins, pigments, antioxidants, bioplastics, and pharmaceuticals

(Ahmad *et al.* 2021). This multi-product strategy not only improves process economics but also supports zero-waste utilization through by-product valorization into biofertilizers or bioethanol, reducing operational costs and enhancing environmental sustainability. The combination of genetic optimization and biorefinery integration positions microalgae as a cornerstone of next-generation renewable energy systems, providing a competitive and sustainable alternative to fossil fuels (Chojnacka 2023).

Artificial Intelligence and Digital Tools for Process Optimization

The adoption of artificial intelligence (AI), automation, and advanced computational tools has significantly improved microalgae cultivation and biodiesel processing efficiency. AI-driven systems analyze real-time physiological, environmental, and biochemical data to support predictive decision-making and autonomous bioprocess adjustment, thereby improving scalability and lowering operational costs. AI-guided nutrient optimization models integrate real-time sensor feedback (*e.g.*, optical density, chlorophyll fluorescence, pH, dissolved oxygen) to regulate nitrogen, phosphorus, and trace element supply dynamically. This approach improves biomass productivity and lipid accumulation while minimizing resource wastage and contamination risk (Ugya *et al*. 2025). Machine-learning algorithms also predict nutrient starvation thresholds that maximize lipid induction efficiency without compromising cellular viability (Kirk *et al.* 2022). Computer-vision-assisted contamination detection using deep-learning imaging platforms can distinguish microalgal cells from bacteria, fungi, and invasive algal species with >95% accuracy, allowing rapid intervention before culture collapse (Abdullah *et al*. 2022). This early detection reduces biomass loss and improves culture stability in large-scale open and semi-open systems. Harvest timing and biomass productivity forecasting are increasingly managed using predictive growth models that integrate climatic data, light-energy conversion efficiency, nutrient flux balances, and lipid accumulation kinetics (Abdur Razzak *et al.* 2024). This ensures harvesting aligns with peak lipid productivity rather than peak biomass. Digital twins, virtual replicas of cultivation systems, simulate operational conditions (light distribution, CO_2 uptake, hydrodynamics, reactor geometry) to evaluate optimal operational strategies without disrupting live cultures (Udugama *et al.* 2021). These models can reduce energy consumption and CO_2 supply costs by up to 18%–30% during scale-up. Machine-learning-based lipid yield prediction models, trained using multi-omics (transcriptomic, proteomic, metabolomic) and environmental datasets, enable targeted strain selection and personalized cultivation strategies for maximizing lipid output.

Overall, the integration of AI aligns microalgal biodiesel production with Industry 4.0, promoting autonomous cultivation platforms with reduced human input and higher reproducibility, ultimately supporting low-cost, continuous biomass production (Kanwal *et al*. 2025).

Microalgae Biodiesel in a Circular Bioeconomy Framework

Integrating microalgae biodiesel production within a circular bioeconomy provides a sustainable and resource-efficient route for renewable fuel generation, environmental remediation, and value-added bioproduct synthesis. In this framework, microalgae cultivation systems are strategically linked with industrial, agricultural, and municipal waste streams to support nutrient recycling, carbon capture, and co-product recovery, thereby improving both ecological performance and economic feasibility (Gaurav *et al*. 2024). One of the most impactful circular strategies involves the use of nutrient-rich wastewater as a cultivation medium. In addition to nutrient-rich wastewater, crude glycerol generated during transesterification can be incorporated into the culture medium as a carbon source for mixotrophic or heterotrophic microalgal cultivation after appropriate pretreatment or dilution to minimize impurities. This integrated approach promotes a circular biorefinery by valorizing biodiesel byproducts, reducing waste generation, and improving the overall sustainability and economic feasibility of microalgal biodiesel production.

