Discover Applied Sciences

Review

Genetically engineered microalgae for enhanced bioactive compounds

Ahmad Hanafi Ahmad Kamal[1,2] · Nor Farahdevah Mohd Hamidi[1] · Muhammad Faiz Zakaria[1,2] · Aziz Ahmad[1] · Mohd Razif Harun[3] · Thirukanthan Chandra Segaran[4] · Malinna Jusoh[1,2]

Received: 26 February 2024 / Accepted: 23 July 2024
Published online: 09 September 2024

Abstract
Microalgae are naturally rich in carbohydrates, lipids, proteins, pigments, minerals, and vitamins. These metabolites make them a renewable and sustainable source of bioactive compounds in nutraceuticals, food, feed for aquaculture, and biofuels. Genetic engineering of the existing strains remains crucial in utilising and upscaling microalgae-based biorefineries. CRISPR/Cas9, TALENs, ZFNs, and genetic transformation are the established tools utilised in microalgae genetic engineering. The common genetic transformation methods include electroporation, particle bombardment (biolistics), glass-bead agitation, and *Agrobacterium*-mediated transformation. Expression vectors and promoters are the prerequisites in genetic engineering. The present accessibility of genome sequences and omics datasets from a diverse array of microalgae species holds promise for catalyzing strategic progress in developing a superior microalgae strain suitable for numerous applications. This paper describes the genetic engineering to enhance microalgae biomass and metabolite production, particularly lipids. The advantages and precautions to fulfil the future application of genetically engineered microalgae are also reviewed and addressed.

Article highlights

- Strategic genetic engineering optimises microalgae strains for enhanced lipid synthesis and biomolecule production.
- CRISPR/Cas9 enables precise genome modifications in microalgae, enhancing their potential for various applications.
- Establishing regulatory frameworks for genetically modified (GM) microalgae is crucial for safe deployment.

Keywords Bioengineering · Biomolecules · Genome · Genetic engineering · Microalgae

✉ Malinna Jusoh, malinna@umt.edu.my | [1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. [2]SATREPS-COSMOS Laboratory, Centre of Research and Field Service, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. [3]Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. [4]Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

1 Introduction

Microalgae, a diverse group of photosynthetic microorganisms, inhabit various aquatic environments, including oceans, rivers, and freshwater systems. These organisms, typically ranging from a few to several hundred micrometres, lack conventional plant structures such as roots, stems, and leaves. Instead, they possess chloroplasts and other cellular organelles that facilitate photosynthesis, converting carbon dioxide into valuable organic compounds. These compounds have potential applications in producing bioactive substances for food, feed, and biofuel industries.

Regarding taxonomy, traditionally, microalgae are broadly classified into prokaryotic and eukaryotic categories. Prokaryotic microalgae are represented by Cyanobacteria (Cyanophyceae), while eukaryotic microalgae encompass a wider diversity, classified into seven distinct phyla: Euglenozoa, Cryptista, Haptophyta, Heterokontophyta, Glaucophyta, Rhodophyta, and Chlorophyta [1, 2]. This rich taxonomic and evolutionary diversity poses unique challenges in cultivation and genetic manipulation.

Microalgae stand at the forefront of sustainable biocompound production, owing to their capability to synthesise a broad spectrum of metabolites such as proteins, carbohydrates, amino acids, lipids, polysaccharides, fatty acids, vitamins, minerals, and pigments, including carotenoids, chlorophyll, and phycocyanin. Their rapid growth rate and minimal land requirements offer a significant advantage over terrestrial plants in producing renewable, sustainable, and cost-effective bioactive compounds. This attribute positions microalgae as a promising, sustainable source of food and non-food products. Recent studies have highlighted their potential in producing nutraceuticals [3–5], aquaculture feeds [6–8], and biofuels [9–11], as depicted in Fig. 1. Cultivating microalgae requires sufficient light, nutrients, and carbon dioxide, but notably, they do not necessitate fertile soil, thereby mitigating land irrigation concerns.

Some microalgae species exhibit robust growth under extreme environmental conditions and limited nutrient availability. The feasibility of cultivating microalgae in wastewater and recycled water further underscores their adaptability. This cultivation approach not only treats wastewater but also generates biomass suitable for biodiesel

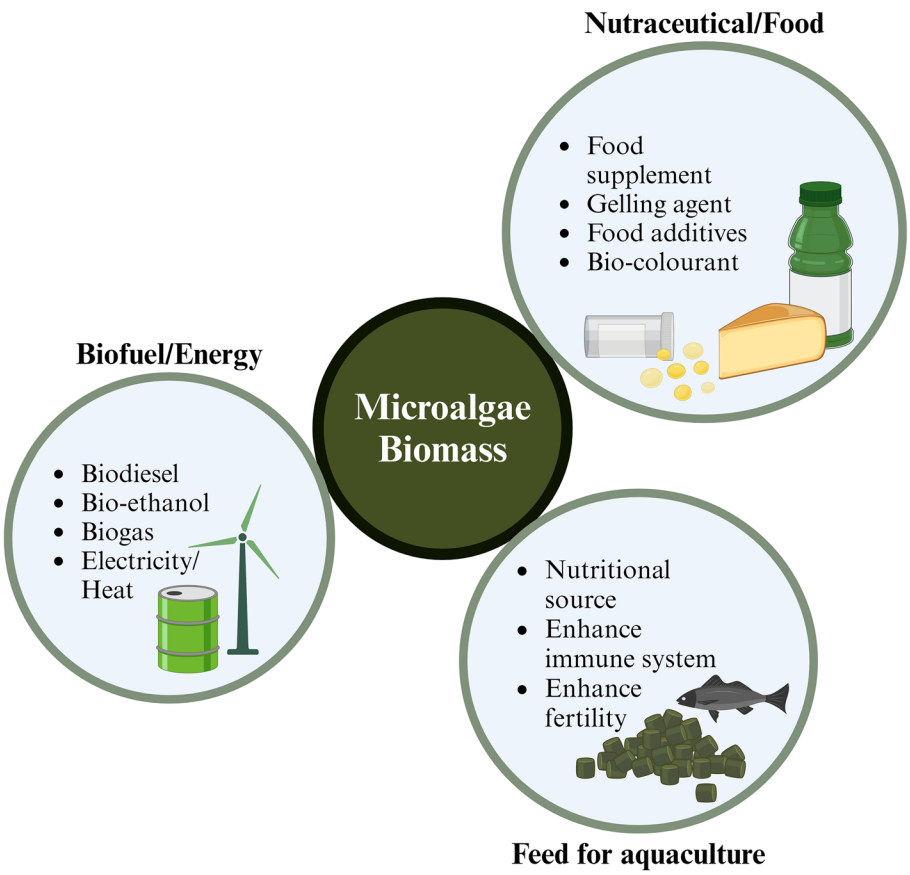


Fig. 1 Potential uses of microalgae in industries

production. Studies have indicated that using recycled water for microalgae cultivation can significantly save water usage by up to 84% and nutrient consumption by 55% [12].

Moreover, microalgae's ability to utilise carbon dioxide from industrial flue gases offers an economical approach to cultivation. [13] have demonstrated that this method can reduce cultivation costs without compromising biomass yield or bio-crude quality. This dual capacity of microalgae to serve both as an effective carbon sink and a source of valuable bioactive compounds highlights their integral role in sustainable biotechnology and environmental management. Despite the myriad benefits of microalgae in producing bioactive compounds, notable challenges impede the commercialisation of microalgal-based products. Key among these is the need for strain improvement to enhance microalgae-derived products' metabolic efficiency and economic viability. Genetic engineering emerges as a pivotal solution to these challenges, enabling the consistent production of high-yield microalgal biomass.

Microalgae produce various biomass, lipids [14], fatty acids, carotenoids, and hydrocarbon profiles [15]. Microalgae are also recognised as the next-generation sustainable feedstock in aquaculture [16] especially due to the high accumulation of lipids. To sustain aquaculture industries, lipids sources are important for the growth and development of aquatic animals. Lipid accumulation in microalgae is usually affected by growth phases [17] and cultivation conditions, including nutrients, light intensity, and temperature. In most microalgae species, lipids are highly synthesised when microalgae achieve a stationary phase of growth [18]. In addition, microalgae lipids are considered renewable energy resources with zero net carbon emissions, as microalgae can convert atmospheric carbon dioxide into biomass and other nutrition compounds through the photosynthesis process [19]. The ability to reproduce fast, higher lipids than terrestrial plants [20] and carbohydrate productivities cause microalgae to have high commercial potential [21, 22].

Genetic engineering, as defined by [23], involves integrating foreign genetic material into the genome of a host organism, resulting in genetic modifications. This technology has proven instrumental in enhancing microalgae strains through sophisticated molecular genetics techniques, significantly boosting productivity. The evolution of genome editing technologies has now made it feasible to target and directly modify the genomic sequences of almost all eukaryotic cells, including those of microalgae.

This review delves into the molecular tools and techniques employed in microalgae to develop genetically engineered strains. It comprehensively covers the range of genetic modifications applied to microalgae, examining their efficacy and potential in strain enhancement. Furthermore, the review addresses the risks linked with genetically modified microalgae, including ecological and health concerns, and outlines strategies for effective risk management. The potential of bioengineering in microalgae, especially in the context of sustainable and efficient production of bioactive compounds, forms a core part of this discussion. This approach holds promise for industrial-scale production and contributes to the broader goal of sustainable biotechnology.

2 Molecular tools for genetic engineering in microalgae

The development of microalgae as a viable model for producing customised bioproducts necessitates a comprehensive molecular toolkit capable of precisely manipulating their genomic architecture. Such advancements are crucial for harnessing the full potential of microalgae in biotechnological applications, enabling the production of superior bioproducts. In contrast to microorganisms like yeast and bacteria, which have a wide array of engineering tools for industrial product synthesis, microalgae require a more specialised set of molecular tools. [24] highlight the disparity in the availability of genetic engineering resources between these organisms. The recent progress in identifying and refining novel genetic elements and transformation techniques positions microalgae as increasingly competitive candidates in the biotechnology sector, as illustrated in Fig. 2. This section underscores the significance of expression vectors and promoters, which are fundamental to the genetic engineering process.

2.1 Expression vectors

In the realm of synthetic biology, the construction of expression vectors is a pivotal process, enabling the manifestation of desired phenotypes and functions in model organisms. These vectors, primarily derived from plasmids, are engineered to drive the expression of target genes, thus demonstrating specific phenotypes and functionalities in model organisms. Highlighting this critical role [25] underscores the foundational impact of synthetic biology in facilitating the creation of these essential tools. The advent of the Golden Gate Modular Cloning toolkit (MoClo), as reported by [26], represents a significant advancement in this domain. This toolkit simplifies the creation of genetic vectors through a library of genetic

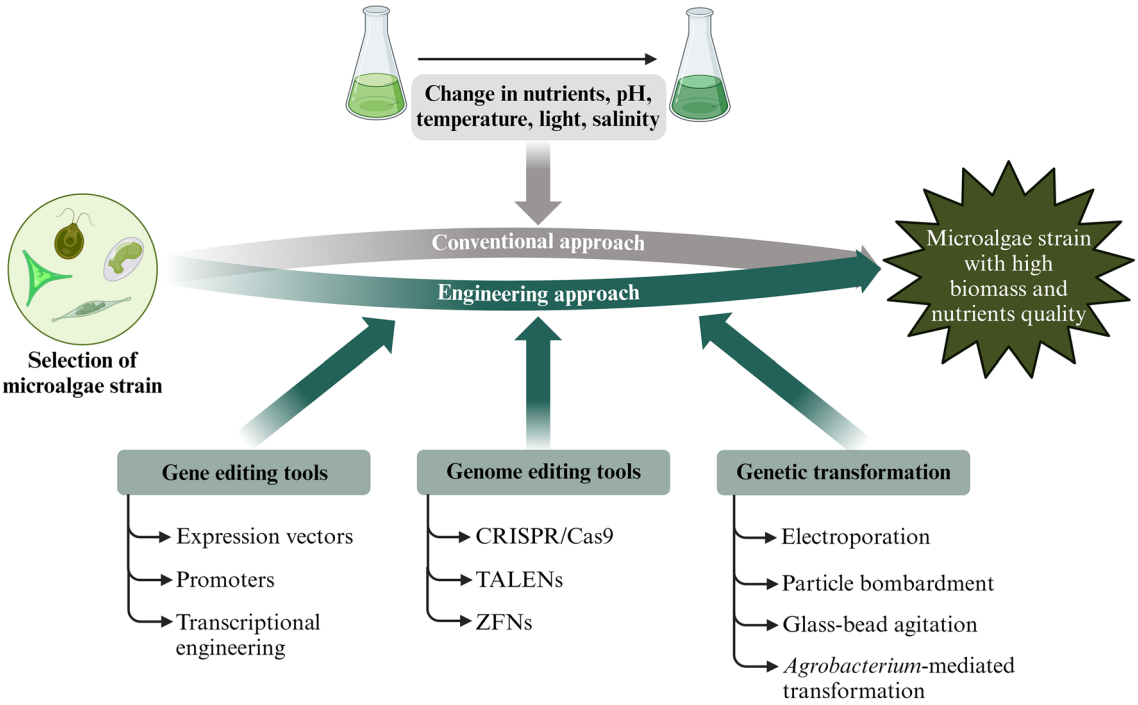


Fig. 2 Overview of tools/techniques used in microalgae genetic engineering

blocks, guiding both the design and assembly processes. Moreover, it provides a robust framework for developing long-term synthetic biology systems.

The impact of such toolkits on the field of microalgae biotechnology has been profound, as indicated by [27, 28]. The MoClo toolkit, first developed for *Chlamydomonas reinhardtii* by [29], incorporates over 100 gene pieces, encompassing a wide range of genetic elements such as terminators, promoters, tags, untranslated regions (UTRs), antibiotic-resistance genes, introns, and reporters. This toolkit allows for the assembly of *C. reinhardtii* codon-optimised gene expression vectors, which are highly effective in gene expression.

