

Review Article

Strain Improvement Through Genetic Engineering and Synthetic Biology for the Creation of Microalgae with Enhanced Lipid Accumulation, Stress Tolerance, and Production of High-value

Alebachew Molla^{1,*} , Gedif Meseret² 

¹Department of Biotechnology, Wolaita Sodo University, Wolaita, Ethiopia

²Department of Biology, Wolaita Sodo University, Wolaita, Ethiopia

Abstract

Microalgae are microscopic, unicellular or simple colony-forming photosynthetic organisms found mainly in freshwater and marine environments. Unlike multicellular macroalgae, microalgae lack complex structures such as roots, stems, and leaves. They perform photosynthesis using pigments like chlorophyll, producing oxygen and serving as primary producers in aquatic ecosystems. Microalgae have emerged as a promising platform for sustainable production of biofuels, high-value biochemicals, and nutraceuticals due to their rapid growth and ability to accumulate lipids. However, natural strains often exhibit limitations in lipid yield, stress tolerance, and metabolic versatility that restrict their industrial application. Strain improvement of microalgae through genetic engineering and synthetic biology involves precise modification of genetic and metabolic pathways to enhance desirable traits such as lipid accumulation, stress tolerance, and production of high-value compounds. This review highlights recent advances in genetic engineering and synthetic biology approaches aimed at enhancing microalgal strains for improved lipid accumulation, stress tolerance, and biosynthesis of high-value compounds. Emphasis is placed on novel transformation methods, genome editing tools such as CRISPR/Cas9, metabolic pathway optimization, and transcriptional regulation strategies. We discuss challenges in strain development, including stability and scalability, as well as future perspectives integrating multi-omics and systems biology to accelerate industrial applications of microalgae for sustainable biofuel and bioproducts production.

Keywords

Microalgae, Strain Improvement, Genetic Engineering, Synthetic Biology, Lipid Accumulation, Stress Tolerance, Metabolic Engineering and CRISPR/Cas9

1. Introduction

Microalgae have emerged as revolutionary sustainable biofactories that represent a paradigm shift in biotechnology

applications [1]. These microscopic photosynthetic organisms, encompassing both prokaryotic cyanobacteria and eukaryotic

*Corresponding author: alexbm2127@gmail.com (Alebachew Molla)

Received: 23 July 2025; **Accepted:** 5 August 2025; **Published:** 27 August 2025



Copyright: © The Author(s), 2025. Published by Science Publishing Group. This is an **Open Access** article, distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

microalgae, have gained recognition for their exceptional ability to serve as living production systems for valuable biomolecules [2, 3]. The concept of microalgae as biofactories stems from their unique characteristics: they are single-cell packages of bioactive molecules that can be cultured to produce high levels of biomass with remarkable efficiency [4].

The evolution of microalgae biotechnology has been fundamentally driven by their dual potential to mitigate atmospheric carbon dioxide and produce a great diversity of high-value compounds [5]. Unlike traditional agricultural crops, microalgae offer several distinctive advantages that position them as next-generation biofactories. They demonstrate higher photosynthetic efficiency than land plants, with carbon fixation rates reported to be 10-50 times higher than terrestrial vegetation [6]. This exceptional photosynthetic capacity allows them to convert CO₂ and nutrients into biomass with unprecedented efficiency [2].

The transition from viewing microalgae as simple organisms to recognizing them as sophisticated biofactories has been gradual but transformative. Early applications focused primarily on whole biomass utilization, but contemporary biotechnology has revealed their potential for producing a vast array of products including foodstuffs, industrial chemicals, compounds with therapeutic applications, and bioremediation solutions [4]. This evolution represents a fundamental shift from exploiting natural microalgal populations to engineering them as programmable cell factories capable of producing designer molecules [7].

The development of microalgae strains with superior traits is critical for realizing their commercial potential and advancing their applications in biofuels, nutraceuticals, and bioproducts [8]. Traditional cultivation using wild-type strains often falls short in meeting industrial demands due to biological and environmental constraints. Consequently, strain improvement has become a cornerstone paradigm in microalgae biotechnology, focusing on tailoring biological characteristics to enhance productivity, stress resilience, and target compound synthesis [9].

This review article aims to provide a comprehensive and up-to-date synthesis of strain improvement strategies for microalgae through genetic engineering and synthetic biology, focusing on enhancing key industrial traits including lipid accumulation, stress tolerance, and production of high-value compounds. By addressing these objectives, the review seeks to offer an authoritative resource for researchers and industry stakeholders advancing sustainable microalgal biotechnologies with practical commercial impact. This aligns with ongoing interests in leveraging microalgae for sustainable applications.

2. Microalgae Biology and Metabolic Foundations

2.1. Cellular Architecture and Metabolism

Microalgae are unicellular photosynthetic organisms equipped with specialized organelles primarily chloroplasts

where photosynthesis takes place [10, 11]. Photosynthesis consists of two major stages: the light reactions and the dark (Calvin) reactions. The light reactions occur in thylakoid membranes, where two photosystems (PSI and PSII) capture and convert light energy into chemical energy (ATP and NADPH), concurrently splitting water molecules to release oxygen [10].

To adapt to varying light intensities, microalgae regulate their photosynthetic apparatus through both short-term and long-term mechanisms. Under prolonged exposure to bright light, they reduce chlorophyll content and increase photo-protective carotenoids and xanthophylls to prevent photo-damage [10, 12]. High-density cultures in photobioreactors often experience self-shading, which limits light availability and affects photosynthetic efficiency; genetic modifications targeting light-harvesting complexes can improve light utilization in such contexts [13].

Microalgae enhance photosynthesis efficiency through a CO₂⁻ concentrating mechanism (CCM), which actively transports inorganic carbon (CO₂ or HCO₃⁻) into the cells to concentrate CO₂ near the enzyme RuBisCO and suppress photorespiration, enhancing carbon fixation even under low CO₂ conditions [10]. Structural adaptations such as carboxysomes (in cyanobacteria) compartmentalize RuBisCO and carbonic anhydrase enzymes for efficient inorganic carbon conversion [10].

Beyond chloroplasts, emerging studies reveal functional interactions between mitochondria and chloroplasts that coordinate energy metabolism, optimize biomass production, and influence synthesis of pigments, lipids, and other metabolites [14]. Light-induced changes increase mitochondrial abundance and organelle contacts, underscoring the integrated subcellular energy management in microalgae.

2.2. High-Value Compound Biosynthesis

Microalgae synthesize a diverse range of carotenoids, which are pigments with essential roles in photosynthesis and cellular protection, as well as high commercial value for nutraceutical, pharmaceutical, and cosmetic industries [15]. The carotenoid biosynthesis pathway primarily proceeds via the methylerythritol phosphate (MEP) pathway in plastids, where the five-carbon isoprenoid precursors isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP) are synthesized [16, 17].

Microalgae are prolific producers of high-value polyunsaturated fatty acids (PUFAs), especially omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [18]. These PUFAs are synthesized through the elongation and desaturation of shorter fatty acid precursors via enzymes like desaturases and elongases. PUFA biosynthesis occurs primarily in plastids and the endoplasmic reticulum, where fatty acid synthase generates saturated acyl chains that are subsequently modified [19]. The highly unsaturated nature of PUFAs renders them essential nutritional

supplements for human and animal health, with microalgal sources offering sustainable alternatives to fish oils. Genetic engineering strategies targeting desaturase gene expression and pathway flux have been employed to increase PUFA content, complementing cultivation conditions optimized to induce PUFA accumulation [20].

Microalgal biomass is rich in proteins and essential amino acids, making it valuable as a nutritional supplement and feedstock [21]. Microalgae can synthesize all essential amino acids, with profiles comparable to conventional protein sources. The protein biosynthesis in microalgae involves transcriptional and translational machinery similar to higher plants and microorganisms [22]. Genetic modifications have aimed to enhance expression of specific proteins or bioactive peptides with antimicrobial or antioxidative properties. Moreover, microalgae's ability to produce bioactive amino acid derivatives and peptides adds functional value to the biomass beyond simple nutrition [23].

3. Genetic Engineering Tools and Technologies

3.1. Overexpression Strategies

Overexpression strategies play a pivotal role in microalgal genetic engineering by enabling the enhancement of desired metabolic pathways and improving the accumulation of valuable compounds [24]. A key factor for successful overexpression is the choice of robust promoter systems that drive high levels of transgene expression. Typically, endogenous promoters derived from highly expressed genes such as *rbcl* (encoding the large subunit of RuBisCO), *psbA* (photosystem II protein D1), and *atpA* (ATP synthase subunit) are employed to ensure strong, constitutive, or inducible expression within microalgal cells [25, 26]. The inclusion of effective 5' untranslated regions (UTRs) further enhances transcript stability and translation efficiency, resulting in improved protein accumulation [27]. In recent studies, novel promoters like the extrinsic protein of PSII (EPPSII) promoter from *Nannochloropsis gaditana* have demonstrated significantly higher expression capabilities compared to conventional promoters, underscoring the importance of promoter selection and optimization.

In addition to promoter choice, metabolic pathway enhancement through targeted gene overexpression focuses on increasing the activity of rate-limiting enzymes within lipid biosynthesis and other valuable metabolite pathways [28]. Overexpressing genes encoding key enzymes such as acetyl-CoA carboxylase (ACC) and acyl carrier protein (ACP) has been shown to increase the flux of metabolites directed toward fatty acid synthesis, thereby boosting lipid accumulation in microalgae [29]. This approach is often complemented by simultaneous suppression of competing pathways to further channel carbon flux toward desired products. Moreover,

the overexpression of specific transcription factors can orchestrate broader metabolic changes by upregulating multiple genes within biosynthetic networks, providing a more coordinated and effective enhancement of metabolic output [30].

3.2. Gene Silencing Technologies

Gene silencing technologies in microalgae primarily leverage RNA interference (RNAi) mechanisms, which enable specific downregulation of target gene expression through sequence-specific mRNA degradation or translational repression [31]. Studies have demonstrated that many microalgal species, including *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Volvox carteri*, and the diatom *Phaeodactylum tricornutum*, possess functional RNAi machinery capable of mediating gene silencing via introduction of double-stranded RNA (dsRNA) or inverted repeat (IR) transgenes [32–34]. This approach has become a customary tool for the functional characterization of genes and as a strategy for metabolic engineering to modulate pathways of interest.