Wastewaters from agro-industrial effluents, food-processing plants, and municipal treatment facilities often contain high concentrations of nitrogen and phosphorus, which microalgae can effectively assimilate to support biomass growth while simultaneously removing up to 80%-90% nitrogen and 70%–85% phosphorus, significantly reducing eutrophication risks and lowering nutrient input costs (Abdelfattah *et al.* 2023). This symbiotic wastewater microalgae system transforms conventional wastewater treatment plants into bioresource recovery platforms. Additionally, microalgae-based systems contribute to carbon circularity through their ability to directly assimilate CO_2 from industrial flue gases. When integrated with emission-heavy industries such as cement plants, breweries, or power stations, microalgae can utilize flue gas streams containing CO_2 concentrations of up to 12%–15%, converting carbon into biomass through photosynthesis and thereby reducing greenhouse gas emissions at the source (Abdelfattah *et al.* 2023). Such integration supports national and global carbon neutrality strategies by establishing a closed-loop carbon utilization cycle.

Beyond biodiesel synthesis, the microalgal biorefinery generates several high-value co-products that significantly enhance process economics. After lipid extraction, the defatted biomass retains substantial quantities of proteins, polysaccharides, vitamins, minerals, and pigments, which can be converted into aquaculture feed supplements, probiotic formulations, biofertilizers, soil biostimulants, or biodegradable polymer precursors, aligning with zero-waste principles (Abdelfattah *et al*. 2023). Integrating these co-product streams diversifies revenue, offsets biodiesel production costs, and supports broader biomanufacturing sectors such as nutraceuticals, agriculture, and biomaterials. Therefore, when CO_2 mitigation, nutrient recycling, and biomass valorization strategies are jointly incorporated, microalgae biodiesel transitions from a single-output energy system to a multi-benefit circular bioresource platform, reinforcing its sustainability and economic viability for future large-scale commercial deployment (Abdelfattah *et al.* 2023).

Microalgal Biorefinery and Recovery of High-Value Bioactive Compounds

Microalgae represent a versatile biorefinery platform capable of producing not only lipids for biodiesel but also a broad spectrum of high-value bioactive compounds, including carotenoids (*e.g.*, astaxanthin, β-carotene), omega-3 polyunsaturated fatty acids (DHA, EPA), phycobiliproteins, sulfated polysaccharides, vitamins, and sterols. These compounds possess potent antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties, making them valuable for nutraceutical, pharmaceutical, cosmetic, and functional food applications (Ahmad *et al.* 2021).

The recovery of these bioactive compounds involves a multistage bioprocessing pipeline, illustrated in Fig. 4. Following cultivation, biomass undergoes cell disruption and extraction using environmentally sustainable methods. Supercritical fluid extraction employing CO_2 enables selective recovery of thermolabile lipids and carotenoids without residual organic solvents (Herzyk *et al.* 2024). Ultrasound-assisted extraction enhances solvent penetration and reduces extraction time through cavitation-induced cell disruption, improving yields of phycocyanin and phenolic compounds (Kumar *et al.* 2021). Enzyme-assisted extraction (EAE) utilizes cell wall-degrading enzymes to release proteins and polysaccharides while preserving structural functionality (Malvis Romero *et al.* 2023).

Post-extraction, downstream separation and purification determine product quality and application potential. Chromatographic techniques, including HPLC, gel filtration, and affinity chromatography, provide high-purity pigments and peptides (Ramraje *et al*. 2020). Membrane-based filtration technologies (ultrafiltration, nanofiltration) offer scalable and energy-efficient separation of proteins and polysaccharides with minimal thermal degradation (Al Sharabati *et al.* 2021). For certain biopolymers and proteins, precipitation methods such as isoelectric precipitation remain economically favorable for large-scale operations.

To enhance stability, bioavailability, and ease of incorporation into commercial formulations, purified compounds are subjected to encapsulation strategies. Spray drying converts bioactive extracts into free-flowing powders with improved oxidation resistance, while microencapsulation using matrices such as maltodextrin, alginate, chitosan, or whey proteins enables controlled release and targeted physiological effects (Mangope *et al.* 2025). These encapsulated microalgal bioingredients are widely incorporated into dietary supplements, cosmeceutical formulations, aquaculture feed enhancers, and functional beverages. The integration of such bioproduct recovery pipelines within the biodiesel production chain supports zero-waste microalgal biorefineries, where lipid extraction for fuel is coupled with value-added metabolite recovery. This model significantly increases the economic viability and sustainability of commercial microalgae cultivation by shifting production from single-output fuel systems toward multi-product circular bioeconomy platforms (Moon *et al.* 2022).