In pathway engineering, where the orchestration of multi-gene transcription is essential for effective biosynthesis, the role of enzymes is critical. Recent advancements in gene stacking technologies have begun to address challenges of predictability and efficiency in metabolic engineering [28]. The integration of sequence optimisation algorithms with computational methods has shown efficacy in boosting the efficiency of gene expression vectors [30]. For instance, the modification of nuclear gene expression in *C. reinhardtii* to produce different synthetic enzymes resulted in expression variability up to 65-fold [28, 31], underscoring the importance of precise codon optimisation for achieving high levels of transgenic expression [31].

The development of the Chlamys Sequence Optimizer (CSO) by [32, 33] is a testament to these advancements. The CSO, which is freely available, enables users to choose from three experimentally validated codon-optimising algorithms. Integrating modular tools with expression vector creation methods significantly accelerates the engineering process, allowing researchers to concentrate on developing and scaling new microalgal products [33]. It also facilitates faster screening and enhances the expression rate of recombinant proteins and natural metabolites, thanks to a standardised set of resources and molecular toolkits [28]. As the repertoire of gene sections expands, it is anticipated that the scientific toolkits for microalgae will continue to evolve, opening new avenues in microalgal biotechnology.

2.2 Promoters

The efficacy of metabolic and recombinant product yields hinges significantly on identifying and utilising cis-regulatory elements, particularly promoters, that can drive optimal expression of transgenes [28]. A promoter, a specific DNA segment, serves as the binding site for RNA polymerase and transcription factors, thereby initiating transcription. High gene expression levels have been observed with the use of promoters from prevalent microalgae production

strains [34], such as the chimeric HSP70/rbcs2 promoter (commonly known as ar1) from *C. reinhardtii* [35] and the fcp promoters in *Phaeodactylum tricornutum*.

However, transgene silencing is a major obstacle in achieving commercially viable amounts of recombinant proteins in microalgae, historically attributed to the organism's defensive mechanisms against viral invasions [36, 37]. In *C. reinhardtii*, for instance, transgene silencing has been linked to various factors, including the gene Mut6p, which is thought to be involved in post-transcriptional silencing through DNA degradation [38]. Additionally, certain protein factors in *C. reinhardtii* can induce chromatin compaction at insertion sites, further contributing to transgene silencing.

Recent advancements in microalgal transgene optimisation have concentrated on refining transformation processes to ensure the insertion of single-copy transgenes, thereby minimising silencing risks. A notable development is the use of the HSP70A promoter, which has been effective in preventing transgene silencing, as reported by [39]. Further enhancements have been achieved using high-expression promoters and flanking sequences derived from endogenous genes in *Chlamydomonas*, such as the Hsp70A-RbcS2 chimeric promoter and various inducible systems including copper-, nitrate-, heat-, light-, and alcohol-inducible promoters [40–44]. Innovative efforts have also led to the development of synthetic microalgal promoters (SAPs) in *C. reinhardtii*, spearheaded by [28, 45]. These SAPs, such as sap11, incorporate novel DNA motifs crucial for promoter function and display enhanced performance over the strongest known endogenous promoters. These advances not only boost the expression levels of transgenes but also offer insights into the structural dynamics of green microalgae promoters, marking significant progress in the field of microalgal biotechnology.

The approach taken by [46] involved using native cis motif elements, shape, and overall nucleotide contents from *C. reinhardtii*'s top gene-generating genes to create these promoters. The potential for future SAPs libraries to yield further advancements in this field is substantial. These libraries have the potential to enhance the precision of gene alterations in microalgae, thereby greatly broadening the variety of promoters accessible in the genetic toolbox for primary microalgal production [47]. This area of research is poised for continued growth, augmenting the capabilities and efficiency of genetic engineering in microalgae.

2.3 Transcriptional engineering

Transcriptional engineering, a vital aspect of genetic manipulation, entails the regulation of multiple genes within a metabolic pathway through the engineering of transcriptional elements, notably transcription factors (TFs). TFs exert control over gene expression by binding to specific DNA sequences and interacting with RNA polymerase, thereby modulating transcription levels either upwards or downwards [48]. Unlike the targeted action of individual genes, TFs have the capacity to concurrently influence various segments of a metabolic pathway, thus offering a more holistic approach to genetic regulation [28].

The potential of transcriptional engineering has been exemplified in vascular plants, where it has been successfully employed to enhance anthocyanin levels [28]. In microalgae, most TFs have been identified serendipitously, with many yet to be characterised in terms of their specific functions [49]. However, insights from RNA sequencing data suggest that transcriptional regulatory networks based on TFs could effectively control multiple elements and binding sites of cellular metabolism [50]. [50] advocate applying this genetic engineering approach to augment the synthesis of various compounds in microalgae.

An example of a key transcription factor group in microalgae is the basic leucine zipper (bZIP) transcription factors, known for regulating metabolic processes like lipid production and contributing to stress responses. Modifying bZIP transcription factors presents a promising strategy for enhancing lipid production in microalgae species such as *Nannochloropsis* sp., as demonstrated through both endogenous and exogenous TFs [51].

In *Arabidopsis thaliana*, the AP2-type transcription factor WRINKLED1 (AtWRI1) is critical in regulating lipid accumulation [52]. When subjected to osmotic stresses, transformed cell lines expressing AtWRI1 exhibited a 44% increase in lipid content compared to wild types and a 36% increase under normal conditions. This finding underscores the potential of employing plant-derived TFs for heterologous gene expression in microalgae, indicating the scope for additional heterologous genetic elements [52].

The advancement of transcriptional engineering in microalgae hinges on the identification of more TFs involved in specific metabolic pathways, understanding their homologs across different microalgae species, and the establishment of standardised protocols for transcriptional engineering across species [53]. These steps are essential for harnessing the full potential of TFs in regulating gene expression, thereby enhancing the biosynthesis of desired products in microalgae.

2.4 Genome editing tools (CRISPR/Cas9, TALENs, and ZFNs)

The expansion and refinement of the biotechnology toolkit, particularly in the realm of genome engineering, represents a monumental leap in the field of microalgae research. Over the past decade, significant strides have been made in enhancing the array of tools available for the genetic engineering of microalgae, thereby bolstering their viability as a model organism for both scientific research and industrial applications.

One of the most pivotal advancements in this domain is the development of various genome editing technologies, each offering unique benefits and limitations, as summarised in Table 1. These include the Clustered Regularly Interspaced Short Palindromic Repeats with associated protein (CRISPR/Cas) systems, Transcription Activator-Like Effector Nucleases (TALENs), and Zinc-Finger Nucleases (ZFNs) [54]. These methods primarily operate by triggering DNA double-strand breaks, which then activate the cell's inherent DNA repair mechanisms. This mechanism can be utilized to either disable targeted genes or introduce donor DNA for transgene integration, as depicted in Fig. 3 [55].

TALENs are engineered tools that combine the FokI cleavage domain with DNA-binding domains from TALE proteins. These transcription activator-like effectors (TALE) proteins have repeat domains, each recognising a single base pair. TALENs create targeted double-strand breaks (DSBs) in DNA, activating repair pathways that allow precise genome editing [54, 56]. Essentially, the TALENs system acts as a custom-made restriction enzyme that can target specific sites on the genome. In a recent study, researchers used both meganucleases and TALE nucleases to transform the diatom *P. tricornutum*. They successfully edited seven genes important for lipid synthesis [57], leading to improved oil production in the modified strain. This demonstrates the potential of TALENs for precise genetic modifications and enhancing desired traits in microalgae.

ZFNs are engineered tools that combine the nonspecific DNA cleavage domain from the FokI restriction enzyme with zinc-finger proteins. Similar to TALENs, ZFNs create targeted DSBs in DNA, activating DNA damage response pathways. The zinc-finger domains are designed to guide ZFNs to specific genomic sites, allowing precise genome editing [54, 56]. By engineering these domains to target precise DNA sequences, researchers successfully applied ZFNs to modify the gene encoding channelrhodopsin-1 in *C. reinhardtii* [58].

Among these, CRISPR/Cas9 has emerged as a particularly transformative tool. It functions as a site-directed gene editor, enabling precise genome modifications in microalgae. This technology is derived from a bacterial immune response, where a single guide RNA (sgRNA) directs the Cas9 protein to target and cleave exogenous DNA sequences. This method has been effectively adapted for genetic engineering in microalgae [23, 59], offering a high degree of control over gene expression and addressing issues such as transgene silencing.

Implementing CRISPR/Cas9 and related technologies in microalgae genetic engineering has led to significant breakthroughs, extending the scope of possible modifications. This technique is applicable to microalgae and a wide range of microorganisms, revolutionising the field of genetic modification [23]. The advancements in CRISPR/Cas9 technology have pushed the boundaries of gene modification and device development, allowing for altering a diverse array of eukaryotic genomes [62].

The application of CRISPR/Cas9 technology in microalgae has marked a significant advancement in genetic engineering, particularly in diatoms like *P. tricornutum*. [63] highlights its critical role in both gene function analysis and the enhancement of industrial traits such as increased lipid content and biomass production. [64] demonstrated how CRISPR/Cas9 can be used to achieve stable genotype alterations in *P. tricornutum*, maintaining the modified genotype through successive generations. Further, the CRISPR-based approach has been employed to target the CpSRP54 gene, which is instrumental in indirect carbon fixation processes in *P. tricornutum*. This modification, as explored in studies including [65], underscores the potential of CRISPR/Cas9 to fine-tune metabolic pathways that are crucial for improving carbon utilisation and storage, thereby enhancing the organism's utility in industrial applications.

However, challenges have arisen in applying CRISPR/Cas9 in microalgae. The initial attempts at employing CRISPR/Cas9 in microalgae in 2014 encountered issues, such as Cas9 toxicity in *C. reinhardtii* when the CRISPR/Cas9 gene was constitutively expressed [66, 67]. Despite these hurdles, [28] reported successful CRISPR-mediated nucleotide replacement in *C. reinhardtii*, albeit with the persistent issue of Cas9 toxicity. Using oligonucleotide-directed mutagenesis (ODM) techniques by [66] also achieved genome modification in *C. reinhardtii*, although the mutagenic rates were low.

CRISPR-mediated gene modification in microalgae has primarily focused on producing gene knockout mutants instead of inserting protein-encoding genes [28]. While it has been a valuable tool in metabolic engineering to boost the production of natural microalgal chemicals, its use in generating high-value recombinant proteins has

Table 1 The advantages and disadvantages of genome editing tools used in microalgae genetic engineering (Adapted from [60, 61])

Genome editing tools	Advantages	Disadvantages
Clustered regularly interspaced short palindromic repeats with associated protein (CRISPR/Cas9)	1. Ease of design and implementation	1. Potential for off-target effects
	2. Cost-effective and straightforward application	2. Risk of unintended mutations
	3. High precision and efficiency in targeting specific genomic sequences. Versatile utility for not only editing but also applications in genome imaging, transcriptional regulation, and epigenetics	
Transcription activator-like effector nucleases (TALENs)	1. The recognition ability of one base instead of 3 bp of TALE proteins together with a fusion of the Fok I DNA cleavage domain served it as an efficient gene editing tool	1. Each target necessitates custom protein engineering for DNA recognition
	2. Greater efficiency and specificity compared to ZFNs, with reduced off-target effects	2. Sensitivity to cytosine methylation can affect targeting
	High specificity minimises off-target alterations	3. Relatively high development costs
Zinc-finger nuclease (ZFNs)	1. Increased targeted homologous recombination in model organisms	1. DNA targeting specificity is protein-dependent, requiring extensive engineering for each site
	2. Economical compared to newer genome editing technologies	2. The process is laborious, with lower specificity and efficiency
		3. Development and application processes are time-consuming

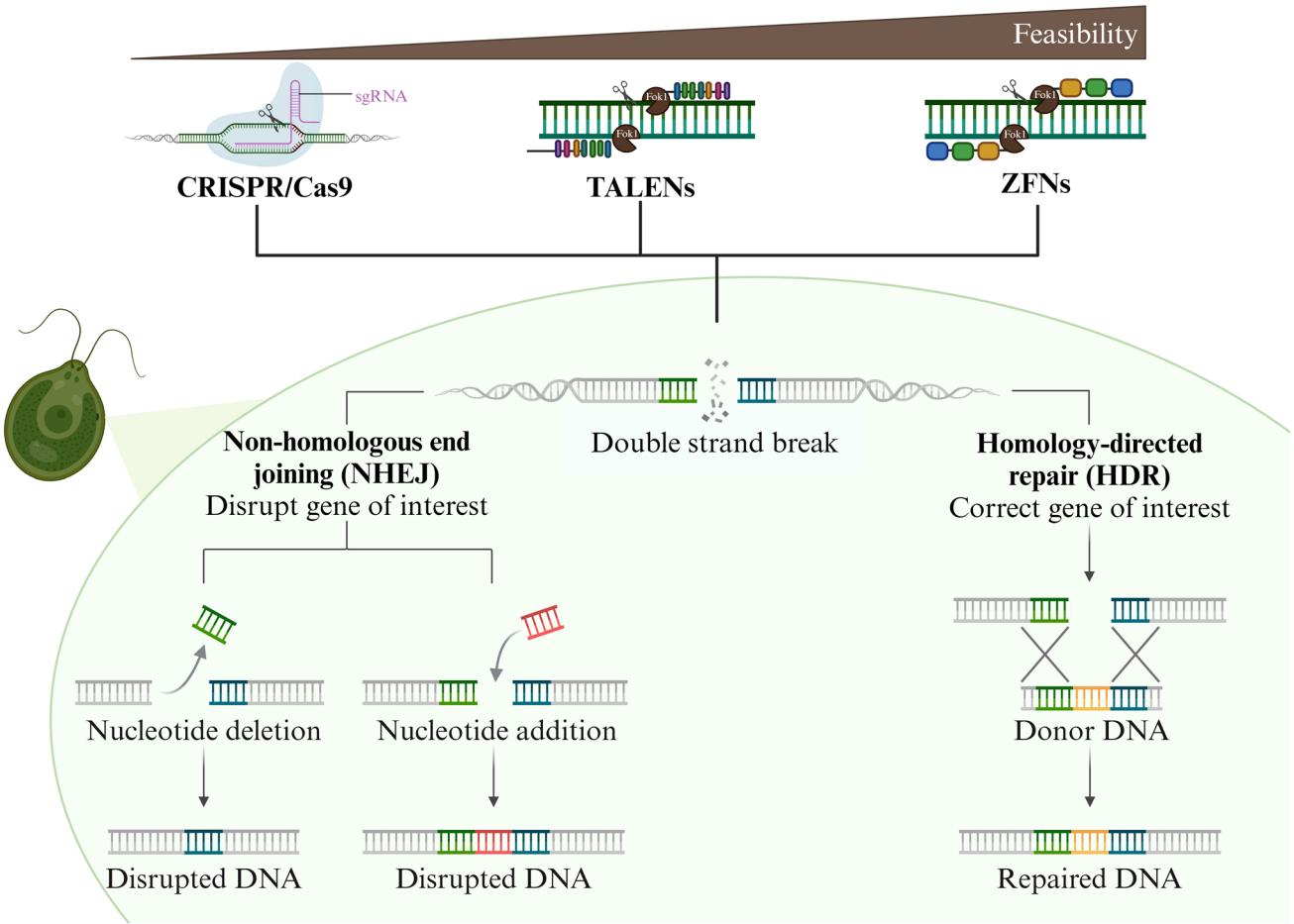


Fig. 3 Simplified working principle of genetic engineering tools-induced genome editing in microalgae

been limited. This limitation stems from *C. reinhardtii*'s (a commonly used strain for producing recombinant proteins) insufficient homologous recombination (HR) pathways for homology-mediated editing [28].