Beyond RNAi, microalgae exhibit additional gene silencing layers, including chromatin modifications such as histone methylation and DNA methylation, which contribute to transposon and transgene repression, although their roles in regulating native gene expression are less understood [35]. RNA-mediated silencing in microalgae operates via both mRNA degradation and translational inhibition, as exemplified in *P. tricornutum*, where gene silencing reduced protein levels without necessarily decreasing mRNA abundance [36]. This dual functionality enhances the versatility of RNAi-based tools.

3.3. Transformation Methods

Transformation methods are fundamental techniques used to introduce foreign DNA into microalgal cells, enabling genetic modification for strain improvement [37]. Among the most widely employed methods are electroporation and biolistic transformation, each offering distinct advantages depending on the species and desired application. Electroporation involves applying short electrical pulses to create transient pores in the cell membrane, allowing exogenous DNA to enter the cytoplasm [38, 39]. This method is favored for its relative simplicity, efficiency, and ability to transform both nuclear and organellar genomes in various microalgae such as *Chlamydomonas reinhardtii* [40]. Biolistic transformation, also known as particle bombardment, uses high-velocity microprojectiles (typically gold or tungsten particles) coated with DNA to physically penetrate cell walls and membranes, delivering DNA directly into the cells [41]. This approach is particularly effective for species with rigid or thick cell walls that are less amenable to electroporation and allows for transformation of chloroplast genomes with high efficiency.

Another valuable method is *Agrobacterium*-mediated

transformation, which utilizes the natural genetic transfer capability of *Agrobacterium tumefaciens* to deliver T-DNA harboring the gene of interest into algal cells [42]. Although originally developed for plants, this technique has been adapted for certain microalgal species, providing an alternative transformation system that can achieve stable nuclear integration with lower copy number insertions and minimal rearrangements compared to physical methods.

Optimization of microparticle bombardment (biolistic transformation) remains a critical focus to maximize transformation efficiency and minimize cellular damage [43]. Factors such as particle size, DNA coating concentration, helium pressure during bombardment, target distance, and vacuum pressure have been systematically refined to improve DNA delivery and cell survival rates [44]. Additionally, using selectable marker genes and optimizing regeneration protocols enhance the recovery of stable transformants.

3.4. CRISPR/Cas9 Systems

The advancement of CRISPR/Cas9 genome editing technology has revolutionized genetic engineering in microalgae, enabling precise and efficient manipulation of their genomes [45]. Adapting CRISPR/Cas9 systems to various microalgal species has involved optimizing delivery methods, expression constructs, and repair pathways tailored to their unique cellular and genomic contexts [45]. For example, in model species such as *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*, CRISPR/Cas9 has been successfully employed to generate targeted gene knockouts through the induction of double-strand breaks followed by error-prone non-homologous end joining (NHEJ), resulting in gene disruption. Furthermore, homology-directed repair (HDR) pathways have been harnessed to introduce precise genetic modifications or knockins, such as insertion of reporter genes or correction of mutations, although HDR efficiency remains relatively low in many microalgae and is an area of active optimization [44].

In recent years, multiplexed genome editing approaches have expanded the capabilities of CRISPR/Cas9 in microalgae, allowing simultaneous targeting of multiple genes or genomic loci within a single transformation event [46]. This multiplexing is achieved by expressing multiple guide RNAs (gRNAs) alongside Cas9, enabling the coordinated modification of complex metabolic pathways or gene families to accelerate strain improvement [47]. Additionally, the development of inducible or tissue-specific promoters and engineered Cas9 variants with altered PAM specificities has further enhanced the versatility and specificity of genome editing in microalgal systems.

Overall, the integration of CRISPR/Cas9 technologies into microalgal biotechnology provides a powerful platform for rapid functional genomics studies and rational design of strains with improved traits such as enhanced lipid production, stress tolerance, and biosynthesis of valuable compounds [45].

Ongoing research focuses on improving editing efficiencies, minimizing off-target effects, and developing novel CRISPR-based tools tailored to the unique biology of diverse microalgal species [48].

3.5. Base Editing and Prime Editing

Base editing and prime editing represent cutting-edge advancements in genome engineering that enable precise nucleotide modifications in microalgae without introducing double-strand DNA breaks, thereby minimizing cellular stress and enhancing editing accuracy [49]. Base editors typically consist of a catalytically impaired Cas protein fused to a deaminase enzyme, allowing for the direct conversion of specific base pairs for example, cytosine to thymine or adenine to guanine at targeted genomic loci [50]. This enables precise correction of point mutations or introduction of beneficial single-nucleotide changes critical for fine-tuning gene function. Prime editing, a more recent innovation, couples a reverse transcriptase to a Cas9 nickase and a specialized prime editing guide RNA (pegRNA), facilitating versatile edits including insertions, deletions, and all twelve types of base substitutions with high specificity [51].

One of the key advantages of these technologies over traditional CRISPR/Cas9 approaches is their reduced off-target effects since they do not rely on the error-prone repair of DNA double-strand breaks, which often lead to unintended mutations or chromosomal rearrangements [52]. This precision is particularly valuable in microalgal metabolic engineering, where even single-nucleotide changes can dramatically impact enzyme activity, regulatory elements, or protein stability thereby affecting metabolite fluxes and product yields [53].

In microalgae, base and prime editing hold great promise for metabolic pathway optimization, such as modifying key enzymes in lipid biosynthesis to increase lipid content or altering regulatory sequences to enhance stress response pathways [49]. By enabling subtle and predictable genetic changes with minimal collateral damage, these tools facilitate the development of improved microalgal strains with enhanced production of biofuels, high-value nutraceuticals, and other industrially relevant compounds [54]. As delivery methods and editing efficiencies continue to improve, base and prime editing are expected to become indispensable components of the synthetic biology toolkit for precise and safe microalgal genome engineering [55].

3.6. Transcriptional Regulation

Transcriptional regulation in microalgae has been significantly advanced by CRISPR-based tools such as CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) systems, which enable targeted upregulation or downregulation of gene expression without altering the DNA sequence [45]. These systems use an enzymatically inactive Cas9 (dCas9) fused to transcriptional activators (like VP64) or

repressors (such as the KRAB domain) to modulate transcription by guiding dCas9 to specific promoter or coding regions via single guide RNAs (sgRNAs) [56]. For instance, in *Chlorella sorokiniana*, CRISPRa enhanced protein content by up to 60%, while CRISPRi increased protein levels to 65% and lipid accumulation, demonstrating practical potential for metabolic reprogramming in microalgae biorefineries [57]. Moreover, multiplexed targeting enabled by Cas12a systems allows simultaneous regulation of multiple genes, expanding the scope of transcriptional control for complex metabolic pathways [58].

Beyond CRISPR-based modulation, epigenetic modifications such as DNA methylation and histone modifications serve as additional layers of gene regulation by altering chromatin accessibility and transcriptional activity without changing the underlying DNA [59, 60]. Although less explored in microalgae, epigenetic marks can provide stable and heritable means of transcriptional control, offering promising avenues to complement CRISPRa/i systems for fine-tuning gene expression [61].

Temporal and spatial control of gene expression further enhances regulatory precision by restricting transcriptional activity to specific developmental stages or cellular compartments [62]. This can be achieved by using inducible or tissue-specific promoters, or coupling CRISPR systems with chemically or light-inducible elements, allowing dynamic, reversible, and localized gene regulation [63]. Such sophisticated control is essential for optimizing metabolic flux in microalgae, avoiding growth penalties, and improving product yields under industrial cultivation conditions.

4. Synthetic Biology Approaches

4.1. Modular Design Principles

Modular design principles in microalgal synthetic biology draw inspiration from proven engineering concepts to create flexible, efficient, and reusable biological systems [64]. Central to this approach is the use of standardized biological parts well-characterized genetic elements such as promoters, terminators, ribosome binding sites, and regulatory sequences that can be assembled predictably and interchangeably [65]. These standardized parts provide a common language and toolkit enabling the construction of complex genetic circuits with reliable function, facilitating the Design-Build-Test (DBT) cycle essential for rapid strain development.

In pathway engineering, modularity is applied by compartmentalizing biosynthetic pathways into discrete, exchangeable units or modules [66]. Each module contains sets of auxiliary enzymes and regulatory elements optimized for the production of a specific metabolite or phenotype. These modules interface seamlessly with a core metabolic "chassis," allowing for plug-and-play assembly and combinatorial testing of pathways [67]. This strategy supports rapid discovery

and optimization of metabolic fluxes toward target molecules like lipids, carotenoids, or other high-value compounds, and enables tailoring biosynthetic routes to different host species or environmental conditions [68].

Chassis engineering refers to the design and construction of minimal or optimized host microalgal strains that provide a robust and stable platform for module integration [69]. The chassis contains essential core metabolic phenotypes and cellular machinery necessary for growth and precursor supply, serving as a consistent foundation upon which diverse production modules can be grafted [33]. By decoupling core metabolism from production functions, chassis engineering enhances strain predictability, reduces metabolic burden, and simplifies strain engineering workflows [70]. This modular architecture mimics the principles of traditional engineering systems, allowing for scalable, reproducible, and adaptable development of microalgal biofactories.

4.2. Systems-Level Engineering

Systems-level engineering in microalgae involves a holistic approach to understanding and manipulating cellular metabolism by integrating comprehensive biological data and computational modeling [71]. A fundamental component of this strategy is metabolic network reconstruction, which entails assembling detailed genome-scale models that map all known metabolic reactions and their associated genes within a microalgal cell [71]. These models enable researchers to simulate and predict metabolic fluxes, identify bottlenecks, and uncover potential targets for genetic intervention. By leveraging tools such as flux balance analysis (FBA), metabolic network models facilitate rational design of engineered strains with optimized pathways for enhanced lipid accumulation, improved stress responses, or increased synthesis of valuable metabolites [72].

Complementing metabolic modeling, multi-omics integration combines data sets from genomics, transcriptomics, proteomics, and metabolomics to provide a comprehensive and dynamic picture of cellular function [73]. By analyzing these layers simultaneously, scientists can correlate gene expression patterns with protein abundances and metabolite concentrations, revealing regulatory mechanisms, metabolic states, and environmental responses with high resolution [44, 74]. This systems-wide insight allows the identification of key regulatory nodes and metabolic hubs that might be invisible when studying single omics layers in isolation.