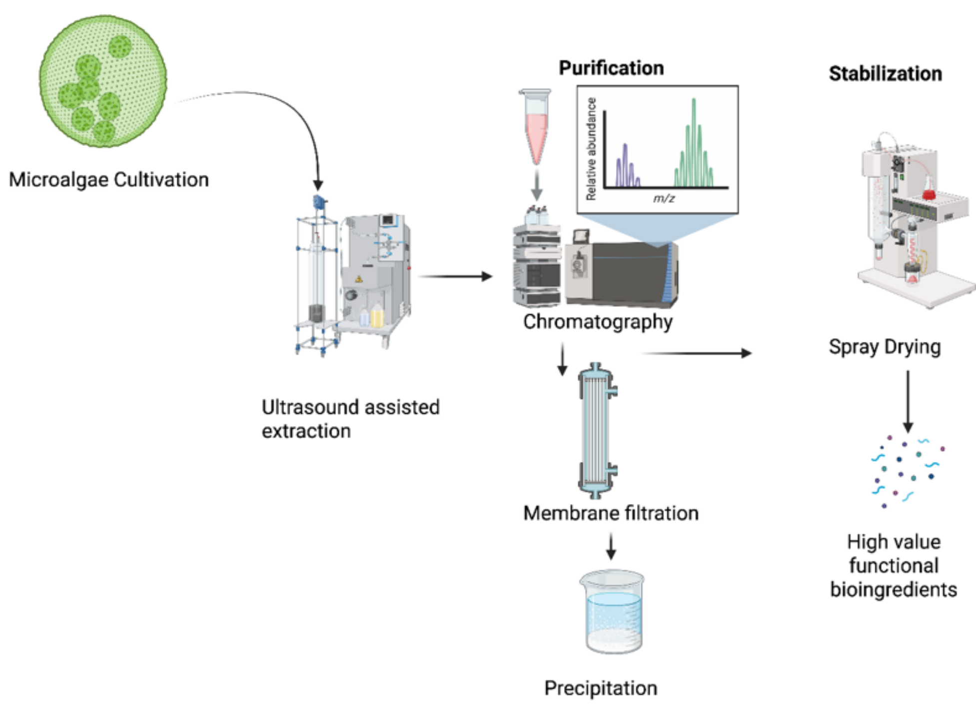

Fig. 4. Integrated bioprocessing workflow for recovery of bioactive compounds from microalgae. Following microalgal cultivation, bioactive compounds are extracted using techniques such as SFE, UAE, and EAE processes. Extracted compounds are purified and subsequently stabilized *via* spray drying or microencapsulation to produce high-value functional bioingredients.

Recent Advances in Microalgae Biodiesel Production Technologies

Significant progress in genetics, cultivation engineering, bioprocess optimization, and circular biorefinery integration has transformed microalgae-derived biodiesel from a laboratory-scale concept into a scalable and economically feasible bioenergy platform. Modern innovations focus on maximizing lipid productivity, improving biomass robustness under outdoor conditions, reducing production costs, and generating multiple high-value co-products to support commercial viability.

Table 4. Recent Technological Innovations and Biorefinery Strategies Enhancing Microalgae-Based Biodiesel Production (2020 to 2025)