Genome editing in microalgae typically involves recombinant nuclear nucleases that recognise specific sequences and create double-strand breaks (DSBs) in the DNA [68]. Non-homologous end joining (NHEJ) repairs these breaks, often resulting in indel mutants within the target genes [69]. On the other hand, HR is a more precise mechanism involving the exchange of information between two identical sequences and is essential for inserting foreign DNA into specific genome locations. Due to the lack of HR in *C. reinhardtii*, NHEJ-mediated gene modification has been more prevalent [70].

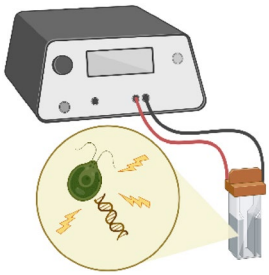
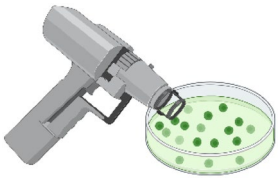
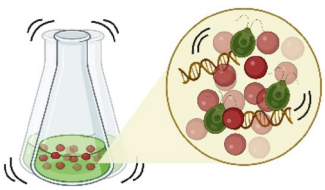
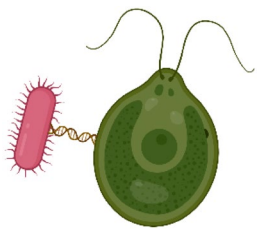
Future research is expected to advance HR-mediated gene editing in *C. reinhardtii* [71]. Such developments would significantly enhance the prospects of protein engineering in microalgae, opening new avenues for producing complex and high-value recombinant proteins, thereby expanding the scope and impact of microalgal biotechnology.

2.5 Genetic transformation techniques

In the field of microalgal genetic engineering, the introduction of optimised expression vectors into the microalgal genome is a critical step. This process requires techniques that ensure high efficiency and minimise the time spent on post-transformational screening [28]. Several genetic transformation techniques have been developed and successfully applied to microalgae, including electroporation, biolistic particle bombardment (biolistics), glass bead agitation, and *Agrobacterium*-mediated transformations [72]. Each of these methods has its own set of advantages and disadvantages, as detailed in Table 2.

Electroporation is the most efficient and commonly used method for nuclear transformations in microalgae, particularly *C. reinhardtii* [73]. This technique is favoured for its relative ease of use and high efficiency, capable of producing a significantly higher number of successful transformants with fewer false positives than other methods [74]. Electroporation

Table 2 The advantages and disadvantages of genetic transformation techniques used in microalgae genetic engineering (Adapted from [73, 80, 81])

Genetic transformation techniques	Advantages	Disadvantages
Electroporation	1. Simple, easy to perform, and very efficient	1. Requires homogenised single-cell preparations
	2. Not affected by cell wall presence	2. Necessitates specialised electroporation devices (e.g., Gene PulserXcell-Biorad, Gemini Systems-BTX)
		3. Exhibits reduced efficacy in cell-wall-bearing organisms
Particle bombardment (Biolistics)	1. Eliminate the need for the production of plant protoplasts or infection by *Agrobacterium*	1. Demands specific target cell diameters
	2. Not affected by cell wall	2. A tendency to generate rearranged and broken transgene copies
	3. Facilitates targeted gene knockouts	3. Potential reduction in cell viability
	4. Highly effective for both nuclear and chloroplast transformations	4. Need specialised equipment (PDS-1000/He apparatus-Biorad)
		5. Risk of vector integration into the microalgae genome
Glass beads agitation	1. Cost-effective, rapid, and straightforward methodology	1. Offers lower transformation efficiency
	2. Utilises simple laboratory equipment	2. Requires cell wall removal
Agrobacterium-mediated transformation	1. Protoplast generation is unnecessary	1. Transformation efficiencies can be low and highly variable
	2. Capable of integrating large DNA segments (> 100 kb)	2. Does not guarantee enhanced gene expression
	3. Yields stable and preferential DNA integration	3. Involves the use of large Ti-Plasmids
		4. Complex equipment and protocols may be required

involves the application of an electric pulse to temporarily permeabilise the cell membrane, allowing foreign DNA to enter the cells. To optimise the electroporation process, researchers have developed tools and methods for fine-tuning the parameters involved. [75] highlighted the importance of optimising this procedure for maximum efficiency. Additionally, [76] identified optimal conditions for electroporation, including light intensities, voltage settings, and cell concentrations. These findings provide valuable insights for determining the most effective experiment conditions.

The integration of the plastid genome via electroporation, coupled with a nuclear-targeted construction, has marked a milestone in the field of microalgal biotechnology, notably with the first successful chloroplast transformation of *Nannochloropsis* sp. [28]. However, the transformation of chloroplasts in *Nannochloropsis* sp. poses challenges due to the organism's small size and the yet-to-be-characterised components of its cell wall, suggesting avenues for further methodological improvements [77]. Despite these challenges, electroporation remains the predominant method for transforming various microalgal species [78]. Nonetheless, alternative transformation methods may be more effective for certain taxa. For instance, [79] reported that haptophytes had been successfully transformed using both biolistic and *Agrobacterium*-mediated approaches, with biolistic yielding the most favourable results.

In the diatom *P. tricornutum*, the biolistic delivery of CRISPR/Cas9 ribonucleoproteins facilitated the creation of double-gene knockouts of a photoreceptor-encoding gene with an efficiency of 65–100%, establishing a DNA-free editing system for the species [82]. Additionally, the glass bead agitation method, which involves vortexing microalgae cells with glass beads, has been employed to transform artificial microRNA into *C. reinhardtii*, aiming to regulate gene expression and enhance fatty acid content. This method is noted for its simplicity and the absence of specialised equipment requirements, although it only applies to cells with compromised cell walls or strains pretreated with autolysin [28].

Agrobacterium-mediated transformation has demonstrated higher success rates in generating transformants compared to the glass bead method, as indicated by [83]. This method, however, requires the co-culture of transgenic bacteria with microalgae. In response, more streamlined techniques, such as electroporation, have been developed to offer simpler, cost-effective alternatives [84]. Although Agrobacterium-mediated transformation generally results in fewer transgene-silenced transformants, it does not significantly outperform electroporation in *Chlamydomonas*, where it is often more labour-intensive and prone to generating false positives [85].

The variety of available transformation techniques underscores the necessity of selecting an appropriate method tailored to the unique requirements and characteristics of the target microalgal species. Ongoing refinement and adaptation of these methods are critical for the progressive capabilities of microalgal genetic engineering, opening new avenues in biotechnological applications.

3 Genetic engineering of microalgae for enhanced biorefinery capabilities

The genetic engineering of microalgae to enhance biorefinery capabilities represents a transformative approach to sustainable biotechnology. Microalgae can be optimised through metabolic engineering to achieve higher productivity and efficiency, improving their economic viability and sustainability. Given the critical role of microalgae in the nutraceutical and food industries, aquaculture feed, and biofuel production, considerable attention has been focused on elucidating and manipulating their biosynthetic pathways [86].

The strategic application of genetic engineering in microalgae targets optimising lipid synthesis, biomolecule production, and photosynthetic efficiency, among other traits. This optimisation is achieved using various genetic engineering techniques tailored to the specific metabolic profiles and biorefinery potentials of different microalgal species. These approaches are crucial for enhancing the efficiency and economic viability of microalgae in biotechnological applications. The following are key examples demonstrating how genetic engineering has effectively improved microalgae for diverse biorefinery purposes:

3.1 Enhancement of lipid production

The advancement of lipid synthesis in microalgae via genetic engineering marks a crucial advancement for multiple sectors, especially in the production of third-generation biofuels and dietary supplements. The ability to boost triacylglycerol (TAG) and polyunsaturated fatty acid (PUFA) production, including omega-3 fatty acids, through metabolic engineering not only promises cost reduction but also presents a sustainable substitute for petroleum-derived goods and fish oils. These traditional sources are restricted by environmental and resource constraints [87–89].

Key enzymes in the lipid biosynthesis pathway, such as malonyl-CoA: ACP transacylase (MAT) and acetyl-CoA synthetase (ACS), have been identified as targets for metabolic engineering to increase the production of microalgal PUFAs to commercially viable levels [28, 90]. ACS plays a crucial role in lipid biosynthesis by converting acetate into acetyl-CoA, a fundamental molecule in fatty acid production [91]. Meanwhile, MAT is involved in forming malonyl-ACP intermediates, essential for both polyketide and fatty acid synthesis in species like *Schizochytrium* sp. [90].

Several innovative genetic strategies have been employed to boost lipid production in microalgae. In *C. reinhardtii*, the overexpression of endogenous chloroplast acyl-CoA synthetase (ACS) genes using the HSP70A and RBCS2 promoters has led to significant increases in ACS transcripts and triacylglycerol (TAG) accumulation, particularly under nitrogen-depleted conditions with acetate supplementation [91, 92]. Additionally, employing the TEF promoter to overexpress malonyl-CoA transacylase (MAT) has notably enhanced docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) yields [28]. Other species have also shown promising results. For instance, targeting ACCase in *C. cryptica* and *N. saprophila* increased enzyme activity, though it did not affect lipid content [93]. Conversely, targeting ACS genes in *Schizochytrium* sp. led to an 11.3% increase in fatty acids and a 29.9% increase in biomass [94]. In *N. oceanica*, manipulation of NoMCAT genes resulted in increases of 36% in total fatty acids and 31% in neutral lipids, alongside improved growth and photosynthetic efficiency [95]. Furthermore, targeting the PtTE gene in *P. tricornutum* enhanced the accumulation of shorter chain-length fatty acids, incorporating 75–90% into TAGs without significant fatty acid secretion [96]. Lastly, in *C. reinhardtii*, targeting DtTE genes led to a substantial increase in neutral lipids and total fatty acids without affecting growth [97].

These breakthroughs in genetic engineering underscore the potential for microalgae to serve as a scalable and sustainable source of essential omega-3 fatty acids like DHA and EPA, which are crucial for human health due to their roles in embryonic development and their anti-inflammatory properties [98]. The advancements in microalgal lipid production through genetic engineering promise to enhance microalgae's economic viability as a source of biofuels and nutraceuticals and contribute to sustainable practices in these industries. Further exploration and optimisation of genetic engineering techniques in microalgae are expected to continue advancing their role in biorefineries, making them an even more valuable resource for producing a wide range of lipid-based products. Examples of genetic engineering for enhanced lipid content in microalgae are shown in Table 3.

3.2 Enhancement of other biomolecules

The enhancement of carotenoid production in microalgae through genetic engineering holds significant promise for various applications, particularly in human health [123]. Carotenoids, a diverse group of pigments found abundantly in microalgae, are not only crucial for efficient photosynthesis by absorbing light energy and protecting chlorophyll but also offer potential health benefits, including the prevention of diseases such as lung cancer [124–126]. Given the well-characterised carotenoid biosynthesis pathway and the identification of its key genes, metabolic engineering techniques have been increasingly applied to boost carotenoid production in microalgae [127].

Phytoene synthase (PSY), which catalyses the initial step in carotenoid biosynthesis, is considered a rate-determining enzyme in the pathway, leading to the production of phytoene [128]. This is followed by the action of enzymes such as ζ-carotene desaturase (ZDS), phytoene desaturase (PDS), and carotene cis–trans isomerase (CRISCO), which convert phytoene into lycopene, a key intermediate for the synthesis of high-value carotenoids [73, 129]. By increasing the expression of genes upstream from lycopene synthesis, such as PSY and PDS, the production of carotenoids can be significantly enhanced. Studies have shown that regulating the PSY gene in microalgae species like *P. tricornutum*, *Haematococcus pluvialis*, and *C. reinhardtii* can increase carotenoid synthesis [130]. Similarly, manipulating PDS gene expression has been demonstrated to boost carotenoid production in various microalgae [131].

The process of converting lycopene into α- or β-type carotene and subsequently into lutein and zeaxanthin involves specific lycopene cyclases and carotene cyclases/hydrolases [132, 133]. While there is a scarcity of publications on the genetic engineering of these enzymes in microalgae, their regulation could potentially increase carotenoid production under certain conditions, such as nitrogen depletion.

Furthermore, the oxidation of β-carotene or zeaxanthin in species like *H. pluvialis* and *C. zofingiensis* can lead to the synthesis of higher-value biocompounds such as canthaxanthin, astaxanthin, or violaxanthin [134]. Enhancing or introducing the β-carotene oxygenase (BKT) enzyme, crucial for astaxanthin production, into model organisms has been a focus of research due to astaxanthin's applications in anti-inflammatory and skincare products [135, 136]. The successful introduction of BKT in *C. reinhardtii*, leading to astaxanthin production, exemplifies the potential of genetic engineering in this area [137].