5. Lipid Accumulation Enhancement

5.1. Metabolic Engineering Strategies

Metabolic engineering strategies in microalgae focus on enhancing lipid production by targeting key steps in fatty acid synthesis, triacylglycerol (TAG) assembly, and minimizing

competing metabolic pathways [6]. One primary approach involves fatty acid synthesis enhancement, where overexpression of pivotal enzymes such as acetyl-CoA carboxylase (ACC), malonyl-CoA acyl carrier protein transacylase (MAT), and fatty acid synthase (FAS) increases the flux of precursors toward fatty acid chains [75]. Elevating the activity of these rate-limiting enzymes boosts the availability of fatty acids, thereby promoting overall lipid biosynthesis [76].

Following fatty acid generation, TAG assembly optimization is crucial, as TAGs constitute the main storage form of lipids in microalgae and are the preferred substrates for biofuel production [6]. Enhancing the expression or activity of enzymes involved in this process such as glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), and diacylglycerol acyltransferase (DGAT) facilitates efficient esterification of fatty acids onto glycerol backbones, improving TAG accumulation [77]. Co-overexpression of these enzymes, as shown in oleaginous species like *Neochloris oleoabundans*, has led to significant increases in lipid content [78].

A complementary strategy is competing pathway inhibition, aiming to reroute carbon flux toward lipid production by suppressing pathways that divert substrates away from fatty acid and TAG biosynthesis [79]. For example, downregulating starch biosynthesis or β -oxidation pathways reduces carbohydrate accumulation and lipid catabolism, respectively, favoring lipid storage. This targeted repression can be achieved through gene knockdown, RNA interference, or gene editing techniques, enhancing lipid yields by channeling metabolic resources into oil biosynthesis [31].

5.2. Regulatory Network Manipulation

Regulatory network manipulation in microalgae involves fine-tuning gene expression at multiple levels to optimize metabolic pathways for enhanced production of lipids and other valuable compounds [80]. At the transcriptional control level, strategies focus on modulating the activity of transcription factors (TFs) and promoter elements that govern the expression of key biosynthetic genes [81]. By overexpressing or engineering TFs that activate lipid biosynthesis-related gene clusters, researchers can coordinately upregulate multiple enzymes within a pathway, resulting in amplified metabolic flux toward desired products [82]. Additionally, the use of stress-responsive or inducible promoters allows dynamic control of gene expression in response to environmental cues, enabling microalgae to balance growth and metabolite accumulation efficiently [83, 84]. Synthetic biology tools such as CRISPR activation (CRISPRa) provide programmable means to precisely enhance transcription of target genes without altering the genome sequence, offering further flexibility in transcriptional regulation [57].

In parallel, post-transcriptional regulation mechanisms including RNA stability, small RNA-mediated gene silencing, and riboswitch-mediated control contribute to the fine-tuning

of gene expression after transcription [85]. MicroRNAs (miRNAs) and small interfering RNAs (siRNAs) can target mRNAs of competing pathways or negative regulators, reducing their translation and thereby redirecting resources toward lipid synthesis [86]. Riboswitches and RNA-binding proteins modulate mRNA stability and translation efficiency in response to intracellular metabolite levels, providing feedback control that adapts metabolic activity to cellular states [44]. Collectively, these post-transcriptional processes add an additional layer of precision, allowing rapid and reversible adjustments to gene expression in response to internal and external signals.

6. Stress Tolerance Engineering

6.1. Abiotic Stress Resistance

Stress tolerance engineering in microalgae focuses on enhancing their ability to withstand various abiotic stresses, which commonly limit productivity in large-scale cultivation [83]. Temperature tolerance is crucial, as microalgae often face fluctuating thermal environments; genetic adaptations such as expression of heat shock proteins and membrane lipid remodeling help maintain cellular integrity and photosynthetic efficiency under heat or cold stress [87]. For example, overexpression of chaperones and genes involved in membrane fluidity adjustment improve thermal resilience, enabling sustained growth and metabolite production [88].

Salinity and osmotic stress pose significant challenges, especially in open pond systems exposed to varying salt concentrations [83]. Microalgae mitigate osmotic stress by accumulating compatible solutes such as proline and glycine betaine, adjusting intracellular ion transporters, and remodeling cell walls to maintain turgor pressure and enzyme function [89]. Genetic engineering strategies enhancing these osmoprotectant pathways have shown promise in improving salt tolerance, thereby expanding microalgae cultivation to marginal water resources [90].

Light stress management addresses issues arising from excessive or fluctuating light intensities that can cause photodamage via reactive oxygen species (ROS) formation [83]. Microalgae employ photoprotective mechanisms including non-photochemical quenching (NPQ), increased synthesis of antioxidant pigments (carotenoids, xanthophylls), and modulation of chlorophyll antenna size to optimize light capture while minimizing excitation energy overload [91]. Engineering these pathways can enhance photoprotection, leading to better growth under intense or variable illumination.

6.2. Oxidative Stress Defense

Oxidative stress defense is a critical aspect of improving microalgal resilience, as exposure to environmental stresses such as high light intensity, nutrient deprivation, and temper-

ature fluctuations often leads to the excessive generation of reactive oxygen species (ROS), which can damage cellular components and impair metabolic functions [92]. Enhancement of the antioxidant system in microalgae involves boosting both enzymatic and non-enzymatic defenses to scavenge and neutralize ROS efficiently. Key antioxidant enzymes include superoxide dismutase (SOD), which converts superoxide radicals into hydrogen peroxide; catalase and peroxidases, which further break down hydrogen peroxide into harmless water and oxygen; and glutathione peroxidase, which reduces lipid hydroperoxides [83]. Genetic engineering strategies targeting the overexpression of these enzymes have demonstrated improved oxidative stress tolerance and sustained photosynthetic activity under challenging conditions [89].

In addition to enzymatic antioxidants, microalgae accumulate non-enzymatic antioxidant molecules such as carotenoids, tocopherols, and glutathione that provide complementary ROS scavenging and membrane protection [84, 93]. For example, carotenoids not only play a role in light harvesting but also protect chloroplast membranes by quenching singlet oxygen and dissipating excess energy.

Effective ROS management involves balancing ROS production and elimination to prevent cellular damage while maintaining their signaling roles [94]. Metabolic flux adjustments during stress conditions can reduce ROS generation, and enhancement of antioxidant pathways ensures rapid detoxification [95]. Advances in synthetic biology have enabled the design of regulatory circuits that dynamically modulate antioxidant gene expression in response to oxidative signals, providing adaptive protection.

7. High-Value Product Enhancement

7.1. Pigment Production

Microalgae are valuable sources of natural pigments, with astaxanthin being one of the most commercially significant carotenoids due to its potent antioxidant properties and applications in nutraceuticals, pharmaceuticals, and aquaculture [96]. The production of astaxanthin has been extensively studied in green microalgae such as *Haematococcus pluvialis* and *Chlorella zofingiensis*, which synthesize astaxanthin via two distinct biosynthetic pathways linked closely to stress responses, photosynthesis, and fatty acid metabolism [97]. These pathways not only confer protection against photooxidative stress but also coordinate with enzymatic defense systems, allowing the cells to accumulate astaxanthin under adverse conditions [98, 99]. Despite its high market value, the large-scale cultivation of *H. pluvialis* faces challenges including slow growth rates, limited light penetration in dense cultures, and inefficient CO₂ transfer, all of which restrict astaxanthin productivity [100]. To overcome these bottlenecks, multifaceted strategies combining strain improvement

(including genetic engineering tools like CRISPR/Cas9), optimized cultivation regimes (such as heterotrophic-phototrophic mixotrophic modes), and enhanced extraction methods are being developed, aiming to lower costs and improve yields for commercial viability [45]. Continuous research is also focused on genome sequencing and developing molecular toolkits for these microalgae, which will accelerate strain engineering and industry expansion.

In addition to astaxanthin, microalgae produce a spectrum of other carotenoids such as beta-carotene, lutein, and zeaxanthin, which have considerable nutritional, antioxidant, and commercial value [24]. These carotenoids share common biosynthetic precursors, and their production can be modulated through both genetic and cultivation strategies to favor specific pigment profiles [101]. Recent advances in two-stage cultivation and nutrient recovery processes have further improved pigment productivity while integrating sustainability goals [102]. Overall, engineering microalgae for enhanced pigment production, particularly astaxanthin and related carotenoids, continues to be a dynamic area combining metabolic, genetic, and process innovations to meet growing market demands.

7.2. Bioactive Compounds

Microalgae are increasingly recognized as a sustainable and versatile source of valuable bioactive compounds, notably omega-3 fatty acids and various pharmaceutical precursors [103]. Omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are produced naturally by many microalgal species and serve as essential nutrients with significant health benefits, including cardiovascular protection and anti-inflammatory effects [104]. Microalgal omega-3s present a sustainable alternative to fish oil, alleviating environmental concerns related to overfishing and offering a scalable platform via controlled cultivation systems [104, 105]. Optimizing cultivation conditions and genetic engineering approaches have enhanced omega-3 yields, facilitating their production for nutraceutical and functional food applications [54].

Beyond omega-3s, microalgae synthesize a broad spectrum of pharmaceutical compounds including polysaccharides, antioxidants, pigments, and other secondary metabolites with antimicrobial, antiviral, anticancer, and anti-inflammatory activities [106, 107]. These bioactives are often intracellular, making extraction techniques a key consideration for commercial viability. Recently, multi-stage and cascade extraction methods have been employed to maximize recovery from biomass, improving process sustainability and cost-efficiency [108, 109]. Advances in cultivation technology, bioreactor design, and metabolic engineering are progressively increasing the yield and diversity of these valuable compounds [110]. Altogether, microalgae offer a promising platform for producing bioactive molecules with applications spanning health, nutrition, and pharmaceuticals, supported by emerging tech-

nologies that enhance extraction and production efficiency.