Innovation / Technology	Key Advancement	Potential Impact	References
CRISPR-Cas9 Genome Editing	Redirected carbon flux from carbohydrate to lipid biosynthesis	30% to 50% increase in lipid yield; improved strain domestication	An *et al.* 2024
Adaptive Laboratory Evolution (ALE)	Gradual stress adaptation for outdoor cultivation stability	High robustness and productivity under variable conditions	Hirasawa and Maeda 2022
Hybrid Cultivation Systems (PBR + Open Pond)	Balances productivity, scalability, and cost	Reduced contamination with lower infrastructure cost	Yun *et al.* 2018
Heterotrophic–Photoautotrophic Two-Stage Cultivation	Rapid biomass accumulation followed by lipid induction	40% to 60% improvement in lipid accumulation efficiency	Zhou *et al.* 2012
AI-Driven Cultivation & Process Control	Machine learning for nutrient dosing, growth prediction, and contamination alerts	10% to 20% higher biomass yield; reduced operational labor and costs	Yang *et al.* 2025
Microalgal–Bacterial Co-Culture Systems	Beneficial microbial symbiosis provides CO_2, vitamins, and biofilm resistance.	Higher stability; lower aeration and nutrient costs	Tong *et al.* 2023
Nano-Fertilizers and Nano-Trace Element Supplementation	Enhanced photosynthetic electron transport and carbon fixation	Increased biomass yield with reduced nutrient input	Mim *et al.* 2025
LED-Based Dynamic Light Modulation	Optimized wavelength/photoperiod to stimulate lipid pathways	20% to 30% increase in lipid productivity	Severes *et al.* 2017
Continuous-Flow Photobioreactors	Real-time biomass withdrawal + controlled light distribution	Higher volumetric productivity; industrial scalability	Acién Fernández *et al.* 1999
Electroporation-Assisted Cell Disruption	Pulsed electric fields weaken the cell wall without solvents	Reduced extraction energy demand; Higher lipid recovery	Lai *et al.* 2014
Microwave + Ultrasound Pretreatment Integration	Enhanced cell wall rupture and solvent penetration	Higher extraction yield with lower energy consumption	Martinez-Guerra *et al.* 2014
Magnetic Nanoparticle-Assisted Harvesting	Magnetic flocculation for rapid biomass separation	Faster harvesting with minimal flocculant residues	Patel *et al.* 2022
CO_2 Flue Gas Utilization	Direct integration of industrial emissions into cultivation	Reduced GHG emissions; cost savings on CO_2 supply	Patel *et al.* 2022
Wastewater-Coupled Cultivation	Recycling nutrient-rich municipal/agricultural effluents	Significant cost reduction + simultaneous wastewater remediation	Yolanda *et al.,* 2025)
Integrated Microalgal Biorefineries	Co-production of biodiesel + feed proteins + pigments + fertilizers	Higher economic feasibility *via* multi-revenue product streams	Wang *et al.* 2022

For example, CRISPR-Cas9-mediated genome editing has enabled targeted modification of carbon flux pathways, enhancing lipid biosynthesis efficiency by 30% to 50% (Zhang *et al.* 2025). Similarly, adaptive laboratory evolution (ALE) strategies have produced strains with improved tolerance to light, salinity, and fluctuating temperatures, supporting industrial-scale outdoor cultivation (LaPanse *et al.* 2021).

Advances in processing automation and digital optimization have also been transformative. AI-driven cultivation control now enables real-time nutrient dosing, growth prediction, and contamination detection, leading to 10% to 20% improvements in biomass productivity while reducing manual supervision (Mana *et al*. 2024). In parallel, two-stage heterotrophic–photoautotrophic systems have demonstrated 40% to 60% increases in lipid accumulation efficiency during induction phases (Lu *et al.* 2018). Moreover, microalgal–bacterial co-cultivation systems have shown enhanced carbon cycling and reduced aeration demand, strengthening process sustainability (Ugya *et al.* 2025).

From a techno-economic perspective, circular resource utilization strategies are now central to commercial biodiesel deployment. The integration of CO_2 flue gas streams from industrial exhausts directly into photobioreactors supports carbon capture while eliminating CO_2 supply costs (Wan Osman *et al.* 2024). Likewise, wastewater-mediated cultivation enables nutrient recovery from municipal and agro-industrial effluents, reducing fertilizer input and contributing to wastewater remediation services (Aryampa *et al.* 2025). Meanwhile, integrated microalgal biorefineries co-produce biodiesel alongside pigments, proteins, polysaccharides, and biofertilizers, generating multi-revenue product streams that boost economic feasibility (Aryampa *et al.* 2025).