Table 3 Examples of genetic engineering for enhanced lipid content in microalgae

Microalgae strains	Approach	Gene/target site	Results	References
Chlamydomonas reinhardtii	Heterologous expression	Diacylglycerol acyltransferase 2 (DGAT2)	Increased α-linolenic acid by more than 12%	[99]
C. reinhardtii	Knockout/CRISPR/Cas9	Phospholipase A2 (PLA2)	Increased lipid by 64.25%	[100]
C. reinhardtii PTS42	Overexpression	Malic enzyme isoform 2 (ME2)	Increased lipid by 23.4%	[101]
C. reinhardtii CC4349	CRISPR-Cas9RNP-mediated knockout method	Zeaxanthin epoxidase (ZEP) and acid glycoprotein (AGP) genes	Increased oil by 81%	[102]
C. reinhardtii	Knocking out of phospholipase	A2 gene	Increased lipid by 64.25%	[103]
C. reinhardtii	Suppression	Phosphoenolpyruvate carboxylase (PEPC)	Improved lipid by 14–28%	[104]
C. reinhardtii	Overexpression	Acetyl-CoA synthetase 2 (ACS2)	Increased TAG by 2.4-fold	[91]
C. reinhardtii	Heterologous expression	DtTE gene	Increased neutral lipid by 69%	[97]
Phaeodactylum tricornutum	Overexpression	Glycerol-3-phosphate acyltransferase (GPAT) and DGAT2 genes	Increased lipid by 2.6-fold	[105]
P. tricornutum	TALENs	ptTES1	Increased TAG by 1.7-fold	[106]
P. tricornutum	Overexpression	Phytochrome A (PhyA)	Increased DHA by 12% and EPA by 18%	[107]
P. tricornutum	Overexpression	Malic enzyme (ME)	Increased lipid by 2.5-folds (31% increase in neutral lipid)	[108]
P. tricornutum	Suppression	Pyruvate dehydrogenase kinase (PDK)	Increased lipid up to 82%	[109]
Nannochloropsis oceanica	Engineering a control-knob gene	AtDXS gene	Increased lipid by ~ 68.6% in nitrogen depletion and ~ 110.6% in high light	[110]
N. oceanica	Transposome complex (mutagenesis)	Transposome	Increased PUFA by 180% and EPA by 40%	[111]
N. oceanica	Upregulation	Malonyl CoA-acyl carrier protein transacylase (MCAT)	Increased lipid by 31%	[112]
N. oceanica	Overexpression	NoMCAT	Increased neutral lipid by 31%	[95]
N. salina	Overexpressed a bZIP TF, NsbZIP1	Basic leucine zipper (bZIP)	Increased lipid by 50%	[113]
N. salina	Upregulation	Basic helix-loop-helix (bHLH)—NsbHLH2	Increased fatty acid methyl ester (FAME) by 33%	[114]
Nannochloropsis sp.	Insertional mutagenesis	In *N. salina* to develop *Nannochloropsis* mutant	Increased lipid (FAME) by 75%	[100]
Neochloris oleoabundans	Co-overexpression	Plastidial lysophosphatidic acid acyltransferase (NeoLPAAT1) and endoplasmic reticulum-located diacylglycerol acyltransferase 2 (NeoDGAT2)	Increased TAG by 2.1-folds and significantly higher production of lipid by 1.6-folds	[110]
Synechocystis sp. PCC 6803	Overexpression	Acyl-ACP synthetase (AAS)	Increased lipid by 5.4%	[115]
Synechocystis sp.	Overexpression	Acetyl-CoA carboxylase (ACC)	Increased lipid by 3.6-fold	[116]
Scenedesmus obliquuss	Upregulated genes	Differential expression genes (DEGs)	Increased lipid by 2.4-fold	[117]
S. quatricauda	Heterologous expression	Acetyl-CoA carboxylase 1 (ACC1)	Increased lipid by 1.6-fold	[118]
Mortierella alpina	Overexpression	Δ6-desaturase from *Micromonaspusilla* CCMP1545	Increased EPA by 26.2-fold	[119]
Schizochytrium sp.	Upregulation	Malonyl-CoA-ACP transacylase (MAT)	Increased PUFA and lipid by 10.1%	[120]
Schizochytrium sp.	Overexpression	MAT	Increased PUFA by 24.5%	[90]

Table 3 (continued)

Microalgae strains	Approach	Gene/target site	Results	References
Chlorella pyrenoidosa	Overexpression	NAD (II) kinase	Increased lipid by 110.4%	[121]
Cyanidioschyzonmerolae	Heterologous expression	Acyl-ACP-reductase	Increased TAG by threefold	[122]

While traditional approaches to increase carotenoid production have often relied on modifying culture conditions or inducing stress, the exploration of genetic engineering offers a more targeted and efficient strategy to enhance carotenoid biosynthesis in microalgae. As research continues to evolve, genetic engineering techniques are expected to play a pivotal role in maximising the production of carotenoids and other valuable biomolecules in microalgae, expanding their applications in health, nutrition, and beyond. Other examples of microalgae genetic engineering for enhanced carotenoids are given in Table 4.

3.3 Improvement of photosynthetic efficiency

The transformation of atmospheric carbon dioxide into energy-rich organic compounds is fundamental for sustaining life on Earth, with carbon fixation playing a pivotal role in this process. Carbon fixation involves the conversion of inorganic carbon from the atmosphere into organic compounds by autotrophic organisms, including photoautotrophic organisms like cyanobacteria, plants, and microalgae. These organisms utilise the Calvin cycle, among other mechanisms, to convert carbon dioxide and water into organic carbon, facilitated by chlorophyll [148–150].

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) is the primary enzyme responsible for carbon fixation, acting at the initial stage of the Calvin cycle. RuBisCo, consisting of large and small subunits encoded by chloroplast and nuclear DNA, catalyses carbon dioxide and ribulose-1,5-bisphosphate conversion into 3-phosphoglycerate. It also catalyses a side reaction converting oxygen and ribulose-1,5-bisphosphate into 2-phosphoglycolate, a metabolite detrimental to cell growth [151–154].

Efforts to genetically modify RuBisCo aim to enhance its selectivity and velocity, reduce unproductive side reactions, and improve overall activity. However, challenges remain in enhancing both selectivity and velocity simultaneously due to the inherent limitations of RuBisCo [155, 156]. The exploration of heterologously expressed RuBisCo variations, particularly in red microalgae, shows promise for boosting carbon fixation efficiency beyond the capabilities of native RuBisCo [157, 158]. In addition to RuBisCo optimisation, controlling metabolic flux and regulating the Calvin cycle pathway is crucial for enhancing carbon fixation. Strategies such as the bypassing of the photorespiratory pathway via the introduction of phosphoglycolate rerouting enzymes can lead to increased carbon fixation rates and biomass formation, highlighting the significance of metabolic flux regulation in optimising photosynthetic efficiency [73, 159, 160].

Furthermore, the overexpression of key Calvin cycle enzymes, such as sedoheptulose-1,7-bisphosphatase, has been identified as a method to boost photosynthetic efficiency and carbon fixation, underscoring the potential for targeted genetic interventions to improve carbon fixation efficiency [161, 162]. However, the enhancement of carbon fixation does not solely rely on modifying individual enzymes within the Calvin Cycle, as the overall balance of flux through the cycle is essential for achieving high microalgae biomass, as shown in Fig. 4. These advances in genetic engineering and metabolic pathway optimisation offer promising avenues for increasing the efficiency of carbon fixation in microalgae, contributing to the development of more sustainable and productive systems for converting atmospheric carbon dioxide into valuable organic compounds.

4 Management of genetic engineering in microalgae

Managing genetic engineering in microalgae needs to be considered to ensure safety, efficacy, and ethical practices. Even though genetic engineering is one of the most powerful technologies in improving the production value of microalgae metabolites, it leads to divergent opinions on the safety of genetically modified microalgae for human health and the environment [163].

Despite the potential of genetic engineering to enhance microalgal performance, there are significant environmental and ecological concerns associated with these technologies that are yet to be fully debated. Globally, the deployment of transgenic and recombinant microalgae is governed by stringent regulations and policies, ensuring careful assessment and management of any associated risks [164, 165]. This regulatory framework is crucial as it not only safeguards biodiversity but also ensures that technological advancements in microalgal research are responsibly aligned with policy developments. Moreover, while international collaborations continue to drive forward the genetic enhancement of microalgae, the translation of these advancements into commercial applications remains restricted. [155] note that, despite extensive research efforts, commercial exploitation of genetically modified microalgae is still largely prohibited, underscoring the need for a balanced approach that weighs innovation against potential environmental impacts.

Table 4 Examples of genetic engineering for enhanced carotenoid content in microalgae

Microalgae strain	Approach	Gene/target site	Result	References
Chlamydomonas reinhardtii	DNA-free CRISPR/Cas9, knock-out mutant	Zeaxanthin epoxidase (ZEP)	Increased zeaxanthin by 56-fold	[138]
C. reinhardtii	Heterologous expression	Bifunctional PBS gene	Increased β-carotene by 38% and lutein by 60%	[139]
C. reinhardtii	Overexpressed via nuclear transformation	DXS and DXR genes	Increased β-carotene by 1.9-fold	[140]
Phaeodactylum tricornutum	Transcriptional upregulation	DXS and Phytoene synthase (PSY) genes	Increased fucoxanthin by 1.8-fold	[141]
P. tricornutum	Transformation and gene expression	PSY gene	Increased fucoxanthin by 1.45-fold	[142]
Haematococcus pluvialis	Codon optimised/overexpressed	Endogenous phytoene desaturase (PDS)	Increased astaxanthin by 67%	[143]
H. pluvialis	Cloning and overexpressed	β-carotene ketolase (BKT)	Increased carotenoids and astaxanthin by 2–threefold	[144]
Dunaliella tertiolecta	Antisense expression and overexpression	Carotenoid biosynthesis-related (CBR)	Increased zeaxanthin by 2.22-fold	[145]
D. tertiolecta	Random mutagenesis	mp3	Increased zeaxanthin by 10–15%	[146]
Chlorella zofingiensis	Overexpression	PDS	Increased carotenoid by 32.1% and astaxanthin by 54.1%	[147]

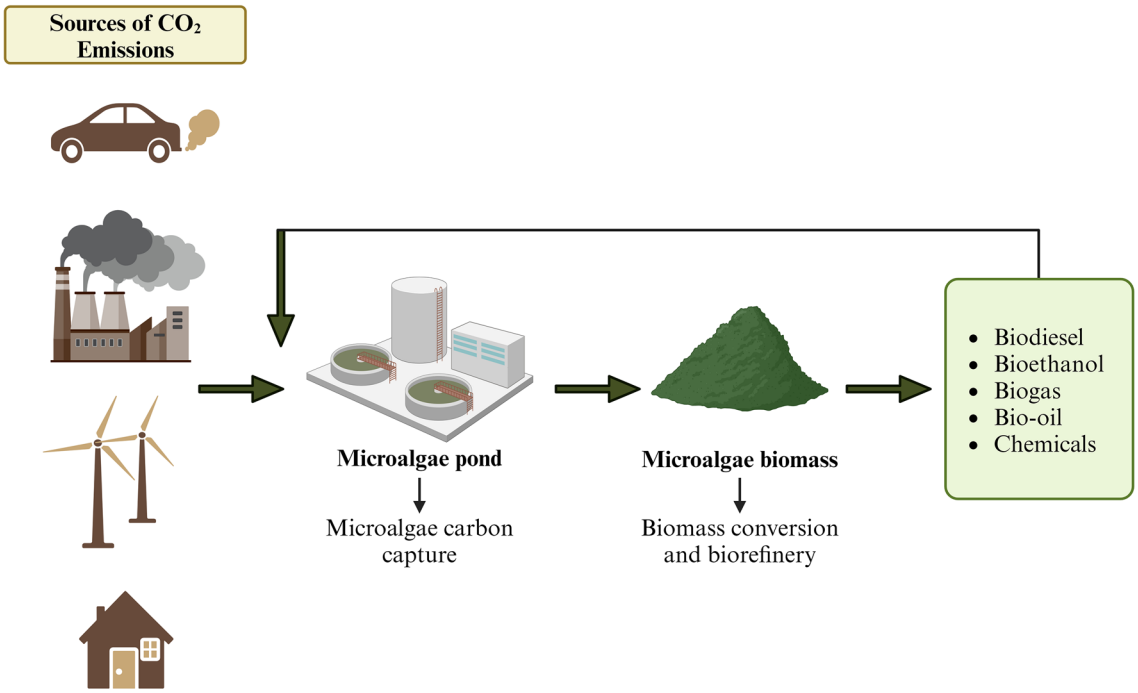


Fig. 4 The schematic of microalgae cultivation, photosynthetic fixation of CO_2 into microalgae biomass and biomass processing

Navigating the realm of genetic engineering in microalgae requires a careful balance of its potential advantages against possible risks, with a strong emphasis on ethical and regulatory considerations. Adopting a cautious and responsible approach towards genetic modifications in microalgae is crucial. The application of genetically modified microalgae in fields like agriculture and biotechnology draws significant attention, yet it simultaneously raises concerns regarding safety for the environment, animals, and humans—all primary consumers of microalgae products [60]. Therefore, establishing rules and regulations concerning genetically modified microalgae is vital for setting clear guidelines to safeguard the environment, animal welfare, and public health. In various countries, these rules and regulations are critical in overseeing genetically modified microalgae research, development, and commercialisation [163]. These regulations typically address risk assessment, labelling, containment, and monitoring strategies, ensuring that genetically modified microalgae are managed carefully to prevent adverse effects.