8. Omics-Based Strain Improvement

8.1. Genomics and Functional Genomics

Genomics and functional genomics have become essential tools for advancing microalgal research and biotechnology. Recent efforts in genome sequencing and assembly have leveraged high-fidelity long-read technologies such as PacBio Revio combined with complementary short-read data to produce high-quality, chromosome-level genome assemblies [111]. For example, the *Nannochloropsis oceanica* C018 genome, approximately 26.4 Mbp in size, was assembled into 30 contigs with extensive coverage and annotated using paired-end transcriptomic reads, enabling the identification of around 8,700 gene models [112]. Such comprehensive genome assemblies provide valuable insights into gene content, metabolic capabilities, and regulatory elements, facilitating targeted genetic manipulation for traits such as biofuel production [113]. De novo assembly approaches integrating technologies like Oxford Nanopore MinION (long reads) and Illumina sequencing (short reads) have also proven effective for middle-sized microalgal genomes, with hybrid assembly pipelines yielding higher quality and completeness essential for comparative and functional genomics studies [114, 115].

In parallel, transcriptomics applications utilize high-throughput RNA sequencing to profile gene expression dynamics under varying environmental and physiological conditions, thereby illuminating functional gene networks and regulatory pathways [116]. Transcriptome data support genome annotation by confirming expressed genes and splice variants and help identify genes responsive to stress, lipid biosynthesis, or pigmentation pathways [117]. Integration of transcriptomic datasets with genomic sequences enables systems biology approaches to model metabolic flux and predict targets for metabolic engineering. Advances in microalgal transcriptomics also aid in understanding stress adaptation, photosynthetic efficiency, and developmental regulation, thus driving functional insights beyond the static genome sequence [118]. Together, genome sequencing, assembly, and transcriptomics form a powerful foundation for microalgal functional genomics, accelerating strain improvement and biotechnological application development.

8.2. Proteomics and Metabolomics

Proteomics and metabolomics are powerful complementary approaches that provide deep insights into microalgal physiology and metabolic states, crucial for optimizing culture conditions and enhancing bioproduct yields [119, 120]. Protein expression profiling through proteomics reveals the dynamic changes in the microalgal proteome in response to environmental factors such as nutrient availability, light in-

tensity, and growth modes [121, 122]. By applying advanced techniques like mass spectrometry-based bottom-up proteomics, researchers have identified hundreds of proteins with industrial relevance, including those involved in photosynthesis, lipid biosynthesis, and stress responses [123]. These studies inform strain optimization and bioprocess design by elucidating pathways that drive rapid growth or desirable metabolite accumulation [44]. However, challenges remain due to the diverse and often complex cell wall compositions of microalgae, requiring tailored protein extraction protocols for effective proteome analysis across different species. Moreover, integrating proteomics data with genomic and transcriptomic information enables a systems-level understanding of cellular regulation, facilitating targeted metabolic engineering.

On the other hand, metabolite profiling through metabolomics characterizes the full complement of small molecules within microalgal cells, capturing real-time metabolic responses to changing conditions and genetic modifications [124]. Metabolomics helps identify key intermediates and bottlenecks in pathways such as fatty acid synthesis, carotenoid production, and stress metabolites, providing critical biomarkers for strain performance [125]. The combination of proteomics and metabolomics contributes to a more complete picture of microalgal metabolism, enabling the development of predictive models that guide rational engineering [119, 126]. Altogether, these omics technologies advance microalgal biotechnology by uncovering molecular mechanisms behind growth, lipid accumulation, and bioactive compound production, ultimately supporting the design of robust, high-yield algal strains for sustainable industrial applications [127].

9. Cultivation Systems and Bioprocess Engineering

9.1. Photobioreactor Design

Photobioreactor design for microalgae cultivation requires careful optimization to maximize biomass productivity while minimizing energy consumption and operational costs [128]. In closed systems, such as cylindrical, tubular, or flat-panel photobioreactors, design efforts focus on maximizing the surface-to-volume ratio to enhance light availability since light is the most critical and energy-intensive input for microalgal growth [128, 129]. These systems often incorporate artificial or natural light sources, with attention to ensuring uniform light distribution to avoid shading and photoinhibition, which can reduce photosynthetic efficiency [130]. Key challenges in closed photobioreactor design include managing light attenuation as cell density increases, controlling temperature and gas exchange (CO₂ supply and oxygen removal), and preventing surface fouling that diminishes light penetration [117]. Advanced designs utilize features like internal

light guides, optimized flow patterns, and membrane-based gas transfer to improve mass transfer and light utilization while minimizing shear stress on cells [131]. Although closed systems enable better control over culture conditions and reduce contamination risk, their higher capital and operational costs currently limit their use primarily to high-value products [132].

In contrast, open systems, such as raceway ponds, offer economically viable cultivation on a large scale but face challenges related to environmental exposure, lower control over culture parameters, and higher contamination risks [133, 134]. Efforts to improve open system performance focus on optimizing pond depth to balance light penetration and temperature control, enhancing mixing for uniform nutrient and gas distribution, and employing cost-effective CO₂ delivery methods [135]. Innovations include hybrid systems that combine beneficial features of closed and open designs to increase productivity and environmental resilience [136]. Additionally, process innovations aim to reduce water and energy consumption while improving biomass quality. Despite their limitations, open systems remain attractive for bulk biomass production due to their simplicity and lower cost, provided that suitable climatic conditions and resource availability exist [137].

9.2. Process Integration

Process integration in microalgal biotechnology encompasses the seamless coordination of upstream and downstream processing steps to enhance overall efficiency, reduce costs, and improve product quality [44, 138]. Upstream processing involves cultivation and biomass production, where optimizing growth conditions such as nutrient supply, light intensity, temperature, and CO₂ availability is critical to maximize cell density and target metabolite accumulation [34]. Advances in photobioreactor design, strain selection, and culture mode (phototrophic, heterotrophic, or mixotrophic) contribute significantly to upstream efficiency [110, 129]. Additionally, strategies like nutrient recycling and environmental monitoring help sustain high productivity while minimizing resource inputs.

Downstream processing focuses on harvesting, cell disruption, extraction, and purification of desired bio-products. Given the often dilute nature of microalgal cultures, efficient biomass recovery methods such as flocculation, centrifugation, or filtration are essential to concentrate cells cost-effectively [139]. Following concentration, effective cell disruption techniques mechanical, chemical, or enzymatic are employed to release intracellular compounds for extraction [140]. Extraction methods are tailored to the target biomolecules, ranging from solvent extraction for lipids and pigments to advanced techniques like supercritical fluid extraction or membrane filtration. Integration of multiple extraction steps can yield diverse value-added products while improving overall biomass utilization [141]. Purification processes then

refine product quality to meet industry standards.

10. Challenges and Future Perspectives

Microalgal biotechnology faces several technical challenges that must be addressed to realize its full commercial potential. These include issues of genetic stability and reproducibility of engineered strains, which complicate consistent production outcomes and long-term cultivation reliability [25]. Low biomass productivity and inefficient harvesting at industrial scales remain major hurdles due to microalgae's small cell size and dilute cultures, driving up processing costs. Additionally, the complexity of optimizing cultivation systems balancing light penetration, nutrient supply, and environmental stresses and scaling photobioreactor designs for cost-effective, contamination-free production pose significant engineering challenges [110, 128]. The intricate interactions between microalgae and their associated microbiomes add another layer of complexity but also offer opportunities for enhanced robustness and productivity when properly managed [142]. Furthermore, the economic feasibility of downstream processing, including biomass recovery and product extraction, remains a bottleneck for large-scale applications.

From a regulatory and ethical perspective, genetic modification techniques used for strain improvement raise public concerns and stringent regulatory scrutiny, particularly around genetically modified organisms (GMOs) [143]. As a result, alternative non-GMO approaches, such as atmospheric and room-temperature plasma (ARTP) mutagenesis, have gained interest for strain development while potentially easing regulatory barriers [144]. Regulatory frameworks across different regions are often fragmented and lack harmonization, complicating market access and technology transfer. Public acceptance also hinges on transparent risk assessments, responsible deployment, and clear communication of environmental and health impacts associated with microalgal products [145]. Ethical considerations extend to ensuring sustainable resource use, preventing unintended ecological consequences, and balancing biotechnological innovation with biodiversity conservation [44].

Looking forward, advances in systems biology, multi-omics integration, and synthetic biology tools promise to overcome many current technical challenges by enabling precise and stable strain engineering, improved cultivation practices, and process integration [146]. Coupled with evolving regulatory landscapes that recognize sustainable and non-GMO methods, these developments are expected to accelerate the transition of microalgal biotechnologies from laboratory to industrial scale. Integrating microbial community management and fostering collaborative innovation will further enhance robustness and scalability [147]. Overall, addressing both technical and socio-regulatory challenges is crucial for harnessing microalgae's potential to contribute to sustainable biofuels, bioproducts, and environmental solutions on a global scale.

11. Conclusions and Future Directions

Microalgae biotechnology stands at a promising crossroads, driven by advances in genetic engineering, multi-omics integration, and cultivation technologies that together enhance their potential as sustainable biofactories for biofuels, nutraceuticals, pharmaceuticals, and high-value chemicals. The vast genetic and metabolic diversity of microalgae offers unprecedented opportunities for precise metabolic pathway engineering and development of novel pigments, omega-3 fatty acids, and bioactive compounds. Despite these advances, technical challenges such as improving biomass productivity, genetic stability, cultivation scalability, and cost-effective downstream processing remain significant hurdles. Emerging strategies like synthetic biology, CRISPR-based genome editing, and systems biology hold promise for overcoming these bottlenecks by enabling targeted and multiplexed genetic modifications and more efficient strain development.

From a regulatory and ethical standpoint, the future of microalgal biotechnology will rely on responsible development practices, clear safety evaluations of genetically modified strains, and transparent communication to gain public acceptance, especially in applications involving pharmaceuticals and food. Non-GMO approaches and the Generally Recognized as Safe (GRAS) status of many microalgal species provide favorable frameworks that can facilitate commercialization.

Looking forward, interdisciplinary integration of advanced molecular tools, smart bioprospecting to uncover novel species, innovative photobioreactor designs, and process integration aiming at circular economy principles will be essential. Continued research must also emphasize ecosystem-level understanding and adaptation to environmental variables to ensure robust and scalable production platforms. Overall, microalgae are poised to become key contributors to future sustainable production systems, addressing global challenges in health, nutrition, energy, and environmental stewardship. The convergence of scientific innovation, technological development, and regulatory readiness will determine the pace at which microalgae transition from promising research subjects to cornerstone industrial biotechnology platforms.