Table 4 summarizes these innovations, highlighting their technical advantages, process improvements, and sustainability benefits. Collectively, these advancements mark a major transition from traditional lipid-only extraction toward scalable, digitally optimized, and circular microalgal biorefinery systems, positioning microalgae biodiesel as a competitive contributor to future low-carbon renewable energy economies.

TIMELINE FOR COMMERCIALISATION

The transition of microalgae-derived biodiesel from laboratory research to competitive industrial deployment requires a phased commercialization strategy that aligns technological advancements, policy support, and market adoption pathways. This roadmap provides a realistic timeline to guide research prioritization, investment decisions, and infrastructural planning, ensuring that innovations outlined progress toward scalable application (Deepika 2025).

Short-term (1–3 years): Pilot Plant Optimization

Near-term efforts should focus on enhancing pilot-scale cultivation efficiency under real environmental conditions. Key targets include optimizing photobioreactor geometry for improved light penetration, integrating CO_2 flue gas and nutrient-rich wastewater streams as feedstocks, and refining low-energy harvesting and lipid extraction methods. Incorporation of AI-enabled monitoring and control systems will improve process stability, contamination resistance, and real-time decision-making (Abdur Razzak *et al.* 2024).

Medium-term (4–7 years): Policy Support and Market Entry

In this phase, supportive policies and economic incentives will be crucial to bridge the gap between pilot-scale and commercial-scale production, in high-value sectors such as sustainable aviation fuel and aquaculture, and achieving positive techno-economic assessments and regulatory credit eligibility (LCFS/RIN). Governments can accelerate deployment by providing tax credits, renewable fuel subsidies, and carbon trading mechanisms that reward low-carbon fuels (Zhou 2023). Public-private partnerships can help de-risk investments in large demonstration plants. During this period, market entry could begin through niche applications, such as high-value co-products, specialty biofuels for aviation, or localized off-grid energy solutions, allowing producers to establish revenue streams before entering the bulk fuel market (Uddin and Wang 2025).

Long-term (8–15 years): Large-scale, Cost-competitive Production

In the longer horizon, fully integrated microalgae biorefineries could operate at an industrial scale, producing biodiesel alongside valuable co-products such as omega-3 fatty acids, pigments, and biofertilizers. Continuous process automation, advanced strain engineering, and large volume cultivation systems, potentially located adjacent to CO_2-emitting industries and wastewater treatment facilities will enable cost reductions through economies of scale (Samoraj *et al.* 2024). With ongoing technological refinement, microalgae biodiesel could achieve parity with petroleum diesel on a per-litre basis, contributing meaningfully to global renewable energy portfolios and helping meet climate targets (Kanwal *et al.* 2025), as illustrated in Fig. 5.

