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*Short Review*

# Metabolic Engineering and Environmental Optimization of Microalgae for Sustainable Biofuel Production: Advances, Strategies, and Prospects

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## Abstract

This review critically examines recent advances in algal biofuel research, focusing on the molecular and biochemical mechanisms of lipid biosynthesis and the application of metabolic engineering strategies to reprogram carbon flux. It further explores the integration of algal systems into sustainable biorefinery platforms, contextualizing algal bioenergy within global decarbonization frameworks and circular bioeconomy models. The review syntheses key findings from the literature, highlighting that microalgae exhibit superior photosynthetic efficiency and high areal productivity compared to terrestrial crops, while simultaneously sequestering $CO_2$. It discusses how genetic modifications targeting key enzymes (e.g., ACCase, DGAT, malic enzyme) have shown promise in enhancing triacylglycerol (TAG) accumulation, alongside the impact of CRISPR/Cas9-based genome editing and systems biology approaches on accelerating strain optimization. Beyond biodiesel, the valorization of algal biomass for bioethanol, biohydrogen, and high-value co-products is reviewed. Furthermore, the environmental benefits confirmed by Life Cycle Assessment (LCA) studies, particularly when coupled with wastewater remediation and flue-gas $CO_2$ utilization, are discussed. Nevertheless, techno-economic analyses highlighting persistent barriers, including energy-intensive harvesting, nutrient supply, and scalability constraints of different cultivation systems, are presented. The review concludes that algal biofuels represent a frontier in renewable energy science, bridging molecular biology, metabolic engineering, and process intensification. Despite current cost prohibitive challenges, the convergence of synthetic biology, bioprocess optimization, and integrated biorefineries positions algal biofuels as a viable contributor to carbon-neutral and potentially carbon-negative energy systems. Future breakthroughs in omics-driven metabolic modeling and closed-loop circular biotechnologies are identified as pivotal for their global deployment.



## 1. Introduction

Algal biofuels have been explored as a renewable and sustainable alternative to fossil-derived fuels for more than seven decades, with seminal studies dating back to Oswald's pioneering work on microalgal cultivation in the 1940s [1]. Early investigations primarily focused on the basic physiology of microalgae, including growth kinetics, photosynthetic efficiency, and nutrient requirements, laying the foundation for subsequent industrial applications [2-4]. By the 1980s, research had shifted toward quantifying lipid accumulation under varying environmental conditions, emphasizing the influence of light intensity, temperature, and nutrient limitation on triacylglycerol (TAG) biosynthesis [5,6].

Microalgae are unique among photosynthetic organisms because of their high areal productivity, rapid doubling times, and ability to sequester $CO_2$ from the atmosphere, which positions them as a promising platform for carbon-neutral energy production [7]. Lipid biosynthesis in microalgae involves Acetyl-CoA carboxylation via the fatty acid synthase (FAS) complex, followed by elongation and desaturation steps cata-

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lyzed by key enzymes such as Acetyl-CoA carboxylase (ACCase), malic enzyme, and diacylglycerol acyltransferase (DGAT) [8,9]. These biochemical pathways have been extensively characterized, providing targets for metabolic engineering aimed at enhancing TAG accumulation under controlled conditions [10,11].

Over the past two decades, genetic engineering strategies, including CRISPR/Cas9-based genome editing, RNA interference, and systems biology approaches, have enabled precise manipulation of carbon flux and lipid metabolism [11,12]. These interventions have been reported to increase lipid yield and productivity, while also improving our understanding of the regulatory networks governing algal lipid biosynthesis [11,12]..

In addition, life cycle assessment (LCA) has become an essential framework for evaluating the environmental sustainability of algal biofuel production. However, the overall feasibility of microalgal biofuels remains constrained by challenges related to cultivation efficiency, harvesting costs, downstream processing, and scalability. Therefore, a comprehensive assessment of recent advances in metabolic engineering, cultivation strategies, and sustainability metrics is necessary to clarify the prospects and limitations of algal biofuel development. This review critically examines the progress made in microalgal biofuel research, with particular emphasis on lipid biosynthesis, genetic engineering, and environmental performance [11,12].