Abbreviations

ACC	Acetyl-CoA Carboxylase
ACC	Acetyl-CoA Carboxylase
ACP	Acyl Carrier Protein
ATP	Adenosine Triphosphate
CCM	CO ₂ -Concentrating Mechanism
CO ₂	Carbon Dioxide
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 Enzyme
DBT	Design-Build-Test
DGAT	Diacylglycerol Acyltransferase
DHA	Docosahexaenoic Acid

DHA	Docosahexaenoic Acid
DMAPP	Dimethylallyl Diphosphate
DSRNA	Double-Stranded RNA
EPA	Eicosapentaenoic Acid
EPPSII	Extrinsic Protein of Psii
FAS	Fatty Acid Synthase
FBA	Flux Balance Analysis
GPAT	Glycerol-3-Phosphate Acyltransferase
GRNAS	Guiding Ribonucleic Acid
HDR	Homology-Directed Repair
IPP	Isopentenyl Pyrophosphate
LPAAT	Lysophosphatidic Acid Acyltransferase
MIRNAS	Micromnas
MRNA	Messenger Ribonucleic Acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NHEJ	Non-Homologous End Joining
NPQ	Non-Photochemical Quenching
PAM	Peptidylglycine Alpha-Amidating Monooxygenase
PSI/PSII	Photosystem I/Photosystem II
PUFAS	Polyunsaturated Fatty Acids,
RBCL	Ribulose-1, 5-Bisphosphate Carboxylase/Oxygenase Large
RNAI	RNA Interference
ROS	Reactive Oxygen Species
RUBISCO	Ribulose-1, 5-Bisphosphate Carboxylase/Oxygenase
SOD	Superoxide Dismutase
TAG	riacylglycerol
T-DNA	Transferred - Deoxyribonucleic Acid
TFS	Transcription Factors

Author Contributions

Alebachew Molla: Conceptualization, Investigation, Validation, Writing - original draft, Writing - review & editing

Gedif Meseret: Conceptualization, Investigation, Supervision, Writing - review & editing

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Funding

This review received no external funding.

Data Availability Statement

No new data were created or analyzed in this review.

Conflicts of Interest

The author declares no conflicts of interest.

References

- [1] M. Fabris *et al.*, “Emerging Technologies in Algal Biotechnology : Toward the Establishment of a Sustainable, Algae-Based Bioeconomy,” vol. 11, no. March, pp. 1-22, 2020, <https://doi.org/10.3389/fpls.2020.00279>
- [2] Sanchez & Monotilla, Microalgae Biodiversity : Sustainable Biofactories for Food, Fuel and Therapeutics,” Vol: 6. pp. 117-156, 2022.
- [3] J. Singh and D. W. Dhar, “Overview of Carbon Capture Technology : Microalgal Biorefinery Concept and State-of-the-Art,” vol. 6, no. February, pp. 1-9, 2019, <https://doi.org/10.3389/fmars.2019.00029>
- [4] G. O. Omokaro, Z. S. Nafula, N. E. Iloabuchi, A. A. Chikukula, O. G. Osayogie, and E. C. Nnoli, “Microalgae as Biofactories for Sustainable Applications : Advancing Carbon Sequestration, Bioenergy, and Environmental Remediation,” *Sustain. Chem. Clim. Action*, p. 100098, 2025, <https://doi.org/10.1016/j.scca.2025.100098>
- [5] Fernandes, T.; Cordeiro, N. Microalgae as Sustainable Biofactories to Produce High-Value Lipids: Biodiversity, Exploitation, and Biotechnological Applications. *Mar. Drugs* 2021, 19, 573. <https://doi.org/10.3390/md19100573>
- [6] S. Wu, K. Ye, X. Zheng, and L. Zhao, “Microalgal Valorization of CO₂ : A Sustainable Pathway to Biofuels and High-Value Chemicals,” 2025.
- [7] Y. Lu, X. Zhang, X. Gu, H. Lin, and A. Melis, “Critical Reviews in Biotechnology Engineering microalgae : transition from empirical design to programmable cells,” *Crit. Rev. Biotechnol.*, vol. 41, no. 8, pp. 1233-1256, 2021, <https://doi.org/10.1080/07388551.2021.1917507>
- [8] S. Piyatilleke, B. Thevarajah, P. H. V Nimarshana, and T. U. Ariyadasa, “Microalgal biofuels : Challenges and prospective in the framework of circular bioeconomy,” *Energy Nexus*, vol. 17, no. October 2024, p. 100338, 2025, <https://doi.org/10.1016/j.nexus.2024.100338>
- [9] K. Jothibas, D. W. Dhar, and S. Rakesh, “Biomass and Bioenergy Recent developments in microalgal genome editing for enhancing lipid accumulation and biofuel recovery,” *Biomass and Bioenergy*, vol. 150, no. January, p. 106093, 2021, <https://doi.org/10.1016/j.biombioe.2021.106093>
- [10] J. Hu, W. Meng, Y. Su, C. Qian, and W. Fu, “Emerging technologies for advancing microalgal photosynthesis and metabolism toward sustainable production,” no. October, pp. 1-20, 2023, <https://doi.org/10.3389/fmars.2023.1260709>
- [11] Sarita, S.; Kalkan, A. E.; Yılmaz, K.; Gurdal, S.; Göksan, T.; Witkowska, A. M.; Lombardo, M.; Karav, S. Biological and Nutritional. Applications of Microalgae. *Nutrients*. 2025, 17, 93. <https://doi.org/10.3390/nu17010093>
- [12] G. Perin, F. Gambaro, and T. Morosinotto, “Knowledge of Regulation of Photosynthesis in Outdoor Microalgae Cultures Is Essential for the Optimization of Biomass Productivity,” vol. 13, no. April, pp. 1-11, 2022, <https://doi.org/10.3389/fpls.2022.846496>
- [13] A. M. Vera-vives, T. Michelberger, T. Morosinotto, and G. Perin, “Plant Physiology and Biochemistry Assessment of photosynthetic activity in dense microalgae cultures using oxygen production,” *Plant Physiol. Biochem.*, vol. 208, no. November 2023, p. 108510, 2024, <https://doi.org/10.1016/j.plaphy.2024.108510>
- [14] D. F. Gomez-casati, J. Barchiesi, and M. V. Busi, “Mitochondria and chloroplasts function in microalgae energy production,” 2022, <https://doi.org/10.7717/peerj.14576>
- [15] A. Patel, U. Rova, P. Christakopoulos, and L. Matsakas, “Microalgal lutein biosynthesis : Recent trends and challenges to enhance the lutein content in microalgal cell factories,” no. October, pp. 1-9, 2022, <https://doi.org/10.3389/fmars.2022.1015419>
- [16] S. Smaoui, M. Barkallah, H. Ben Hlima, I. Fendri, and A. M. Khaneghah, “Microalgae Xanthophylls : From Biosynthesis Pathway and,” 2021.
- [17] Y. Ren, H. Sun, J. Deng, J. Huang, and F. Chen, “Carotenoid Production from Microalgae : Biosynthesis, Salinity Responses and Novel Biotechnologies,” 2021.
- [18] S. Dubey and C. Wen, “Development in health - promoting essential polyunsaturated fatty acids production by microalgae : a review,” *J. Food Sci. Technol.*, vol. 61, no. 5, pp. 847-860, 2024, <https://doi.org/10.1007/s13197-023-05785-1>
- [19] Y. Xin, S. Wu, C. Miao, T. Xu, and Y. Lu, “Towards Lipid from Microalgae : Products, Biosynthesis, and Genetic Engineering,” pp. 1-17, 2024.
- [20] M. Lati, F. J. Bidgoli, and H. Hajihassani, “Recent advances and future directions on GLA-producing organisms,” no. July, pp. 1-31, 2025, <https://doi.org/10.3389/fbioe.2025.1567840>
- [21] J. A. M. Prates, “Unlocking the Functional and Nutritional Potential of Microalgae Proteins in Food Systems : A Narrative Review,” pp. 1-19, 2025.
- [22] S. Park and Y. J. Lee, “Efficient secretory production of recombinant proteins in microalgae using an exogenous signal peptide,” no. June, 2025, <https://doi.org/10.3389/fmicb.2025.1603204>
- [23] R. Fernando, X. Sun, and H. P. V. Rupasinghe, “Production of Bioactive Peptides from Microalgae and Their Biological Properties Related to Cardiovascular Disease,” pp. 582-596, 2024.
- [24] B. Coleman, E. Vereecke, K. Van Laere, and L. Novoveska, “Genetic Engineering and Innovative Cultivation Strategies for Enhancing the Lutein Production in Microalgae,” pp. 1-24, 2024.