Regulatory frameworks overseeing genetically modified microalgae vary significantly among different nations, particularly concerning their use in food or feed and their release into the environment. In the United States, the oversight of genetically modified microalgae is overseen by three primary agencies: the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). These agencies are responsible for ensuring public health and environmental safety in relation to genetically modified organisms (GMOs). Conversely, in the European Union (EU), regulation is managed by the European Commission, the European Food Safety Authority (EFSA), and individual EU Member States. India's regulatory approach to GMOs is led by the Genetic Engineering Approval Committee (GEAC), situated within the Ministry of Environment, Forest and Climate Change (MoEFCC) [166, 167].

Further illustrating the global landscape (Table 5), countries like Australia, India, Brazil, and Argentina have established dedicated authorities for managing GMOs, including genetically modified microalgae. Meanwhile, nations like Malaysia, while not having specific legislation for genetically modified microalgae, manage GMOs within the broader context of the National Biosafety Board's biosafety and environmental protection frameworks [168]. These regulatory measures are foundational to ensuring the responsible development of genetically modified microalgae and their integration into biotechnological and sustainable development initiatives.

As the field of genetic engineering continues to evolve, so will international regulatory frameworks. These adjustments are essential for addressing both the emerging challenges and opportunities presented by GM microalgae, ensuring their ethical advancement and application in a manner that aligns with global standards for safety and sustainability.

Table 5 Country-specific rules and regulations for GM microalgae. Adapted from [166, 167]

Country	Competent authorities/key legislation	
United States	United States Department of Agriculture (USDA)	Oversees GM plants, including specific types of GM microalgae, as potential agricultural products
	Food and Drug Administration (FDA)	Regulates GMOs, including microalgae, within the broader context of biotechnology products intended for food, food additives, animal feeds, or dietary supplements. Pre-market consultation is recommended
	Environmental Protection Agency (EPA)	Manages GM microalgae-producing pesticides or engineered for insect resistance, requiring appropriate permits
European Union (EU)	European Food Safety Authority (EFSA)	Conducts risk assessments and provides scientific advice on GM food and feed, including microalgae, to inform EU regulatory decisions
	Directive 2001/18/EC	Sets the EU framework for the environmental release of GMOs
	Directive (EU) 2015/412	Governs environmental release of GMOs, excluding commercial purposes
	Regulation (EC) No 1829/2003	Pertains to the use of GM food and feed, including microalgae-derived products
	Regulation (EC) No 1830/2003	Ensures supply chain transparency through traceability and labelling of GMOs and derived products
Australia	Office of the Gene Technology Regulator (OGTR)	Manages GMOs, including regulatory responsibilities
	Gene Technology Act 2000	Primary legislation for GMO regulation, establishing a comprehensive framework for dealing with GMOs
	Gene Technology Regulations 2001	Details procedural and licensing requirements for GMO-related activities
Malaysia	National Biosafety Act 2007	Provides a legal framework for the safe management of GMOs, including microalgae
	National Biosafety Board (NBB)	Oversees GMO-related activities, focusing on application evaluation, risk assessment, and compliance monitoring
Japan	Ministry of Health, Labour and Welfare (MHLW)	Evaluates GM food and food additive safety
	Ministry of Agriculture, Forestry, and Fisheries (MAFF)	Regulates GM agricultural products, including organisms used in agriculture
	Food Sanitation Act	Addresses safety evaluation and labelling of GM products, including those derived from microalgae
	Biodiversity Act	Manages GMO use to protect the environment and biodiversity, covering release and handling
China	Ministry of Agricultural and Rural Affairs (MARA)	Responsible for GMO development, research, and commercial application in agriculture
Canada	Canadian Food Inspection Agency (CFIA)	Regulates GMOs affecting the environment and agriculture, including crop and other agricultural organisms
	Health Canada	Oversees the safety assessment of GMOs in terms of human health
India	Genetic Engineering Approval Committee (GEAC)	Evaluates GMO development, release, and research application
	Rules for Manufacture, Use, Import, Export, and Storage of Hazardous Microorganisms/ Genetically Engineered Organisms or Cells (1989 Rules)	Establish guidelines for the safe handling, transport, and storage of GMOs, including microalgae
Brazil	National Technical Commission on Biosafety (CTNBio)	Main authority for GMO oversight, including risk evaluation and authorisation of GMO-related activities, ensuring biosafety compliance
	Biosafety Law (Law No. 11,105/2005)	Legal framework for GMO regulation, covering development, commercial release, and research

Table 5 (continued)

Country	Competent authorities/key legislation	
Argentina	National Advisory Commission on Agricultural Biotechnology (CONABIA)	Evaluates and regulates GMOs, including risk assessment and application evaluation
	Resolution 387/2017	Defines the regulatory framework for GMOs, detailing evaluation and approval processes

5 Risk and disadvantages

The exploration of genetically modified microalgae as a solution for bioindustrial challenges must be approached with caution, given the substantial risks associated with their proliferation and interaction with natural ecosystems. The absence of widespread outdoor cultivation of genetically modified microalgae likely stems from concerns over the unpredictability of their effects in open cultivation systems, a caution underscored by research from [155, 169]. The primary apprehensions involve the potential for genetically modified microalgae to outcompete native species due to their enhanced adaptability, leading to possible ecological disruptions [170]. This competitive edge necessitates implementing stringent biosecurity measures to mitigate the risks of introducing genetically modified strains into local environments [169].

Moreover, the risk of gene flow between genetically modified microalgae and sexually compatible wild species introduces additional complexities, potentially resulting in unforeseen environmental impacts [171]. Therefore, legislation related to genetically modified microalgae must prioritise the protection of local ecosystems through comprehensive biosecurity protocols that guard against the release of foreign species. The ecological considerations extend beyond the mere presence of genetically modified strains, demanding an analysis of their interaction with and impact on the stability of native microalgal communities [172].

A cost–benefit analysis is needed to understand better the use of genetically modified microalgae [173]. Biosafety regulations for genetically modified microalgae must include strict monitoring and assessment of cultivation, handling, and health risks [174]. Genetically modified microalgae can be seen as a solution for the techno-economic problems of the microalgal industry. Therefore, it is essential that all parties, including legislators and business advocates, work out a plan for future genetically modified microalgae use. According to [175], various federal governments worldwide must establish laws and regulations that govern the safe use of genetically modified microalgae for human and ecological benefit. In summary, while genetic engineering in microalgae holds great promise, it is crucial to proceed cautiously and carefully consider the potential risks and disadvantages before deploying this technology in real-world applications.

6 Conclusion and future prospects

Microalgae represent a promising, sustainable biomanufacturing platform for producing economically valuable compounds with potential for long-term viability. The business model surrounding microalgae necessitates the development of processes that are not only financially appealing but also environmentally sustainable, alongside achieving higher levels of technological readiness. The genetic engineering aimed at producing robust microalgal strains is at the heart of enhancing the economic viability of microalgal processes.

Advances in high-throughput technology and molecular biology tools have aided the biotechnological approach to improve the performance of microalgae strains. New advancements have significantly expanded the molecular toolkit and simplified the engineering process, enabling more rapid study and development in this sector. Improved strains of eukaryotic microalgae are being developed for the cost-effective generation of biofuels and bioproducts. Combining microalgal multi-omics datasets and modern molecular techniques provides a strategic roadmap for strain enhancement. Progress in this research will inevitably result in strains that can be used to make beneficial industrial products. However, the economic feasibility of genetically altered strains will be decided by their safety for human health and the environment once they are produced. As a result, strict controls and monitoring are advised to evaluate the environmental and human health concerns of using GM microalgae.

Acknowledgements Ahmad Hanafi Ahmad Kamal received the Sultan Ismail Nasiruddin Shah Terengganu Scholarship, and Muhammad Faiz Zakaria received the Universiti Malaysia Terengganu Scholarship (BUMT) award. The graphical figures were created with https://www.biorender.com.

Authors contributions Conceptualisation: Malinna Jusoh and Aziz Ahmad; writing original draft: Ahmad Hanafi Ahmad Kamal, Nor Farahdevah Mohd Hamidi and Muhammad Faiz Zakaria; preparing figures and tables: Ahmad Hanafi Ahmad Kamal; review and editing: Malinna Jusoh, Aziz Ahmad, Mohd Razif Harun and Thirukanthan Chandra Segaran; funding acquisition: Malinna Jusoh.

Funding This research was funded by TAPE-RG (Grant no. TAPERG/2022/UMT/1441).

Data availability The authors confirm that the data supporting the findings of this study are available in the article.

Declarations

Competing interests The authors declare no competing interests.

References

1. Levasseur W, Perré P, Pozzobon V. A review of high value-added molecules production by microalgae in light of the classification. Biotechnol Adv. 2020;41: 107545. https://doi.org/10.1016/j.biotechadv.2020.107545.
2. Ruggiero MV, Sarno D, Barra L, Kooistra WHCF, Montresor M, Zingone A. Diversity and temporal pattern of *Pseudo-nitzschia* species (Bacillariophyceae) through the molecular lens. Harmful Algae. 2015;42:15–24. https://doi.org/10.1016/j.hal.2014.12.001.
3. Andrade LM, Andrade CJ, Dias M, Nascimento CA, Mendes MA. Chlorella and Spirulina microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. MOJ Food Process Technol. 2018;6(1):45–8. https://doi.org/10.15406/mojfpt.2018.06.00144.
4. Matos J, Cardoso CL, Falé P, Afonso CM, Bandarra NM. Investigation of nutraceutical potential of the microalgae *Chlorella vulgaris* and *Arthrospira platensis*. Int J Food Sci Technol. 2020;55(1):303–12. https://doi.org/10.1111/ijfs.14278.
5. Patel A, Matsakas L, Hrůzová K, Rova U, Christakopoulos P. Biosynthesis of nutraceutical fatty acids by the oleaginous marine microalgae *Phaeodactylum tricornutum* utilizing hydrolysates from organosolv-pretreated birch and spruce biomass. Mar Drugs. 2019;17(2):119. https://doi.org/10.3390/md17020119.
6. Ashour M, Elshobary ME, El-Shenody R, Kamil AW, Abomohra AEF. Evaluation of a native oleaginous marine microalga *Nannochloropsis oceanica* for dual use in biodiesel production and aquaculture feed. Biomass Bioenergy. 2019;120:439–47. https://doi.org/10.1016/j.biombioe.2018.12.009.
7. Kasan NA, Hashim FS, Haris N, Zakaria MF, Mohamed NN, Rasdi NW, Wahid MEA, Katayama T, Takahashi K, Jusoh M. Isolation of freshwater and marine indigenous microalgae species from Terengganu water bodies for potential uses as live feeds in aquaculture industry. Int Aquat Res. 2020;12(1):74–83. https://doi.org/10.22034/iar(20).2020.671730.
8. Nagappan S, Das P, AbdulQuadir M, Thaher M, Khan S, Mahata C, Al-Jabri H, Vatland AK, Kumar G. Potential of microalgae as a sustainable feed ingredient for aquaculture. J Biotechnol. 2021;341:1–20. https://doi.org/10.1016/j.jbiotec.2021.09.003.
9. Collotta M, Champagne P, Mabee W, Tomasoni G. Wastewater and waste CO_2 for sustainable biofuels from microalgae. Algal Res. 2018;29:12–21. https://doi.org/10.1016/j.algal.2017.11.013.
10. Huang Z, Zhang J, Pan M, Hao Y, Hu R, Xiao W, Li G, Lyu T. Valorisation of microalgae residues after lipid extraction: pyrolysis characteristics for biofuel production. Biochem Eng J. 2022;179. https://doi.org/10.1016/j.bej.2021.108330.
11. Zabed HM, Akter S, Yun J, Zhang G, Awad FN, Qi X, Sahu JN. Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew Sustain Energy Rev. 2019;105:105–28. https://doi.org/10.1016/j.rser.2019.01.048.
12. Lu Z, Loftus S, Sha J, Wang W, Park MS, Zhang X, Johnson ZI, Hu Q. Water reuse for sustainable microalgae cultivation: current knowledge and future directions. Resour Conserv Recycl. 2020;161. https://doi.org/10.1016/j.resconrec.2020.104975.
13. Zieliński M, Dębowski M, Kazimierowicz J, Świca I. Microalgal carbon dioxide (CO_2) capture and utilization from the European Union perspective. Energies. 2023;16(3):1446. https://doi.org/10.3390/en16031446.
14. Chhandama MVL, Satyan KB, Changmai B, Vanlalveni C, Rokhum SL. Microalgae as a feedstock for the production of biodiesel: a review. Bioresour Technol Rep. 2021;15: 100771. https://doi.org/10.1016/j.biteb.2021.100771.
15. Huang GH, Chen F, Wei D, Zhang XW, Chen G. Biodiesel production by microalgal biotechnology. Appl Energy. 2010;87(1):38–46. https://doi.org/10.1016/j.apenergy.2009.06.016.
16. Pérez-López P, González-García S, Jeffryes C, Agathos SN, McHugh E, Walsh D, Murray P, Moane S, Feijoo G, Moreira MT. Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. J Clean Prod. 2014;64:332–44. https://doi.org/10.1016/j.jclepro.2013.07.011.
17. Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 2008;54(4):621–39. https://doi.org/10.1111/j.1365-313x.2008.03492.x.
18. Li Y, Horsman M, Wang B, Wu N, Lan CQ. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl Microbiol Biotechnol. 2008;81(4):629–36. https://doi.org/10.1007/s00253-008-1681-1.
19. Stepan DJ, Shockey RE, Moe TA, Dorn R. Carbon dioxide sequestering using microalgal systems. Pittsburgh, PA, and Morgantown, WV; 2002 Feb. https://doi.org/10.2172/882000.
20. Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007;25(3):294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001.