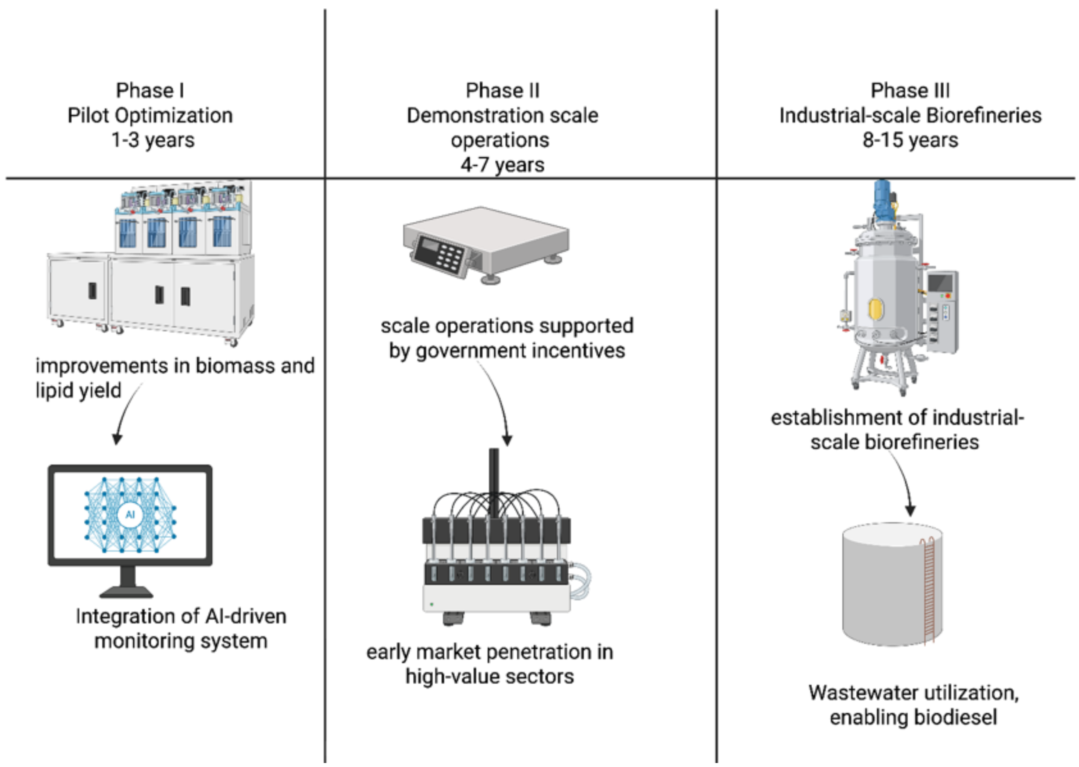

Fig. 5. Roadmap for the commercialization of microalgae-based biodiesel: Phase I (1 to 3 years) focuses on pilot optimization; Phase II (4 to 7 years) emphasizes demonstration-scale operations supported by government incentives, early market penetration; and Phase III (8 to 15 years) involves the establishment of industrial-scale biorefineries integrated with CO_2 capture and wastewater utilization.

The most suitable size scale for microalgae biodiesel production will likely vary depending on the availability of feedstock, transportation arrangements, and local infrastructure. The economies of scale, advanced process integration and efficient recovery of various high-value co-products of large centralized biorefinery can play a vital role in economy. But, if biomass can be produced in decentralized or regional production facilities in close proximity to wastewater treatment plants, industrial CO_2 sources, or sites with agricultural residues, transportation expenses can be decreased and resources can be used more efficiently. A distributed network of medium-scale integrated biorefineries may therefore be more viable and economically viable for future commercialization than very small facilities and/or highly centralized plants.

Table 5. Potential Co-Products from Microalgae Biorefineries and their Market Applications

Co-Product	Primary Applications	Estimated Market Value (USD/kg)	Economic Significance	References
Astaxanthin (*Haematococcus pluvialis*)	Nutraceuticals, anti-ageing cosmetics, and aquaculture pigmentation	2,000 to 7,000	High-value pigment; key profitability driver in biorefineries	Shah *et al.* 2016
β-Carotene (*Dunaliella salina*)	Natural food coloring, dietary supplements	300 to 500	Strong demand in food and pharmaceutical markets	Pourkarimi *et al.* 2020
EPA and DHA (*Nannochloropsis oculata*, *Phaeodactylum tricornutum*)	Functional foods, infant formulas, brain/cardiac health supplements	40 to 100	Replaces fish-derived omega-3 oils; expanding global demand	Liu *et al.* 2022)
Phycocyanin (*Arthrospira*/*Spirulina*)	Natural blue colorant, pharmaceuticals, antioxidant formulations	300 to 500	FDA-approved; preferred natural colorant worldwide	Minić *et al.* 2024
Protein-Rich Defatted Biomass	Poultry feed, aquaculture feed, pet food	1 to 5	Bulk ingredient reduces biorefinery waste	Nagappan *et al.* 2021
Biofertilizers (Residual Biomass Digestate)	Soil nutrient replenishment, organic farming	0.5 to 2	Supports circular nutrient recycling	Siol *et al.* 2023
Bioplastic Precursors (PHA, Algal Polymers)	Biodegradable packaging, eco-materials	3 to 6	Growing market for sustainable polymers	(Zhao *et al.* 2023)
Sulfated Polysaccharides	Antiviral, anticoagulant, and wound healing applications	250 to 800	Pharmaceutical-grade extracts with rising clinical interest	(Hans *et al.* 2021)
Chlorophyll and Chlorophyllin	Antioxidant nutraceuticals, detox supplements	80 to 200	Expanding health supplement market	Sun *et al.* 2024
Lutein (*Chlorella*, *Scenedesmus*)	Eye health supplements, pigment stabilizers	150 to 350	High-value carotenoid with increasing global demand	Erdoğan *et al.* 2022
Fucoxanthin (*Phaeodactylum tricornutum*)	Anti-obesity, anti-diabetic, anti-cancer supplements	200 to 500	One of the fastest-growing marine nutraceuticals	Gille *et al.* 2019
Hydrocarbon Fractions (*Botryococcus braunii*)	Aviation biofuels, specialty lubricants	3 to 8	Direct drop-in fuel precursor with high energy density	Jin *et al.* 2016
Exopolysaccharides (EPS)	Cosmetics, moisturizers, wound dressings	40 to 120	Premium bio-based polymer for dermatological products	Chinjoo and Golzary 2025