- [25] A. Gerszberg, L. Kolek, and K. Hnatuszko-konka, "In Vitro Culture, Genetic Transformation and the Production of Biopharmaceuticals in Microalgae," pp. 1-26, 2025.
- [26] A. Gupta *et al.*, "Harnessing genetic engineering to drive economic bioproduct production in algae," no. January, pp. 1-20, 2024, <https://doi.org/10.3389/fbioe.2024.1350722>
- [27] "Improving recombinant protein production in the *Chlamydomonas reinhardtii* chloroplast using vivid Verde Fluorescent Protein as a reporter," pp. 1289-1297, 2015, <https://doi.org/10.1002/biot.201400566>
- [28] N. Son and I. J. Goldberg, "Intracellular lipase and regulation of the lipid droplet," vol. 35, no. 2, pp. 85-92, 2025, <https://doi.org/10.1097/MOL.0000000000000918.Intracellular>
- [29] R. Rengel, R. T. Smith, R. P. Haslam, O. Sayanova, M. Vila, and R. León, "Overexpression of acetyl-CoA synthetase (ACS) enhances the biosynthesis of neutral lipids and starch in the green microalga *Chlamydomonas reinhardtii*," *Algal Res.*, vol. 31, no. February, pp. 183-193, 2018, <https://doi.org/10.1016/j.algal.2018.02.009>
- [30] C. Shang, B. Pang, H. Yu, S. Gan, and Y. Li, "Identification of Targets of Transcription Factor WRINKLED1-Like Related to Lipid Biosynthesis From Marine Microalga *Dunaliella parva*," vol. 8, no. February, pp. 1-14, 2022, <https://doi.org/10.3389/fmars.2021.807493>
- [31] H. G. Koh, K. Park, S. Park, M. Kim, and N. K. Kang, "Enhancing lipid production in *Nannochloropsis salina* via RNAi-mediated downregulation of carbohydrate biosynthesis," no. May, pp. 1-10, 2025, <https://doi.org/10.3389/fmicb.2025.1601691>
- [32] H. Cerutti, X. Ma, J. Msanne, and T. Repas, "RNA-Mediated Silencing in Algae : Biological Roles and Tools for Analysis of Gene Function," vol. 10, no. 9, pp. 1164-1172, 2011, <https://doi.org/10.1128/EC.05106-11>
- [33] A. Einhaus and T. Baier, "Molecular design of microalgae as sustainable cell factories," *Trends Biotechnol.*, vol. 42, no. 6, pp. 728-738, 2024, <https://doi.org/10.1016/j.tibtech.2023.11.010>
- [34] A. K. Sharma, S. Jaryal, S. Sharma, A. Dhyani, and B. S. Tewari, "Biofuels from Microalgae : A Review on Microalgae Cultivation, Biodiesel Production Techniques and Storage Stability," pp. 1-26, 2025.
- [35] E. Kim, X. Ma, and H. Cerutti, "Bioresource Technology Gene silencing in microalgae : Mechanisms and biological roles," *Bioresour. Technol.*, 2014, <https://doi.org/10.1016/j.biortech.2014.10.119>
- [36] E. Grypioti, H. Richard, N. Kryovrysaniaki, M. Jaubert, and A. Falcatore, "Dicer-dependent heterochromatic small RNAs in the model diatom species *Phaeodactylum tricornutum*," pp. 811-826, 2024, <https://doi.org/10.1111/nph.19429>
- [37] A. E. Sproles, F. J. Fields, T. N. Smalley, C. H. Le, A. Badary, and P. Stephen, "Version of Record: 0310262," pp. 1-83, <https://www.sciencedirect.com/science/article/pii/S221192642>
- [38] C. F. Muñoz *et al.*, "Improved DNA / protein delivery in microalgae - A simple and reliable method for the prediction of optimal electroporation settings," vol. 33, no. March, pp. 448-455, 2018, <https://doi.org/10.1016/j.algal.2018.06.021>
- [39] D. Poveda-huertes, P. Patwari, J. Günther, M. Fabris, and J. Andersen-ranberg, "Novel transformation strategies improve efficiency up to 10-fold in stramenopile algae," *Algal Res.*, vol. 74, no. May, p. 103165, 2023, <https://doi.org/10.1016/j.algal.2023.103165>
- [40] M. Nkg, "Genetic Transformation of the Marine Oleaginous Microalga," *Mar. Biotechnol.*, vol. 2050, 2025, <https://doi.org/10.1007/s10126-025-10473-6>
- [41] E. François, S. Youn-seb, and J. Fengying, "Biocatalysis and Agricultural Biotechnology Engineering the haploid genome of microspores," pp. 10-13, 2013, <https://doi.org/10.1016/j.bcab.2013.11.002>
- [42] M. Biotechnology, "An Efficient Protocol for the Agrobacterium-mediated Genetic Transformation An Efficient Protocol for the Agrobacterium -mediated Genetic Transformation of Microalga *Chlamydomonas reinhardtii*," no. November 2013, 2017, <https://doi.org/10.1007/s12033-013-9720-2>
- [43] G. Engineering, "Gene Delivery Technologies with Applications in Microalgal," 2021.
- [44] C. Zhang *et al.*, "Recent advances in biotechnology and bioengineering for efficient microalgal biofuel production," *Fuel Process. Technol.*, vol. 270, no. February, p. 108199, 2025, <https://doi.org/10.1016/j.fuproc.2025.108199>
- [45] D. Dhokane, A. Shaikh, A. Yadav, and N. Giri, "CRISPR-based bioengineering in microalgae for production of industrially important biomolecules," no. October, pp. 1-15, 2023, <https://doi.org/10.3389/fbioe.2023.1267826>
- [46] K. Chen *et al.*, "CRISPR-Cas9-based one-step multiplexed genome editing through optimizing guide RNA processing strategies in *Pichia pastoris*," vol. 10, no. January, pp. 484-494, 2025, <https://doi.org/10.1016/j.synbio.2025.01.005>
- [47] M. Ghribi, S. B. Nouemssi, F. Meddeb-mouelhi, and I. Desgagn, "Genome Editing by CRISPR-Cas : A Game Change in the Genetic Manipulation of *Chlamydomonas*," pp. 1-21, 2020.
- [48] A. P. Nievergelt *et al.*, "Report Efficient precision editing of endogenous *Chlamydomonas reinhardtii* genes with CRISPR-Cas II Efficient precision editing of endogenous *Chlamydomonas reinhardtii* genes with CRISPR-Cas," *Cell Reports Methods*, vol. 3, no. 8, p. 100562, 2023, <https://doi.org/10.1016/j.crmeth.2023.100562>
- [49] B. Jeong, J. Jang, and E. Jin, "Bioresource Technology Genome engineering via gene editing technologies in microalgae," *Bioresour. Technol.*, vol. 373, no. February, p. 128701, 2023, <https://doi.org/10.1016/j.biortech.2023.128701>
- [50] S. S. Bharat, S. Li, J. Li, L. Yan, and L. Xia, "ScienceDirect Base editing in plants : Current status and challenges," *Crop J.*, vol. 8, no. 3, pp. 384-395, 2019, <https://doi.org/10.1016/j.cj.2019.10.002>

- [51] R. Article, "Prime editing: therapeutic advances and mechanistic insights," no. April, 2024, <https://doi.org/10.1038/s41434-024-00499-1>
- [52] A. Kantor and M. E. McClements, "CRISPR-Cas9 DNA Base-Editing and Prime-Editing," pp. 1-21, 2020.
- [53] J. De Saeger, E. C. Vandellannoote, H. Lee, J. Park, J. Blomme, and J. Blomme, "Genome editing in macroalgae : advances and challenges," no. March, pp. 1-9, 2024, <https://doi.org/10.3389/fgeed.2024.1380682>
- [54] Z. Huang and G. Liu, "Current advancement in the application of prime editing," no. February, pp. 1-15, 2023, <https://doi.org/10.3389/fbioe.2023.1039315>
- [55] F. Chen *et al.*, "Recent advances of CRISPR- based genome editing for enhancing staple crops," no. September, pp. 1-21, 2024, <https://doi.org/10.3389/fpls.2024.1478398>
- [56] R. M. Genga and N. A. Kearns, "HHS Public Access," pp. 36-42, 2017, <https://doi.org/10.1016/j.ymeth.2015.10.014.Controlling>
- [57] Q. Tran, T. T. Le, D. Choi, D. Cho, H. Kim, and Y. J. Lee, "Progress and challenges in CRISPR / Cas applications in microalgae," no. March, 2025, <https://doi.org/10.71150/jm.2501028>
- [58] H. Xie, B. Bourgade, K. Stensjö, and P. Lindblad, "dCas12a - mediated CRISPR interference for multiplex gene repression in cyanobacteria for enhanced isobutanol and 3 - methyl - 1 - butanol production," *Microb. Cell Fact.*, 2025, <https://doi.org/10.1186/s12934-025-02727-8>
- [59] F. Naselli *et al.*, "First evidence of epigenetic modulation of human gene methylation by microalga *Aphanizomenon flos - aquae* (AFA) in inflammation - related pathways in intestinal cells," *Clin. Epigenetics*, 2025, <https://doi.org/10.1186/s13148-025-01849-5>
- [60] A. Herdean, M. Bedoya, M. Kim, N. Poddar, L. Hoch, and P. J. Ralph, "Phenotypic plasticity can be modulated by epigenetic means via DNA methylation in the microalgae *Desmodesmus armatus*," *Algal Res.*, vol. 90, no. October 2024, p. 104206, 2025, <https://doi.org/10.1016/j.algal.2025.104206>
- [61] R. Bacova, M. Kolackova, B. Klejdus, V. Adam, and D. Huska, "Epigenetic mechanisms leading to genetic flexibility during abiotic stress responses in microalgae : A review," *Algal Res.*, vol. 50, no. July, p. 101999, 2020, <https://doi.org/10.1016/j.algal.2020.101999>
- [62] A. H. Cardona, M. M. Peixoto, T. Borjigin, and T. Gregor, "Bridging spatial and temporal scales of developmental gene regulation," *Curr. Opin. Genet. Dev.*, vol. 92, p. 102328, 2025, <https://doi.org/10.1016/j.gde.2025.102328>
- [63] G. L. Butterfield, S. J. Reisman, N. Iglesias, and C. A. Gersbach, "Gene regulation technologies for gene and cell therapy," *Mol. Ther.*, vol. 33, no. 5, pp. 2104-2122, 2025, <https://doi.org/10.1016/j.ymthe.2025.04.004>
- [64] A. Jaramillo *et al.*, "Outlook on Synthetic Biology-Driven Hydrogen Production : Lessons from Algal Photosynthesis Applied to Cyanobacteria," 2025, <https://doi.org/10.1021/acs.energyfuels.4c04772>
- [65] Ramírez Rojas AA, Swidah R and Schindler D, Microbes of traditional fermentation processes as synthetic biology chassis to tackle future food challenges. *Front. Bioeng. Biotechnol.* 2022, 10: 982975. <https://doi.org/10.3389/fbioe.2022.982975>
- [66] C. Ignea, "Natural Product Reports," vol. 40, no. 12, 2023, <https://doi.org/10.1039/d3np00005b>
- [67] X. Xu *et al.*, "Trimming the genomic fat : minimising and re-functionalising genomes using synthetic biology," pp. 1-11, 2023, <https://doi.org/10.1038/s41467-023-37748-7>
- [68] M. Marsafari, F. Azi, S. Dou, and P. Xu, "Modular co - culture engineering of *Yarrowia lipolytica* for amorphaadiene biosynthesis," *Microb. Cell Fact.*, pp. 1-10, 2022, <https://doi.org/10.1186/s12934-022-02010-0>
- [69] X. Lu, M. Hagemann, J. Liu, P. Shukla, and X. Tan, "Editorial : Engineering microalgal chassis cells".
- [70] S. Della Valle, W. Tu, and W. E. Huang, "ScienceDirect Systems Biology Construction of microbial platform chassis for CO₂ utilisation," *Curr. Opin. Syst. Biol.*, vol. 37, p. 100489, 2024, <https://doi.org/10.1016/j.coisb.2023.100489>
- [71] J. Tamburro and N. R. Boyle, "Charting the state of GEMs in microalgae : progress, challenges, and innovations," no. June, pp. 1-14, 2025, <https://doi.org/10.3389/fpls.2025.1614397>
- [72] S. Shin, H. G. Koh, and K. Park, "Increasing lipid production in *Chlamydomonas reinhardtii* through genetic introduction for the overexpression of," no. April, 2024, <https://doi.org/10.3389/fbioe.2024.1396127>
- [73] H. Lu *et al.*, "Biotechnology for Biofuels Time - resolved multi - omics analysis reveals the role of nutrient stress - induced resource reallocation for TAG accumulation in oleaginous fungus *Mortierella alpina*," *Biotechnol. Biofuels*, pp. 1-17, 2020, <https://doi.org/10.1186/s13068-020-01757-1>
- [74] Y. Wu, "AI-driven multi-omics integration for multi-scale predictive modeling of genotype-environment-phenotype relationships," *Comput. Struct. Biotechnol. J.*, vol. 27, no. January, pp. 265-277, 2025, <https://doi.org/10.1016/j.csbj.2024.12.030>
- [75] E. L. Sanchez and M. Lagunoff, "Viral activation of cellular metabolism," *Virology*, vol. 479-480, pp. 609-618, 2015, <https://doi.org/10.1016/j.virol.2015.02.038>
- [76] L. Xu, X. Cheng, and Q. Wang, "Enhanced Lipid Production in *Chlamydomonas reinhardtii* by Co-culturing With *Azotobacter chroococcum*," vol. 9, no. June, pp. 1-13, 2018, <https://doi.org/10.3389/fpls.2018.00741>
- [77] S. Zou *et al.*, "Microalgal glycerol-3-phosphate acyltransferase role in galactolipids and high-value storage lipid biosynthesis Research Article," *Plant Physiol.*, vol. 192, no. 1, pp. 426-441, 2023, <https://doi.org/10.1093/plphys/kiad091>