21. Doan TTY, Sivaloganathan B, Obbard JP. Screening of marine microalgae for biodiesel feedstock. Biomass Bioenergy. 2011;35(7):2534–44. https://doi.org/10.1016/j.biombioe.2011.02.021.
22. Hempel N, Petrick I, Behrendt F. Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. J Appl Phycol. 2012;24:1407–18. https://doi.org/10.1007/s10811-012-9795-3.
23. Jeon S, Lim JM, Lee HG, Shin SE, Kang NK, Park Y, Oh HM, Jeong WJ, Jeong BR, Chang YK. Current status and perspectives of genome editing technology for microalgae. Biotechnol Biofuels. 2017;10:267. https://doi.org/10.1186/s13068-017-0957-z.
24. Adegboye MF, Ojuederie OB, Talia PM, Babalola OO. Bioprospecting of microbial strains for biofuel production: metabolic engineering, applications, and challenges. Biotechnol Biofuels. 2021;14:5. https://doi.org/10.1186/s13068-020-01853-2.
25. Berla BM, Saha R, Immethun CM, Maranas CD, Moon TS, Pakrasi HB. Synthetic biology of cyanobacteria: unique challenges and opportunities. Front Microbiol. 2013;4:246. https://doi.org/10.3389/fmicb.2013.00246.
26. Celińska E, Ledesma-Amaro R, Larroude M, Rossignol T, Pauthenier C, Nicaud JM. Golden gate assembly system dedicated to complex pathway manipulation in *Yarrowia lipolytica*. Microb Biotechnol. 2017;10(2):450–5. https://doi.org/10.1111/1751-7915.12605.
27. Poliner E, Clark E, Cummings C, Benning C, Farre EM. A high-capacity gene stacking toolkit for the oleaginous microalga, *Nannochloropsis oceanica* CCMP1779. Algal Res. 2020;45: 101664. https://doi.org/10.1016/j.algal.2019.101664.
28. Sproles AE, Fields FJ, Smalley TN, Le CH, Badary A, Mayfield SP. Recent advancements in the genetic engineering of microalgae. Algal Res. 2021;53: 102158. https://doi.org/10.1016/j.algal.2020.102158.
29. Crozet P, Navarro FJ, Willmund F, Mehrshahi P, Bakowski K, Lauersen KJ, Pérez-Pérez ME, Auroy P, Gorchs Rovira A, Sauret-Gueto S, Niemeyer J, Spaniol B, Theis J, Trösch R, Westrich LD, Vavitsas K, Baier T, Hübner W, De Carpentier F, Cassarini M, Danon A, Henri J, Marchand CH, De Mia M, Sarkissian K, Baulcombe DC, Peltier G, Crespo JL, Kruse O, Jensen PE, Schroda M, Smith AG, Lemaire SD. Birth of a photosynthetic chassis: a MoClo toolkit enabling synthetic biology in the microalga *Chlamydomonas reinhardtii*. ACS Synth Biol. 2018;7(9):2074–86. https://doi.org/10.1021/acssynbio.8b00251.
30. Chai LE, Loh SK, Low ST, Mohamad MS, Deris S, Zakaria Z. A review on the computational approaches for gene regulatory network construction. Comput Biol Med. 2014;48:55–65. https://doi.org/10.1016/j.compbiomed.2014.02.011.
31. Weiner I, Atar S, Schweitzer S, Eilenberg H, Feldman Y, Avitan M, Blau M, Danon A, Tuller T, Yacoby I. Enhancing heterologous expression in *Chlamydomonas reinhardtii* by transcript sequence optimization. Plant J. 2018;94(1):22–31. https://doi.org/10.1111/tpj.13836.
32. Cutolo EA, Mandalà G, Dallosto L, Bassi R. Harnessing the algal chloroplast for heterologous protein production. Microorganisms. 2022;10(4):743. https://doi.org/10.3390/microorganisms10040743.
33. Weiner I, Feldman Y, Shahar N, Yacoby I, Tuller T. CSO – a sequence optimization software for engineering chloroplast expression in *Chlamydomonas reinhardtii*. Algal Res. 2020;46: 101788. https://doi.org/10.1016/j.algal.2019.101788.
34. Barahimipour R, Strenkert D, Neupert J, Schroda M, Merchant SS, Bock R. Dissecting the contributions of GC content and codon usage to gene expression in the model alga *Chlamydomonas reinhardtii*. Plant J. 2015;84(4):704–17. https://doi.org/10.1111/tpj.13033.
35. Schroda M, Blöcker D, Beck CF. The HSP70A promoter as a tool for the improved expression of transgenes in *Chlamydomonas*. Plant J. 2000;21(2):121–31. https://doi.org/10.1046/j.1365-313x.2000.00652.x.
36. Potvin G, Zhang Z. Strategies for high-level recombinant protein expression in transgenic microalgae: a review. Biotechnol Adv. 2010;28(6):910–8. https://doi.org/10.1016/j.biotechadv.2010.08.006.
37. Gong Y, Hu H, Gao Y, Xu X, Gao H. Microalgae as platforms for production of recombinant proteins and valuable compounds: progress and prospects. J Ind Microbiol Biotechnol. 2011;38(12):1879–90. https://doi.org/10.1007/s10295-011-1032-6.
38. Wu-Scharf D, Jeong BR, Zhang C, Cerutti H. Transgene and transposon silencing in *Chlamydomonas reinhardtii* by a DEAH-Box RNA helicase. Science. 2000;290(5494):1159–62. https://doi.org/10.1126/science.290.5494.1159.
39. Strenkert D, Schmollinger S, Schroda M. Heat shock factor 1 counteracts epigenetic silencing of nuclear transgenes in *Chlamydomonas reinhardtii*. Nucleic Acids Res. 2013;41(10):5273–89. https://doi.org/10.1093/nar/gkt224.
40. Quinn JM, Merchant S. Two copper-responsive elements associated with the *Chlamydomonas* Cyc6 gene function as targets for transcriptional activators. Plant Cell. 1995;7(5):623–8. https://doi.org/10.1105/tpc.7.5.623.
41. Richard C, Ouellet H, Guertin M. Characterization of the LI818 polypeptide from the green unicellular alga *Chlamydomonas reinhardtii*. Plant Mol Biol. 2000;42(2):303–16. https://doi.org/10.1023/a:1006340308077.
42. Fischer N, Rochaix JD. The flanking regions of PsaD drive efficient gene expression in the nucleus of the green alga *Chlamydomonas reinhardtii*. Mol Genet Genomics. 2001;265(5):888–94. https://doi.org/10.1007/s004380100485.
43. Koblenz B, Lechtreck KF. The NIT1 promoter allows inducible and reversible silencing of centrin in *Chlamydomonas reinhardtii*. Eukaryot Cell. 2005;4(11):1959–62. https://doi.org/10.1128/ec.4.11.1959-1962.2005.
44. Lee S, Lee YJ, Choi S, Park SB, Tran QG, Heo J, Kim HS. Development of an alcohol-inducible gene expression system for recombinant protein expression in *Chlamydomonas reinhardtii*. J Appl Phycol. 2018;30:2297–304. https://doi.org/10.1007/s10811-018-1480-8.
45. Scranton MA, Ostrand JT, Georgianna DR, Lofgren SM, Li D, Ellis RC, Carruthers DN, Dräger A, Masica DL, Mayfield SP. Synthetic promoters capable of driving robust nuclear gene expression in the green alga *Chlamydomonas reinhardtii*. Algal Res. 2016;15:135–42. https://doi.org/10.1016/j.algal.2016.02.011.
46. Brown AJ, Sweeney B, Mainwaring DO, James DC. Synthetic promoters for CHO cell engineering. Biotechnol Bioeng. 2014;111(8):1638–47. https://doi.org/10.1002/bit.25227.
47. Ranjbar S, Malcata FX. Challenges and prospects for sustainable microalga-based oil: a comprehensive review, with a focus on metabolic and genetic engineering. Fuel. 2022;324(Part A). https://doi.org/10.1016/j.fuel.2022.124567.
48. Bajhaiya AK, Ziehe Moreira J, Pittman JK. Transcriptional engineering of microalgae: prospects for high-value chemicals. Trends Biotechnol. 2017;35(2):95–9. https://doi.org/10.1016/j.tibtech.2016.06.001.
49. Thiriet-Rupert S, Carrier G, Chénais B, Trottier C, Bougaran G, Cadoret JP, Schoefs B, Saint-Jean B. Transcription factors in microalgae: genome-wide prediction and comparative analysis. BMC Genomics. 2016;17:282. https://doi.org/10.1186/s12864-016-2610-9.
50. Gargouri M, Park JJ, Holguin FO, Kim MJ, Wang H, Deshpande RR, Shachar-Hill Y, Hicks LM, Gang DR. Identification of regulatory network hubs that control lipid metabolism in *Chlamydomonas reinhardtii*. J Exp Bot. 2015;66(15):4551–66. https://doi.org/10.1093/jxb/erv217.
51. Ma XN, Chen TP, Yang B, Liu J, Chen F. Lipid production from *Nannochloropsis*. Mar Drugs. 2016;14(4):61. https://doi.org/10.3390/md14040061.

52. Kang NK, Kim EK, Kim YU, Lee B, Jeong WJ, Jeong BR, Chang YK. Increased lipid production by heterologous expression of AtWRI1 transcription factor in *Nannochloropsis salina*. Biotechnol Biofuels. 2017;10:231. https://doi.org/10.1186/s13068-017-0919-5.
53. Uprety BK, Morrison EN, Emery RJN, Farrow SC. Customizing lipids from oleaginous microbes: leveraging exogenous and endogenous approaches. Trends Biotechnol. 2022;40:482–508. https://doi.org/10.1016/j.tibtech.2021.09.004.
54. Gaj T, Gersbach CA, Barbas CF. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397–405. https://doi.org/10.1016/j.tibtech.2013.04.004.
55. Malzahn A, Lowder L, Qi Y. Plant genome editing with TALEN and CRISPR. Cell Biosci. 2017;7:21. https://doi.org/10.1186/s13578-017-0148-4.
56. Kumar A, Perrine Z, Stroff CL, Postier BA, Coury DT, Sayre R, Thomas Allnutt FC. Molecular tools for bioengineering eukaryotic microalgae. Curr Biotechnol. 2016;5(2):93–108. https://doi.org/10.2174/2211550105666160127002147.
57. Daboussi F, Leduc S, Maréchal A, Dubois G, Guyot V, Perez-Michaut C, Amato A, Falciatore A, Juillerat A, Beurdeley M, Voytas DF, Cavarec L, Duchateau P. Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. Nat Commun. 2014;5. https://doi.org/10.1038/ncomms4831.
58. Sizova I, Greiner A, Awasthi M, Kateriya S, Hegemann P. Nuclear gene targeting in *Chlamydomonas* using engineered zinc-finger nucleases. Plant J. 2013;73(5):873–82. https://doi.org/10.1111/tpj.12066.
59. Zhang YT, Jiang JY, Shi TQ, Sun XM, Zhao QY, Huang H, Ren LJ. Application of the CRISPR/Cas system for genome editing in microalgae. Appl Microbiol Biotechnol. 2019;103:3239–48. https://doi.org/10.1007/s00253-019-09726-x.
60. Baldia A, Rajput D, Kumar A, Pandey A, Dubey KK. Engineering microalgae as the next-generation food. Syst Microbiol Biomanufacturing. 2023;3:166–78. https://doi.org/10.1007/s43393-022-00144-1.
61. Cebrailoglu N, Yildiz AB, Akkaya O, Ozden Ciftci Y. CRISPR-Cas: removing boundaries of the nature. Eur J Biol. 2019;78(2):157–64. https://doi.org/10.26650/eurjbiol.2019.0024.
62. Adli M. The CRISPR tool kit for genome editing and beyond. Nat Commun. 2018;9:1911. https://doi.org/10.1038/s41467-018-04252-2.
63. Patel VK, Soni N, Prasad V, Sapre A, Dasgupta S, Bhadra B. CRISPR–Cas9 system for genome engineering of photosynthetic microalgae. Mol Biotechnol. 2019;61:541–61. https://doi.org/10.1007/s12033-019-00185-3.
64. Nymark M, Sharma AK, Sparstad T, Bones AM, Winge P. A CRISPR/Cas9 system adapted for gene editing in marine algae. Sci Rep. 2016;6:24951. https://doi.org/10.1038/srep24951.
65. Lee TM, Lin JY, Tsai TH, Yang RY, Ng IS. Clustered regularly interspaced short palindromic repeats (CRISPR) technology and genetic engineering strategies for microalgae towards carbon neutrality: a critical review. Bioresour Technol. 2023;368: 128350. https://doi.org/10.1016/j.biortech.2022.128350.
66. Jiang WZ, Dumm S, Knuth ME, Sanders SL, Weeks DP. Precise oligonucleotide-directed mutagenesis of the *Chlamydomonas reinhardtii* genome. Plant Cell Rep. 2017;36:1001–4. https://doi.org/10.1007/s00299-017-2138-8.
67. Jiang WZ, Weeks DP. A gene-within-a-gene Cas9/sgRNA hybrid construct enables gene editing and gene replacement strategies in *Chlamydomonas reinhardtii*. Algal Res. 2017;26:474–80. https://doi.org/10.1016/j.algal.2017.04.001.
68. Petolino JF. Genome editing in plants via designed zinc finger nucleases. In Vitro Cell Dev Biol - Plant. 2015;51:1–8. https://doi.org/10.1007/S11627-015-9663-3.
69. Momose T, Concordet JP. Diving into marine genomics with CRISPR/Cas9 systems. Mar Genomics. 2016;30:55–65. https://doi.org/10.1016/j.margen.2016.10.003.
70. Slaninová M, Hroššová D, Vlček D, Mages W. Is it possible to improve homologous recombination in *Chlamydomonas reinhardtii*? Biologia. 2008;63:941–6. https://doi.org/10.2478/s11756-008-0146-4.
71. George J. Exploring genetic engineering strategies to enable heterologous monoterpenoid production in model microalgae, *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* [PhD Thesis]. Open Publications of UTS Scholars. University of Technology Sydney; 2021.
72. Jinkerson RE, Jonikas MC. Molecular techniques to interrogate and edit the *Chlamydomonas* nuclear genome. Plant J. 2015;82(3):393–412. https://doi.org/10.1111/tpj.12801.
73. Ng IS, Tan SI, Kao PH, Chang YK, Chang JS. Recent developments on genetic engineering of microalgae for biofuels and bio-based chemicals. Biotechnol J. 2017;12(10). https://doi.org/10.1002/biot.201600644.
74. Shimogawara K, Fujiwara S, Grossman A, Usuda H. High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation. Genetics. 1998;148(4):1821–8. https://doi.org/10.1093/genetics/148.4.1821.
75. Potter H, Heller R. Transfection by electroporation. Curr Protoc Mol Biol. 2010;121(1). https://doi.org/10.1002/cpmb.48.
76. Muñoz CF, de Jaeger L, Sturme MHJ, Lip KYF, Olijslager JWJ, Springer J, Wolbert EJH, Martens DE, Eggink G, Weusthuis RA, Wijffels RH. Improved DNA/protein delivery in microalgae – a simple and reliable method for the prediction of optimal electroporation settings. Algal Res. 2018;33:448–55. https://doi.org/10.1016/j.algal.2018.06.021.
77. Gan Q, Jiang J, Han X, Wang S, Lu Y. Engineering the chloroplast genome of oleaginous marine microalga *Nannochloropsis oceanica*. Front Plant Sci. 2018;9. https://doi.org/10.3389/fpls.2018.00439.
78. Chen Z, Lee WG. Electroporation for microalgal biofuels: a review. Sustain Energy Fuels. 2019;3(11):2954–67. https://doi.org/10.1039/c9se00087a.
79. Velmurugan N, Deka D. Transformation techniques for metabolic engineering of diatoms and haptophytes: current state and prospects. Appl Microbiol Biotechnol. 2018;102:4255–67. https://doi.org/10.1007/s00253-018-8925-5.
80. Gutiérrez S, Lauersen KJ, Fabris M, Abbriano R. Gene delivery technologies with applications in microalgal genetic engineering. Biology. 2021;10(4). https://doi.org/10.3390/biology10040265.
81. Sreenikethanam A, Raj S, Gugulothu P, Bajhaiya AK. Genetic engineering of microalgae for secondary metabolite production: recent developments, challenges, and future prospects. Front Bioeng Biotechnol. 2022;10. https://doi.org/10.3389/fbioe.2022.836056.
82. Serif M, Dubois G, Finoux AL, Teste MA, Jallet D, Daboussi F. One-step generation of multiple gene knock-outs in the diatom *Phaeodactylum tricornutum* by DNA-free genome editing. Nat Commun. 2018;9:3924. https://doi.org/10.1038/S41467-018-06378-9.
83. Kumar SV, Misquitta RW, Reddy VS, Rao BJ, Rajam MV. Genetic transformation of the green alga - *Chlamydomonas reinhardtii* by *Agrobacterium tumefaciens*. Plant Sci. 2004;166(3):731–8. https://doi.org/10.1016/j.plantsci.2003.11.012.