## 2. Methodological Approaches in Microalgal Biofuel Research

Previous research on microalgal biofuel production has employed diverse experimental and analytical approaches, which can be broadly categorized into cultivation optimization, lipid extraction, genetic engineering, and life-cycle assessment. This section provides an overview of the methods and strategies reported in the literature, highlighting common trends, representative species, and key findings from previous studies rather than describing new experimental work [13,14].

### *2-1. Cultivation and Growth Optimization*

Studies have commonly focused on model microalgal species such as *Chlorella vulgaris*, *Nannochloropsis gaditana*, and *Scenedesmus obliquus*, selected for their high lipid content and adaptability to variable environmental conditions [5,15,16]. Growth performance is influenced by light intensity, photoperiod, temperature, nutrient supply, and $CO_2$ availability. Both open raceway ponds and closed photobioreactors have been investigated to compare productivity and cost-efficiency. Nutrient limitation, especially nitrogen or phosphorus deprivation, is widely reported to trigger lipid accumulation through metabolic stress responses [17,18].

### *2-2. Lipid Extraction and Analysis*

Lipid extraction is a key step in evaluating biofuel potential. Commonly reported methods include solvent-based extraction using the Bligh and Dyer technique, Soxhlet extraction, and supercritical $CO_2$ extraction. These approaches differ in efficiency, solvent consumption, and environmental impact. Lipid yields are typically reported as a percentage of dry biomass, and fatty acid profiles are characterized by conversion to fatty acid methyl esters (FAMEs) followed by gas chromatography–mass spectrometry (GC–MS). Reported trends indicate that cultivation stress markedly influences lipid composition, with higher proportions of saturated and monounsaturated fatty acids observed under nutrient limitation conditions (Table 1) [19,20].

### *2-3. Genetic and Metabolic Engineering Strategies*

Numerous studies have applied genetic tools to enhance microalgal lipid biosynthesis.

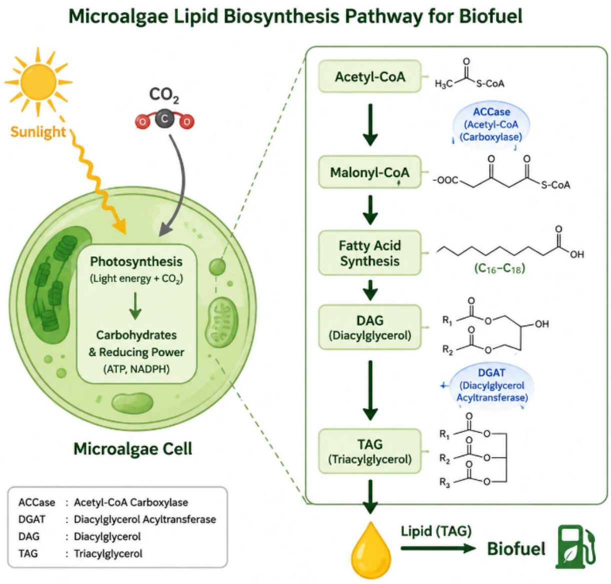


**Fig. 1.** Schematic overview of lipid biosynthesis and triacylglycerol (TAG) accumulation pathways in microalgae, highlighting the roles of acetyl-CoA, ACCase, fatty acid synthesis, and DGAT in biofuel precursor formation.

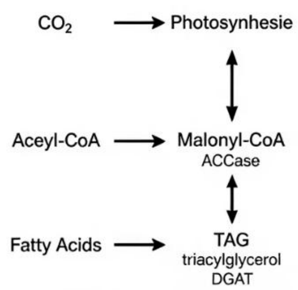


**Fig. 2.** Comparison of open raceway ponds and closed photobioreactors for microalgae cultivation in terms of cost, contamination risk, environmental control, and biomass productivity.