The economic viability of microalgae biodiesel production is strongly dependent on the co-recovery of high-value bioproducts alongside lipids, forming an integrated biorefinery approach. These co-products, including pigments, omega-3 fatty acids, proteins, polysaccharides, and biodegradable polymers possess substantial commercial value in nutraceutical, pharmaceutical, aquaculture, cosmetic, and sustainable materials markets. For instance, astaxanthin from *Haematococcus pluvialis* is valued at USD 2,000 to 7,000 per kg due to its antioxidant and anti-inflammatory properties, making it one of the key revenue drivers in algae-based production systems (Kumar *et al.* 2022). Similarly, β-carotene from *Dunaliella salina* and phycocyanin from *Arthrospira* are widely used in natural food coloring and cosmetics, demonstrating strong, stable market demand (Sathasivam *et al.* 2019). The integration of such co-product streams subsidizes biodiesel production costs and supports a zero-waste circular bioeconomy, positioning microalgae biorefineries as a competitive and sustainable model. Table 5 summarizes key co-products, their primary applications, estimated market values, and economic significance.

CONCLUSIONS

Microalgae represent one of the most promising and sustainable feedstocks for next-generation biodiesel production due to their superior lipid productivity, rapid growth rates, and ability to utilize non-arable land, industrial CO_2 emissions, and nutrient-rich wastewater streams. Over the past decade, significant advancements in strain engineering (*e.g.*, CRISPR/Cas9 and adaptive laboratory evolution), cultivation system optimization, and energy-efficient harvesting and extraction technologies have addressed major biological and technical bottlenecks that previously limited large-scale deployment. The integration of AI-driven process monitoring, machine learning-based growth prediction, and digital-twin-assisted system control is emerging as a transformative approach to enhance process stability, reduce operational costs, and accelerate commercialization readiness.

A key shift highlighted in this study is the transition from single-product biodiesel systems toward integrated microalgal biorefineries, where high-value co-products, such as astaxanthin, EPA/DHA, phycocyanin, pigments, biopolymers, and biofertilizers, play a critical role in improving economic feasibility. Coupling microalgae cultivation with industrial CO_2 capture and wastewater remediation further strengthens the sustainability profile, enabling circular resource utilization and reduced environmental footprint. However, challenges remain in achieving cost-competitive large-scale production, mainly regarding outdoor cultivation stability, seasonality, and energy inputs associated with dewatering and lipid recovery.

To achieve industrial scale and market competitiveness, coordinated efforts are vital across technology optimization, supportive policy frameworks, carbon credit mechanisms, and targeted market entry strategies. As outlined in the commercialization roadmap, pilot-scale validation, demonstration plant deployment, and progressive biorefinery integration are essential next steps. With continued innovation and policy alignment, microalgae-based biodiesel has the potential to become a key contributor to global decarbonization goals, advancing a sustainable and resilient renewable energy future.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Use of Generative AI

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, San Francisco, CA, USA) solely for language editing, including improvement of grammar, sentence clarity, readability, and overall presentation. The scientific design, experimental data, statistical analysis, interpretation of results, conclusions, and verification of references were conducted and critically reviewed by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the final manuscript.

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