- [78] E. C. Goncalves, A. C. Wilkie, M. Kirst, and B. Rathinasabapathi, "Metabolic regulation of triacylglycerol accumulation in the green algae: identification of potential targets for engineering to improve oil yield," pp. 1649-1660, 2016, <https://doi.org/10.1111/pbi.12523>
- [79] A. Alboresi *et al.*, "Light Remodels Lipid Biosynthesis in *Nannochloropsis gaditana* by Modulating Carbon Partitioning between Organelles 1 [OPEN]," vol. 171, no. August, pp. 2468-2482, 2016, <https://doi.org/10.1104/pp.16.00599>
- [80] B. Jia, "Increased Lipids in *Chlamydomonas reinhardtii* by Multiple Regulations of DOF, LACS2, and CIS1," 2022.
- [81] P. Liu *et al.*, "Discovery of mannose as an alternative non-nutrient-deficient regulator of lipid accumulation in microalgae," *J. Adv. Res.*, no. xxxx, 2025, <https://doi.org/10.1016/j.jare.2025.03.019>
- [82] Y. Song, F. Wang, L. Chen, and W. Zhang, "Engineering Fatty Acid Biosynthesis in Microalgae: Recent Progress and Perspectives," 2024.
- [83] B. Vangenechten, B. De Coninck, and J. Ceusters, "How to improve the potential of microalgal biostimulants for abiotic stress mitigation in plants?," no. April, 2025, <https://doi.org/10.3389/fpls.2025.1568423>
- [84] A. Koletti, D. Skliros, I. Dervisi, and A. Roussis, "Oxidative Stress Responses in Microalgae: Modern Insights into an Old Topic," pp. 1-18, 2025.
- [85] P. Babitzke, Y. Lai, A. J. Renda, and T. Romeo, "by Bacterial RNA-Binding Proteins," pp. 43-67, 2022, <https://doi.org/10.1146/annurev-micro-020518-115907.Posttranscription>
- [86] J. K. W. Lam, M. Y. T. Chow, Y. Zhang, and S. W. S. Leung, "siRNA Versus miRNA as Therapeutics for Gene Silencing," no. July, 2015, <https://doi.org/10.1038/mtna.2015.23>
- [87] C.- Trebouxiphyceae *et al.*, "Mechanisms of temperature acclimatisation in the psychrotolerant green alga *Coccomyxa subellipsoidea*," no. June, pp. 1-14, 2023, <https://doi.org/10.1111/pp.14034>
- [88] M. Janssen and M. J. Barbosa, "Growth and fatty acid distribution over lipid classes in *Nannochloropsis oceanica* acclimated to different temperatures," no. February, pp. 1-16, 2023, <https://doi.org/10.3389/fpls.2023.1078998>
- [89] L. Fang *et al.*, "De novo transcriptomic profiling of *Dunaliella salina* reveals concordant flows of glycerol metabolic pathways upon reciprocal salinity change," *Algal Res.*, vol. 23, pp. 135-149, 2017, <https://doi.org/10.1016/j.algal.2017.01.017>
- [90] M. Biostimulants, "Enhancing Sustainability by Improving Plant Salt Tolerance through Macro- and," pp. 1-21, 2020.
- [91] A. Isiaka, S. Thuthukani, and M. Erasmus, "Journal of Hazardous Materials Advances Harnessing the potential of microalgae in sequestration of CO₂ emissions: Removal mechanisms, optimization strategies, and bioenergy production," *J. Hazard. Mater. Adv.*, vol. 18, no. March, p. 100722, 2025, <https://doi.org/10.1016/j.hazadv.2025.100722>
- [92] Z. Sun, X. Mao, H. Gerken, X. Wang, W. Yang, and J. Liu, "Multiomics analysis reveals a distinct mechanism of oleaginousness in the emerging model alga *Chromochloris zofingiensis*," pp. 1060-1077, 2019, <https://doi.org/10.1111/tpj.14302>
- [93] M. J. Rao *et al.*, *Antioxidant Defense System in Plants: Reactive Oxygen Species Production, Signaling, and Scavenging During Abiotic Stress-Induced Oxidative Damage*. 2025.
- [94] C. Han and P. Wang, "Reactive oxygen species: Multidimensional regulators of plant adaptation to abiotic stress and development," 2023, <https://doi.org/10.1111/jipb.13601>
- [95] S. Ibragi, "The Good, the Bad, and the Epigenetic: Stress-Induced Metabolite Regulation and Transgenerational Effects," pp. 1-27, 2025.
- [96] S. Villar, M. Ciardi, A. Morillas-españa, S. Ana, G. Aci, and T. Lafarga, "Trends and Industrial Use as Food," pp. 1-17, 2021.
- [97] J. F. Qiu *et al.*, "Synergistic and stepwise treatment of resveratrol and catechol in *Haematococcus pluvialis* for the overproduction of biomass and astaxanthin," *Biotechnol. Biofuels Bioprod.*, pp. 1-14, 2024, <https://doi.org/10.1186/s13068-024-02527-z>
- [98] B. Y. Chung, S. H. Park, S. Y. Yun, D. S. Yu, and Y. B. Lee, "Astaxanthin Protects Ultraviolet B-Induced Oxidative Stress and Apoptosis in Human Keratinocytes via Intrinsic Apoptotic Pathway," vol. 34, 2022.
- [99] J. Sun, Z. Zhang, L. Gao, and F. Yang, "Advances and trends for astaxanthin synthesis in *Phaffia rhodozyma*," no. 2025, pp. 1-19, 2026.
- [100] L. Peng, Z. Zhang, Q. Li, and H. Yang, "Current Challenges and Issues in the Application of Astaxanthin," pp. 1-19, 2025.
- [101] B. Panahi, N. Hosseinzadeh, and H. Mohammadzadeh, "Harnessing systems biology approach for characterization of carotenoid biosynthesis pathways in microalgae," *Biochem. Biophys. Reports*, vol. 39, no. June, p. 101759, 2024, <https://doi.org/10.1016/j.bbrep.2024.101759>
- [102] X. Huang *et al.*, "Influence of Light Regimes on Production of Beneficial Pigments and Nutrients by Microalgae for Functional Plant-Based Foods," pp. 1-26, 2025.
- [103] J. L. Guil-guerrero, "Microalgae Bioactives for Functional Food Innovation and Health Promotion," pp. 1-27, 2025.
- [104] J. Qin, E. Kurt, T. Lbassi, and L. Sa, "Biotechnological production of omega-3 fatty acids: current status and future perspectives," no. November, 2023, <https://doi.org/10.3389/fmicb.2023.1280296>
- [105] M. Silva, P. Geada, R. N. Pereira, and A. Teixeira, "Microalgae biomass - A source of sustainable dietary bioactive compounds towards improved health and well-being," vol. 6, no. February, 2025, <https://doi.org/10.1016/j.focha.2025.100926>
- [106] C. Magnabosco and G. Santaniello, "Microalgae: A Promising Source of Bioactive Polysaccharides for Biotechnological Applications," 2025.