**Table 1.** Representative comparative summary of reported extraction approaches

| Extraction Method | Reported Advantages | Limitations | Ref |
|---|---|---|---|
| Bligh & Dyer | High efficiency; simple procedure | Uses chlorinated solvents | Hu et al., 2008 |
| Soxhlet | Suitable for larger biomass samples | High energy consumption | Chisti, 2007 |
| Supercritical $CO_2$ | Non-toxic, solvent-free method | High operational cost | Li et al., 2020 |

Reported approaches include CRISPR/Cas9 genome editing, RNA interference, and overexpression of key metabolic enzymes. Target genes such as *Acetyl-CoA carboxylase* (*ACCase*), *diacylglycerol acyltransferase* (*DGAT*), and *malic enzyme* have been shown to play pivotal roles in triacylglycerol synthesis and carbon flux regulation. Omics-based studies and metabolic flux analyses further clarify how carbon partitioning between lipids, carbohydrates, and proteins can be balanced to improve overall biofuel productivity [21,22]. (Table 2).

### *2-4. Life Cycle Assessment and Sustainability*

In recent years, life cycle assessment (LCA) has become essential for evaluating the sustainability of microalgal biofuel systems. Studies typically follow ISO 14040 standards and consider factors such as energy demand, nutrient usage, $CO_2$ fixation, and greenhouse gas emissions throughout the production stages. Comparative LCA analyses reveal that cultivation, harvesting, and lipid extraction are major contributors to environmental impact. Integration of wastewater treatment, $CO_2$ capture, and co-production of valuable bioproducts is widely recommended to improve overall process viability [23,24].

Reported LCAs consistently highlight the need for multifunctional biorefineries that combine fuel and co-product generation, thus improving economic and environmental performance [25,26].

## 3. Results and Discussion

### *3-1. Growth Performance: Insights from Previous Studies*

A wide range of studies have evaluated the growth behaviour of microalgal species such as *Chlorella vulgaris*, *Nannochloropsis gaditana*, and *Scenedesmus obliquus* under different cultivation systems, including open ponds and closed photobioreactors. Reported findings consistently indicate that growth performance is strongly dependent on light regime, temperature control, aeration, and nutrient availability [27, 28]. Photobioreactors generally support higher biomass productivity due to improved control of environmental parameters, whereas open ponds remain economically attractive for large-scale applications despite their exposure to environmental fluctuations [29,30].

Nutrient stress, particularly nitrogen deprivation, has been widely documented to induce metabolic shifts that initially slow biomass accumulation but subsequently activate lipid biosynthesis pathways. These physiological responses have been observed across many species and represent a well-established strategy for enhancing biofuel-relevant metabolite production [31,32].

### *3-2. Lipid Accumulation and Profiling*

Variations in total lipid content and fatty acid composition among microalgal species have been extensively reported in the literature. Under nutrient-replete conditions, many green microalgae accumulate moderate lipid levels, while nutrient-limited cultures typically exhibit substantial increases in triacylglycerol (TAG) production [8,33]. Species such as Nannochloropsis are frequently highlighted for their high proportions of long-chain polyunsaturated fatty acids (PUFAs), including EPA, which provide added value for biorefinery applications [34].

Fatty acid methyl ester (FAME) analyses reported in previous studies show that stress conditions tend to favor the accumulation of saturated and monounsaturated fatty acids, which are desirable for biodiesel due to improved oxidative stability and suitable fuel properties. These trends align with the broader understanding of microalgal metabolic adaptation to environmental stressors [35].

### *3-3. Genetic Modification Effects*

A growing body of research has demonstrated the potential of metabolic engineering to enhance lipid biosynthesis in microalgae. *CRISPR/Cas9*-mediated genome editing, overexpression of key enzymes such as diacylglycerol *acyltransferase* (*DGAT*) and acetyl-CoA carboxylase (*ACCase*), and knockdown of competing pathways have been reported to redirect carbon flux toward lipid production [12,36].