84. Kong F, Yamaoka Y, Ohama T, Lee Y, Li-Beisson Y. Molecular genetic tools and emerging synthetic biology strategies to increase cellular oil content in *Chlamydomonas reinhardtii*. Plant Cell Physiol. 2019;60(6):1184–96. https://doi.org/10.1093/pcp/pcz022.
85. Mini P, Demurtas OC, Valentini S, Pallara P, Aprea G, Ferrante P, Giuliano G. *Agrobacterium*-mediated and electroporation-mediated transformation of *Chlamydomonas reinhardtii*: a comparative study. BMC Biotechnol. 2018;18. https://doi.org/10.1186/s12896-018-0416-3.
86. Schuhmann H, Lim DKY, Schenk PM. Perspectives on metabolic engineering for increased lipid contents in microalgae. Biofuels. 2012;3(1):71–86. https://doi.org/10.4155/bfs.11.147.
87. Mofijur M, Rasul MG, Hassan NMS, Nabi MN. Recent development in the production of third generation biodiesel from microalgae. Energy Procedia. 2019;156:53–8. https://doi.org/10.1016/j.egypro.2018.11.088.
88. Barta DG, Coman V, Vodnar DC. Microalgae as sources of omega-3 polyunsaturated fatty acids: biotechnological aspects. Algal Res. 2021;58: 102410. https://doi.org/10.1016/j.algal.2021.102410.
89. Tibbetts SM, Scaife MA, Armenta RE. Apparent digestibility of proximate nutrients, energy and fatty acids in nutritionally-balanced diets with partial or complete replacement of dietary fish oil with microbial oil from a novel *Schizochytrium* sp. (T18) by juvenile Atlantic salmon (*Salmo salar* L.). Aquaculture. 2020;520:735003. https://doi.org/10.1016/j.aquaculture.2020.735003.
90. Li Z, Meng T, Ling X, Li J, Zheng C, Shi Y, Chen Z, Li Z, Li Q, Lu Y, He N. Overexpression of malonyl-CoA: ACP transacylase in *Schizochytrium* sp. to improve polyunsaturated fatty acid production. J Agric Food Chem. 2018;66(21):5382–91. https://doi.org/10.1021/acs.jafc.8b01026.
91. Rengel R, Smith RT, Haslam RP, Sayanova O, Vila M, León R. Overexpression of acetyl-CoA synthetase (ACS) enhances the biosynthesis of neutral lipids and starch in the green microalga *Chlamydomonas reinhardtii*. Algal Res. 2018;31:183–93. https://doi.org/10.1016/j.algal.2018.02.009.
92. Einhaus A, Baier T, Rosenstengel M, Freudenberg RA, Kruse O. Rational promoter engineering enables robust terpene production in microalgae. ACS Synth Biol. 2021;10(4):847–56. https://doi.org/10.1021/acssynbio.0c00632.
93. Dunahay TG, Jarvis EE, Dais SS, Roessler PG. Manipulation of microalgal lipid production using genetic engineering. Appl Biochem Biotechnol. 1996;57:223–31. https://doi.org/10.1007/bf02941703.
94. Yan J, Cheng R, Lin X, You S, Li K, Rong H, Ma Y. Overexpression of acetyl-CoA synthetase increased the biomass and fatty acid proportion in microalga *Schizochytrium*. Appl Microbiol Biotechnol. 2013;97:1933–9. https://doi.org/10.1007/s00253-012-4481-6.
95. Chen JW, Liu WJ, Hu DX, Wang X, Balamurugan S, Alimujiang A, Yang WD, Liu JS, Li HY. Identification of a malonyl CoA-acyl carrier protein transacylase and its regulatory role in fatty acid biosynthesis in oleaginous microalga *Nannochloropsis oceanica*. Biotechnol Appl Biochem. 2017;64(5):620–6. https://doi.org/10.1002/bab.1531.
96. Radakovits R, Eduafo PM, Posewitz MC. Genetic engineering of fatty acid chain length in *Phaeodactylum tricornutum*. Metab Eng. 2011;13(1):89–95. https://doi.org/10.1016/j.ymben.2010.10.003.
97. Tan KWM, Lee YK. Expression of the heterologous *Dunaliella tertiolecta* fatty acyl-ACP thioesterase leads to increased lipid production in *Chlamydomonas reinhardtii*. J Biotechnol. 2017;247:60–7. https://doi.org/10.1016/j.jbiotec.2017.03.004.
98. Kannan N, Rao AS, Nair A. Microbial production of omega-3 fatty acids: an overview. J Appl Microbiol. 2021;131(5):2114–30. https://doi.org/10.1111/jam.15034.
99. Ahmad I, Sharma AK, Daniell H, Kumar S. Altered lipid composition and enhanced lipid production in green microalga by introduction of brassica diacylglycerol acyltransferase 2. Plant Biotechnol J. 2015;13(4):540–50. https://doi.org/10.1111/pbi.12278.
100. Shin YS, Jeong J, Nguyen THT, Kim JYH, Jin ES, Sim SJ. Targeted knockout of phospholipase A2 to increase lipid productivity in *Chlamydomonas reinhardtii* for biodiesel production. Bioresour Technol. 2019;271:368–74. https://doi.org/10.1016/j.biortech.2018.09.121.
101. Kim J, Kwak HS, Sim SJ, Jin ES. Overexpression of malic enzyme isoform 2 in *Chlamydomonas reinhardtii* PTS42 increases lipid production. Bioresour Technol Rep. 2019;7: 100239. https://doi.org/10.1016/j.biteb.2019.100239.
102. Song I, Kim S, Kim J, Oh H, Jang J, Jeong SJ, Baek K, Shin WS, Sim SJ, Jin ES. Macular pigment-enriched oil production from genome-edited microalgae. Microb Cell Fact. 2022;21:27. https://doi.org/10.1186/s12934-021-01736-7.
103. Li DW, Balamurugan S, Yang YF, Zheng JW, Huang D, Zou LG, Yang WD, Liu JS, Guan Y, Li HY. Transcriptional regulation of microalgae for concurrent lipid overproduction and secretion. Sci Adv. 2019. https://doi.org/10.1126/sciadv.aau3795.
104. León-Saiki GM, Remmers IM, Martens DE, Lamers PP, Wijffels RH, van der Veen D. The role of starch as transient energy buffer in synchronized microalgal growth in *Acutodesmus obliquus*. Algal Res. 2017;25:160–7. https://doi.org/10.1016/j.algal.2017.05.018.
105. Zou LG, Chen JW, Zheng DL, Balamurugan S, Li DW, Yang WD, Liu JS, Li HY. High-efficiency promoter-driven coordinated regulation of multiple metabolic nodes elevates lipid accumulation in the model microalga *Phaeodactylum tricornutum*. Microb Cell Fact. 2018;17:54. https://doi.org/10.1186/s12934-018-0906-y.
106. Hao X, Luo L, Jouhet J, Rébeillé F, Maréchal E, Hu H, Pan Y, Tan X, Chen Z, You L, Chen H, Wei F, Gong Y. Enhanced triacylglycerol production in the diatom *Phaeodactylum tricornutum* by inactivation of a Hotdog-fold thioesterase gene using TALEN-based targeted mutagenesis. Biotechnol Biofuels. 2018;11:312. https://doi.org/10.1186/s13068-018-1309-3.
107. Pudney A, Gandini C, Economou CK, Smith R, Goddard P, Napier JA, Spicer A, Sayanova O. Multifunctionalizing the marine diatom *Phaeodactylum tricornutum* for sustainable co-production of omega-3 long chain polyunsaturated fatty acids and recombinant phytase. Sci Rep. 2019;9:11444. https://doi.org/10.1038/s41598-019-47875-1.
108. Anand V, Kashyap M, Samadhiya K, Kiran B. Strategies to unlock lipid production improvement in algae. Int J Environ Sci Technol. 2019;16:1829–38. https://doi.org/10.1007/s13762-018-2098-8.
109. Gimpel JA, Henríquez V, Mayfield SP. In metabolic engineering of eukaryotic microalgae: potential and challenges come with great diversity. Front Microbiol. 2015;6:1376. https://doi.org/10.3389/fmicb.2015.01376.
110. Han X, Song X, Li F, Lu Y. Improving lipid productivity by engineering a control-knob gene in the oleaginous microalga *Nannochloropsis oceanica*. Metab Eng Commun. 2020;11. https://doi.org/10.1016/j.mec.2020.e00142.
111. Osorio H, Jara C, Fuenzalida K, Rey-Jurado E, Vásquez M. High-efficiency nuclear transformation of the microalgae *Nannochloropsis oceanica* using Tn5 transposome for the generation of altered lipid accumulation phenotypes. Biotechnol Biofuels. 2019;12:134. https://doi.org/10.1186/s13068-019-1475-y.
112. Shi H, Chen H, Gu Z, Zhang H, Chen W, Chen YQ. Application of a delta-6 desaturase with α-linolenic acid preference on eicosapentaenoic acid production in *Mortierella alpina*. Microb Cell Fact. 2016;15:117. https://doi.org/10.1186/s12934-016-0516-5.