- [107] M. Kaur, S. Bhatia, U. Gupta, and E. Decker, *Microalgal bioactive metabolites as promising implements in nutraceuticals and pharmaceuticals: inspiring therapy for health benefits*, vol. 7. Springer Netherlands, 2023. <https://doi.org/10.1007/s11101-022-09848-7>
- [108] G. Kopp and C. Lauritano, “Greener Extraction Solutions for Microalgal Compounds,” pp. 1-34, 2025.
- [109] M. Osathanunkul *et al.*, “Diversity of Bioactive Compounds in Microalgae: Key Classes and Functional Applications,” pp. 1-28, 2025.
- [110] S. Abdur *et al.*, “Green Chemical Engineering Microalgae cultivation in photobioreactors: sustainable solutions for a greener future,” *Green Chem. Eng.*, vol. 5, no. 4, pp. 418-439, 2024, <https://doi.org/10.1016/j.gce.2023.10.004>
- [111] A. Estrada-graf, H. Koneru, J. Arnold, S. Calhoun, I. V. Grigoriev, and Z. I. Johnson, “Genome assembly and annotation of microalga *Nannochloropsis oceanica* C018,” vol. 1, no. 9, pp. 12-14.
- [112] E. Corteggiani, A. Telatin, N. Vitulo, C. Forcato, and M. D. Angelo, “Chromosome Scale Genome Assembly and Transcriptome Profiling of *Nannochloropsis gaditana* in Nitrogen Depletion,” no. August, 2013, <https://doi.org/10.1093/mp/sst120>
- [113] M. S. Roth, S. J. Cokus, S. D. Gallaher, A. Walter, D. Lopez, and E. Erickson, “Chromosome-level genome assembly and transcriptome of the green alga *Chromochloris zofingiensis* illuminates astaxanthin production,” 2017, <https://doi.org/10.1073/pnas.1619928114>
- [114] R. Minei, R. Hoshina, and A. Ogura, “De novo assembly of middle-sized genome using MinION and Illumina sequencers,” pp. 1-12, 2018.
- [115] X. Li, Y. Li, C. Zhong, J. Li, S. Li, and J. Li, “Chromosome-level genome assembly for the ecologically and economically important alga *Saccharina japonica*,” pp. 1-7, 2025, <https://doi.org/10.1038/s41597-025-04620-w>
- [116] O. Cheregi *et al.*, “Transcriptome analysis reveals insights into adaptive responses of two marine microalgae species to Nordic seasons,” vol. 74, no. August, 2023, <https://doi.org/10.1016/j.algal.2023.103222>
- [117] Z. Zhou, E. Jiawei, Q. Shi, W. Zhang, L. Sun, and J. Fan, “Chromosome-level genome provides novel insights into the starch metabolism regulation and evolutionary history of *Tetraselmis helgolandica*,” *J. Adv. Res.*, no. xxxx, 2025, <https://doi.org/10.1016/j.jare.2025.03.022>
- [118] B. Zhang, C. Deng, S. Wang, and Q. Deng, “The RNA landscape of *Dunaliella salina* in response to short-term salt stress,” no. December, pp. 1-16, 2023, <https://doi.org/10.3389/fpls.2023.1278954>
- [119] A. Mishra, K. Medhi, P. Malaviya, and I. S. Thakur, “Bioresource Technology Omics approaches for microalgal applications: Prospects and challenges,” *Bioresour. Technol.*, vol. 291, no. July, p. 121890, 2019, <https://doi.org/10.1016/j.biortech.2019.121890>
- [120] D. P. Yee, C. Juery, A. Catacora-grundy, C. Lekieffre, D. Wangpraseurt, and J. Decelle, “Tansley review Physiology and metabolism of eukaryotic microalgae involved in aquatic photosymbioses,” pp. 71-89, 2025, <https://doi.org/10.1111/nph.70190>
- [121] A. Yunusa, H. Chen, and Q. Wang, “Materials Today Sustainability Enhancing the sustainability of microalgae cultivation through biosensing technology,” *Mater. Today Sustain.*, vol. 31, no. January, p. 101139, 2025, <https://doi.org/10.1016/j.mtsust.2025.101139>
- [122] T. Selvaratnam, S. Sivanantharajah, and K. Sriram, “Beyond Biomass: Reimagining Microalgae as Living Environmental Nano-Factories,” 2025.
- [123] N. T. Tran, M. P. Padula, C. R. Evenhuis, A. S. Commault, P. J. Ralph, and B. Tamburic, “Proteomic and biophysical analyses reveal a metabolic shift in nitrogen deprived *Nannochloropsis oculata*,” *ALGAL*, vol. 19, pp. 1-11, 2016, <https://doi.org/10.1016/j.algal.2016.07.009>
- [124] D. K. Saini, G. Manchanda, and R. G. Chaudhary, “Microalgal Metabolomes and Recent Biotechnological Advances for Their Industrial Application,” pp. 2056-2069, 2024.
- [125] L. I. Yuanxiang, C. A. I. Xuehua, G. U. Wenhui, and W. Guangce, “Transcriptome analysis of carotenoid biosynthesis in *Dunaliella salina* under red and blue light Transcriptome analysis of carotenoid biosynthesis in *Dunaliella salina* under red and blue light *,” no. January, 2020, <https://doi.org/10.1007/s00343-019-9064-2>
- [126] F. R. Pinu *et al.*, “Systems Biology and Multi-Omics Integration: Viewpoints from the Metabolomics Research Community,” pp. 1-31, 2019.
- [127] R. Carrasco-reinado, M. Bermudez-sauco, A. Escobar-niño, and M. Cantoral, “Development of the ‘Applied Proteomics’ Concept for Biotechnology Applications in Microalgae: Example of the Proteome Data in *Nannochloropsis gaditana*,” 2022.
- [128] K. A. Rihihaio, L. Lind, M. L. Calderini, V. Halonen, and I. Pölönen, “Photobioreactor design utilizing procedural three-dimensional modelling and ray tracing,” 2025.
- [129] F. Ortenzi *et al.*, “Bioresource Technology Sono-photobioreactor: Design and application of characterized and modifiable reactor to optimize microalgal production with ultrasounds,” *Bioresour. Technol.*, vol. 434, no. March, p. 132810, 2025, <https://doi.org/10.1016/j.biortech.2025.132810>
- [130] B. Amanna, P. A. Bahri, G. Zhang, and N. R. Moheimani, “Enhancing *Arthrospira platensis* productivity by optimizing mixing rates in a self-cooling flat plate photobioreactor,” *Algal Res.*, vol. 88, no. February, p. 104035, 2025, <https://doi.org/10.1016/j.algal.2025.104035>
- [131] K. Azizi, M. Keshavarz, and H. Hassanzadeh, “Consideration of inclined mixers embedded inside a photobioreactor for microalgae cultivation using computational fluid dynamic and particle image velocimetry measurement,” *J. Clean. Prod.*, vol. 195, pp. 753-764, 2018, <https://doi.org/10.1016/j.jclepro.2018.05.253>

- [132] Deprá M. C.; Dias, R. R.; Zepka, L. Q.; Jacob-Lopes, E. Tackling Old Challenges in Microalgal Biotechnology: The Role of Photobioreactors to Advance the Technology Readiness Level. *Processes*, 2025, 13, 51. <https://doi.org/10.3390/pr13010051>
- [133] I. Skifa, N. Chauchat, P. Cocquet, and Y. Le Guer, "Microalgae cultivation in raceway ponds: Advances, challenges, and hydrodynamic considerations," *EFB Bioeconomy J.*, vol. 5, no. December 2024, p. 100073, 2025, <https://doi.org/10.1016/j.bioeco.2024.100073>
- [134] R. R. Narala, S. Garg, K. K. Sharma, and S. R. Thomas-hall, "Comparison of Microalgae Cultivation in Photobioreactor, Open Raceway Pond, and a Two-Stage Hybrid System," vol. 4, no. August, pp. 1-10, 2016, <https://doi.org/10.3389/fenrg.2016.00029>
- [135] S. Ren *et al.*, "Optimization and synergistic enhancement of microalgae productivity in laboratory raceway ponds via co-regulation of automated light-supplemented mixers and electric field system," *Biotechnol. Biofuels Bioprod.*, 2025, <https://doi.org/10.1186/s13068-025-02658-x>
- [136] A. A. Kubar, S. Mehmood, M. Schagerl, S. Kumar, X. Hu, and F. Zhu, "Integrated hybrid Nested-bottled photobioreactor for enhanced mixing, mass transfer, and CO₂ fixation in *Arthrospira platensis* raceway pond cultivation systems," pp. 1-10, 2025.
- [137] Q. Zhang, Y. Guan, Z. Zhang, S. Dong, T. Yuan, and Z. Ruan, "Sustainable microalgae cultivation: a comprehensive review of open and enclosed systems for biofuel and high value compound production," vol. 01008, 2024.
- [138] R. Yasu, "Upstream and Downstream Processing in Pharmaceutical Bioprocessing," vol. 140766, no. 4, pp. 210-211, 2024, [https://doi.org/10.37532/2048-9145.2024.12\(4\).210-211](https://doi.org/10.37532/2048-9145.2024.12(4).210-211)
- [139] K. Shiong, K. Wayne, G. Yong, W. Hong, and Y. Ho, "Bioresource Technology Recent advances in downstream processing of microalgae lipid recovery for biofuel production," *Bioresour. Technol.*, vol. 304, no. February, p. 122996, 2020, <https://doi.org/10.1016/j.biortech.2020.122996>
- [140] S. T. Taj-liad, R. Raviadaran, and D. Chandran, "Results in Engineering Cell disruption and extraction for microalgae biorefinery: Focus on deep eutectic solvents moving towards aqueous biphasic system," *Results Eng.*, vol. 26, no. January, p. 105066, 2025, <https://doi.org/10.1016/j.rineng.2025.105066>
- [141] Rathod NB, Nirmal NP, Abdullah S, Surasani VKR, Ranveer RC, Kumar S, Chunhavacharatorn P, Benjakul S and Al-Asmari F. Extraction of natural bioactive compounds using clean label technologies and their application as muscle food preservatives. *Front. Sustain. Food Syst.* 2023, 7: 1207704. <https://doi.org/10.3389/fsufs.2023.1207704>
- [142] H. Koneru, S. Bamba, A. Bell, A. A. Estrada-graf, and Z. I. Johnson, "Integrating microbial communities into algal biotechnology: a pathway to enhanced commercialization," 2022.
- [143] A. S. Bawa & K. R. Anilakumar, Genetically modified foods: safety, risks and public concerns, a review. *J Food Sci Technol.* 2013, 50(6): 1035-1046 <https://doi.org/10.1007/s13197-012-0899-1>
- [144] P. Rozas, E. I. K. Pérez, and C. Martínez, "Genetically modified organisms: adapting regulatory frameworks for evolving genome editing technologies," *Biol. Res.*, pp. 1-14, 2022, <https://doi.org/10.1186/s40659-022-00399-x>
- [145] A. Paula, F. De Oliveira, A. Pavesi, and A. Bragotto, "Microalgae-based products: Food and public health," vol. 6, no. June, 2022, <https://doi.org/10.1016/j.fufo.2022.100157>
- [146] E. Stavridou *et al.*, "Landscape of microalgae omics and metabolic engineering research for strain improvement: An overview Maria," vol. 587, no. March, 2024, <https://doi.org/10.1016/j.aquaculture.2024.740803>
- [147] B. Barati *et al.*, "Biomass and Bioenergy Recent progress in genetically modified microalgae for enhanced carbon dioxide sequestration," *Biomass and Bioenergy*, vol. 145, no. December 2020, p. 105927, 2021, <https://doi.org/10.1016/j.biombioe.2020.105927>