Transcriptomic and metabolomic analyses in these studies reveal that engineered strains often exhibit improved accumulation of metabolic intermediates associated with fatty acid biosynthesis. Genetically optimized strains of Nannochloropsis and Chlorella have shown enhanced production of high-value lipids under controlled environmental conditions, reinforcing the feasibility of integrating synthetic biology tools into microalgal biofuel strategies [37].

### *3-4. Metabolic Flux Analysis*

Metabolic flux analysis using approaches such as $^{13}C$-labeling, NMR spectroscopy, and computational flux balance modeling has been widely applied to elucidate carbon distribution between lipid, carbohydrate, and protein pathways. These studies report that nutrient stress commonly down-regulates glycolysis while enhancing TCA cycle flux toward lipid precursors, explaining the observed increases in TAG accumulation under limiting conditions [38].

Flux modeling has also identified bottlenecks in acetyl-CoA and malonyl-CoA availability, with several studies showing that targeted overexpression of associated enzymes can partially alleviate these constraints. Such mechanistic insights support the development of rational metabolic engineering designs for improving biofuel productivity [39].

**Table 2.** Summary of representative genetic targets reported in recent studies.

| Target Enzyme | Genetic Strategy | Reported Outcome | Ref. |
|---|---|---|---|
| ACCase | Overexpression | Increased lipid synthesis | Ng et al., 2017 |
| DGAT | CRISPR-mediated activation | Enhanced triacylglycerol accumulation | Li-Beisson et al., 2015 |
| Malic enzyme | Co-expression with ATP citrate lyase | Improved NADPH supply | Sun et al., 2019 |

### *3-5. Comparison with Previous Studies and Broader Implications*

Across the literature, consistent trends emerge regarding the influence of environmental factors, genetic engineering, and metabolic regulation on microalgal lipid productivity [5,8]. Integrating these findings with advances in photobioreactor design, flux analysis, and life cycle assessment underscores the potential for developing sustainable and economically feasible microalgal biofuel systems.

Enhanced lipid production in engineered strains, combined with optimized cultivation and environmental control, aligns with the goals of modern biorefineries. Co-production of high-value metabolites such as pigments, antioxidants, and PUFAs further strengthens the economic viability of microalgal platforms. Collectively, these insights highlight the importance of coupling strain selection, cultivation optimization, metabolic engineering, and sustainability assessment to maximize both biomass yield and lipid productivity in future microalgal biofuel applications [40].

## 4. Conclusion

This review provides an integrated synthesis of current knowledge on microalgal growth performance, lipid biosynthesis, metabolic engineering strategies, and environmental adaptation mechanisms relevant to sustainable biofuel development. Studies across diverse species, including *Chlorella vulgaris*, *Nannochloropsis gaditana*, and *Scenedesmus obliquus*, consistently indicate that light intensity, nutrient availability, and cultivation system design play central roles in determining both biomass productivity and lipid accumulation [41].

Previous research has demonstrated that genetic and metabolic engineering approaches—such as overexpression of lipid-biosynthetic enzymes, suppression of competing pathways, and CRISPR-based genome editing—can enhance carbon flux toward triacylglycerol formation. These strategies, supported by molecular analyses and metabolomic profiling reported in the literature, reveal the potential of targeted interventions to improve lipid yields in various microalgal species [10].

Metabolic flux studies further highlight the dynamic redistribution of carbon under nutrient limitation and environmental stress, providing valuable mechanistic insights into the regulation of central metabolic pathways. Collectively, these findings emphasize the necessity of integrating genetic optimization with environmental control to maximize lipid productivity [27].

Future research should prioritize the development of advanced cultivation platforms, large-scale photobioreactor optimization, nutrient recycling through industrial waste stream integration, and techno-economic evaluation to support scalable biofuel production. Continued integration of systems biology, metabolic engineering, and sustainability assessment will be essential for realizing the full potential of microalgae as a viable and environmentally responsible bioenergy resource [32].

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