113. Kwon S, Kang NK, Koh HG, Shin SE, Lee B, Jeong BR, Chang YK. Enhancement of biomass and lipid productivity by overexpression of a bZIP transcription factor in *Nannochloropsis salina*. Biotechnol Bioeng. 2018;115(2):331–40. https://doi.org/10.1002/bit.26465.
114. Xue J, Niu YF, Huang T, Yang WD, Liu JS, Li HY. Genetic improvement of the microalga *Phaeodactylum tricornutum* for boosting neutral lipid accumulation. Metab Eng. 2015;27:1–9. https://doi.org/10.1016/j.ymben.2014.10.002.
115. Eungrasamee K, Miao R, Incharoensakdi A, Lindblad P, Jantaro S. Improved lipid production via fatty acid biosynthesis and free fatty acid recycling in engineered *Synechocystis* sp. PCC 6803. Biotechnol Biofuels. 2019;12:8. https://doi.org/10.1186/s13068-018-1349-8.
116. Fathy W, Essawy E, Tawfik E, Khedr M, Abdelhameed MS, Hammouda O, Elsayed K. Recombinant overexpression of the *Escherichia coli* acetyl-CoA carboxylase gene in *Synechocystis* sp. boosts lipid production. J Basic Microbiol. 2021;61(4):330–8. https://doi.org/10.1002/jobm.202000656.
117. Xi Y, Yin L, Chi Z, Luo G. Characterization and RNA-seq transcriptomic analysis of a *Scenedesmus obliqnus* mutant with enhanced photosynthesis efficiency and lipid productivity. Sci Rep. 2021;11. https://doi.org/10.1038/s41598-021-88954-6.
118. Gomma AE, Lee SK, Sun SM, Yang SH, Chung G. Improvement in oil production by increasing malonyl-CoA and glycerol-3-phosphate pools in *Scenedesmus quadricauda*. Indian J Microbiol. 2015;55:447–55. https://doi.org/10.1007/s12088-015-0546-4.
119. Chen CY, Kao AL, Tsai ZC, Chow TJ, Chang HY, Zhao XQ, Chen PT, Su HY, Chang JS. Expression of type 2 diacylglycerol acyltransferse gene DGTT1 from *Chlamydomonas reinhardtii* enhances lipid production in *Scenedesmus obliquus*. Biotechnol J. 2016;11(3):336–44. https://doi.org/10.1002/biot.201500272.
120. Matthijs M, Fabris M, Obata T, Foubert I, Franco-Zorrilla JM, Solano R, Fernie AR, Vyverman W, Goossens A. The transcription factor bZIP14 regulates the TCA cycle in the diatom *Phaeodactylum tricornutum*. EMBO J. 2017;36(11):1559–76. https://doi.org/10.15252/embj.201696392.
121. Ravindran B, Kurade MB, Kabra AN, Jeon BH, Gupta SK. Recent advances and future prospects of microalgal lipid biotechnology. In: Gupta S, Malik ABF, editors. Algal Biofuels. Springer, Cham; 2017. p. 1–37. https://doi.org/10.1007/978-3-319-51010-1_1.
122. Sumiya N, Kawase Y, Hayakawa J, Matsuda M, Nakamura M, Era A, Tanaka K, Kondo A, Hasunuma T, Imamura S, Miyagishima SY. Expression of cyanobacterial Acyl-ACP reductase elevates the triacylglycerol level in the red alga *Cyanidioschyzon merolae*. Plant Cell Physiol. 2015;56(10):1962–80. https://doi.org/10.1093/pcp/pcv120.
123. Sirohi P, Verma H, Singh SK, Singh VK, Pandey J, Khusharia S, Kumar D, Kaushalendra TP, Kumar A. Microalgal carotenoids: therapeutic application and latest approaches to enhance the production. Curr Issues Mol Biol. 2022;44(12):6257–79. https://doi.org/10.3390/cimb44120427.
124. Ahmad MT, Shariff M, Md Yusoff F, Goh YM, Banerjee S. Applications of microalga *Chlorella vulgaris* in aquaculture. Rev Aquac. 2020;12(1):328–46. https://doi.org/10.1111/raq.12320.
125. Cezare-Gomes EA, Mejia-da-Silva L del C, Pérez-Mora LS, Matsudo MC, Ferreira-Camargo LS, Singh AK, de Carvalho JCM. Potential of microalgae carotenoids for industrial application. Appl Biochem Biotechnol. 2019;188:602–34. https://doi.org/10.1007/s12010-018-02945-4.
126. Woodside JV, McGrath AJ, Lyner N, McKinley MC. Carotenoids and health in older people. Maturitas. 2015;80:63–8. https://doi.org/10.1016/j.maturitas.2014.10.012.
127. Armstrong GA, Hearstt JE. Genetics and molecular biology of carotenoid pigment biosynthesis. FASEB J. 1996;10(2):228–37. https://doi.org/10.1096/fasebj.10.2.8641556.
128. Tsai TH, Lin JY, Ng IS. Cooperation of phytoene synthase, pyridoxal kinase and carbonic anhydrase for enhancing carotenoids biosynthesis in genetic *Chlamydomonas reinhardtii*. J Taiwan Inst Chem Eng. 2022;137: 104184. https://doi.org/10.1016/j.jtice.2021.104184.
129. Sun H, Zhao W, Mao X, Li Y, Wu T, Chen F. High-value biomass from microalgae production platforms: strategies and progress based on carbon metabolism and energy conversion. Biotechnol Biofuels. 2018;11:227. https://doi.org/10.1186/s13068-018-1225-6.
130. Couso I, Vila M, Rodriguez H, Vargas MA, León R. Overexpression of an exogenous phytoene synthase gene in the unicellular alga *Chlamydomonas reinhardtii* leads to an increase in the content of carotenoids. Biotechnol Prog. 2011;27(1):54–60. https://doi.org/10.1002/btpr.527.
131. Varela JC, Pereira H, Vila M, León R. Production of carotenoids by microalgae: achievements and challenges. Photosynth Res. 2015;125:423–36. https://doi.org/10.1007/s11120-015-0149-2.
132. Cordero BF, Couso I, Leon R, Rodriguez H, Vargas MA. Isolation and characterization of a lycopene ε-cyclase gene of *Chlorella (Chromochloris) zofingiensis*. regulation of the carotenogenic pathway by nitrogen and light. Mar Drugs. 2012;10(9):2069–88. https://doi.org/10.3390/md10092069.
133. Sathasivam R, Ki JS. A review of the biological activities of microalgal carotenoids and their potential use in healthcare and cosmetic industries. Mar Drugs. 2018;16(1):26. https://doi.org/10.3390/md16010026.
134. Fan L, Vonshak A, Gabbay R, Hirshberg J, Cohen Z, Boussiba S. The biosynthetic pathway of astaxanthin in a green alga *Haematococcus pluvialis* as indicated by inhibition with diphenylamine. Plant Cell Physiol. 1995;36(8):1519–24. https://doi.org/10.1093/oxfordjournals.pcp.a078916.
135. Ye VM, Bhatia SK. Pathway engineering strategies for production of beneficial carotenoids in microbial hosts. Biotechnol Lett. 2012;34:1405–14. https://doi.org/10.1007/s10529-012-0921-8.
136. Davinelli S, Nielsen ME, Scapagnini G. Astaxanthin in skin health, repair, and disease: a comprehensive review. Nutrients. 2018;10(4):522. https://doi.org/10.3390/nu10040522.
137. Huang JC, Zhong YJ, Liu J, Sandmann G, Chen F. Metabolic engineering of tomato for high-yield production of astaxanthin. Metab Eng. 2013;17:59–67. https://doi.org/10.1016/j.ymben.2013.02.005.
138. Baek K, Yu J, Jeong J, Sim SJ, Bae S, Jin ES. Photoautotrophic production of macular pigment in a *Chlamydomonas reinhardtii* strain generated by using DNA-free CRISPR-Cas9 RNP-mediated mutagenesis. Biotechnol Bioeng. 2018;115(3):719–28. https://doi.org/10.1002/bit.26499.
139. Rathod JP, Vira C, Lali AM, Prakash G. Metabolic engineering of *Chlamydomonas reinhardtii* for enhanced β-carotene and lutein production. Appl Biochem Biotechnol. 2020;190:1457–69. https://doi.org/10.1007/s12010-019-03194-9.

140. Morikawa T, Uraguchi Y, Sanda S, Nakagawa S, Sawayama S. Overexpression of DnaJ-like chaperone enhances carotenoid synthesis in *Chlamydomonas reinhardtii*. Appl Biochem Biotechnol. 2018;184:80–91. https://doi.org/10.1007/s12010-017-2521-5.
141. Eilers U, Bikoulis A, Breitenbach J, Büchel C, Sandmann G. Limitations in the biosynthesis of fucoxanthin as targets for genetic engineering in *Phaeodactylum tricornutum*. J Appl Phycol. 2016;28:123–9. https://doi.org/10.1007/s10811-015-0583-8.
142. Kadono T, Kira N, Suzuki K, Iwata O, Ohama T, Okada S, Nishimura T, Akakabe M, Tsuda M, Adachi M. Effect of an introduced phytoene synthase gene expression on carotenoid biosynthesis in the marine diatom *Phaeodactylum tricornutum*. Mar Drugs. 2015;13(8):5334–57. https://doi.org/10.3390/md13085334.
143. Galarza JI, Gimpel JA, Rojas V, Arredondo-Vega BO, Henríquez V. Over-accumulation of astaxanthin in *Haematococcus pluvialis* through chloroplast genetic engineering. Algal Res. 2018;31:291–7. https://doi.org/10.1016/j.algal.2018.02.024.
144. Kathiresan S, Chandrashekar A, Ravishankar GA, Sarada R. Regulation of astaxanthin and its intermediates through cloning and genetic transformation of β-carotene ketolase in *Haematococcus pluvialis*. J Biotechnol. 2015;196–197:33–41. https://doi.org/10.1016/j.jbiotec.2015.01.006.
145. Zhang JY, Jeon H, Sim SJ, Lee Y, Jin ES. Homologous sense and antisense expression of a gene in *Dunaliella tertiolecta*. Planta. 2015;242(4):1051–8. https://doi.org/10.1007/s00425-015-2369-2.
146. Kim M, Ahn J, Jeon H, Jin ES, Cutignano A, Romano G. Development of a *Dunaliella tertiolecta* strain with increased zeaxanthin content using random mutagenesis. Mar Drugs. 2017;15(6):189. https://doi.org/10.3390/md15060189.
147. Liu J, Sun Z, Gerken H, Huang J, Jiang Y, Chen F. Genetic engineering of the green alga *Chlorella zofingiensis*: a modified norflurazon-resistant phytoene desaturase gene as a dominant selectable marker. Appl Microbiol Biotechnol. 2014;98:5069–79. https://doi.org/10.1007/s00253-014-5593-y.
148. Raven JA. Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments. Aquat Microb Ecol. 2009;56:177–92. https://doi.org/10.3354/ame01315.
149. Rosgaard L, de Porcellinis AJ, Jacobsen JH, Frigaard NU, Sakuragi Y. Bioengineering of carbon fixation, biofuels, and biochemicals in cyanobacteria and plants. J Biotechnol. 2012;162(1):134–47. https://doi.org/10.1016/j.jbiotec.2012.05.006.
150. Berg IA. Ecological aspects of the distribution of different autotrophic CO_2 fixation pathways. Appl Environ Microbiol. 2011;77(6):1925–36. https://doi.org/10.1128/aem.02473-10.
151. Matsuda Y, Kroth PG. Carbon fixation in diatoms. In: Hohmann-Marriott M, editor. The structural basis of biological energy generation advances in photosynthesis and respiration. Springer, Dordrecht; 2014. p. 335–62. https://doi.org/10.1007/978-94-017-8742-0_18.
152. Guan Z, Wang W, Yu X, Lin W, Miao Y. Comparative proteomic analysis of coregulation of CIPK14 and WHIRLY1/3 mediated pale yellowing of leaves in *Arabidopsis*. Int J Mol Sci. 2018;19(8):2231. https://doi.org/10.3390/ijms19082231.
153. Feller U, Anders I, Mae T. Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. J Exp Bot. 2008;59(7):1615–24. https://doi.org/10.1093/jxb/erm242.
154. Hagemann M, Fernie AR, Espie GS, Kern R, Eisenhut M, Reumann S, Bauwe H, Weber APM. Evolution of the biochemistry of the photorespiratory C2 cycle. Plant Biol. 2013;15(4):639–47. https://doi.org/10.1111/j.1438-8677.2012.00677.x.
155. Kumar G, Shekh A, Jakhu S, Sharma Y, Kapoor R, Sharma TR. Bioengineering of microalgae: recent advances, perspectives, and regulatory challenges for industrial application. Front Bioeng Biotechnol. 2020. https://doi.org/10.3389/fbioe.2020.00914.
156. Muthukrishnan L. Bio-engineering of microalgae: challenges and future prospects toward industrial and environmental applications. J Basic Microbiol. 2022;62(3–4):310–29. https://doi.org/10.1002/jobm.202100417.
157. Ducat DC, Silver PA. Improving carbon fixation pathways. Curr Opin Chem Biol. 2012;16(3–4):337–44. https://doi.org/10.1016/j.cbpa.2012.05.002.
158. Whitney SM, Houtz RL, Alonso H. Advancing our understanding and capacity to engineer nature's CO_2-sequestering enzyme. Rubisco Plant Physiol. 2011;155(1):27–35. https://doi.org/10.1104/pp.110.164814.
159. Yang B, Liu J, Ma X, Guo B, Liu B, Wu T, Jiang Y, Chen F. Genetic engineering of the Calvin cycle toward enhanced photosynthetic CO_2 fixation in microalgae. Biotechnol Biofuels. 2017;10:229. https://doi.org/10.1186/s13068-017-0916-8.
160. Díaz-Santos E. Towards the genetic manipulation of microalgae to improve the carbon dioxide fixation and the production of biofuels: present status and future prospect. In: Alam MWZ, editor. Microalgae biotechnology for development of biofuel and wastewater treatment. Springer, Singapore; 2019. p. 135–46. https://doi.org/10.1007/978-981-13-2264-8_7
161. Gerotto C, Norici A, Giordano M. Toward enhanced fixation of CO_2 in aquatic biomass: focus on microalgae. Front Energy Res. 2020;8:213. https://doi.org/10.3389/fenrg.2020.00213.
162. Raines CA. Increasing photosynthetic carbon assimilation in C3 plants to improve crop yield: current and future strategies. Plant Physiol. 2011;155(1):36–42. https://doi.org/10.1104/pp.110.168559.
163. Beacham TA, Sweet JB, Allen MJ. Large scale cultivation of genetically modified microalgae: a new era for environmental risk assessment. Algal Res. 2017;25:90–100. https://doi.org/10.1016/j.algal.2017.04.028.
164. Spicer A, Molnar A. Gene editing of microalgae: Scientific progress and regulatory challenges in Europe. Biology. 2018;7(1):21. https://doi.org/10.3390/biology7010021.
165. Dattaroy T, Badami P, Dasgupta S. Biosafety and regulatory issues related to genetically modified microalgae. In: Shekh A, Schenk P, Sarada R, editors. Microalgal Biotechnology: recent advances, market potential, and sustainability. Royal Society of Chemistry; 2021. p. 305–24. https://doi.org/10.1039/9781839162473-00305.
166. Campden BRI (Chipping Campden) Ltd. Comparing international approaches to food safety regulation of GM and novel foods. https://doi.org/10.46756/sci.fsa.rdg239.
167. Glass DJ. Government regulation of the uses of genetically modified algae and other microorganisms in biofuel and bio-based chemical production. In: Prokop A, Bajpai RZM, editors. Algal Biorefineries. Springer, Cham; 2015. p. 23–60. https://doi.org/10.1007/978-3-319-20200-6_2.
168. Abdullah MFF, Anthonysamy MA. Malaysia—genetic modifications and synthetic biology regulations and biodiversity – a legal perspective for Malaysia. In: Chaurasia A, Hawksworth DLP de MM, editors. GMOs topics in biodiversity and conservation. Springer, Cham; 2020. p. 577–83. https://doi.org/10.1007/978-3-030-53183-6_33.

169. Nethravathy MU, Mehar JG, Mudliar SN, Shekh AY. Recent advances in microalgal bioactives for food, feed, and healthcare products: commercial potential, market space, and sustainability. Compr Rev Food Sci Food Saf. 2019;18(6):1882–97. https://doi.org/10.1111/1541-4337.12500.
170. Usher PK, Ross AB, Camargo-Valero MA, Tomlin AS, Gale WF. An overview of the potential environmental impacts of large-scale microalgae cultivation. Biofuels. 2014;5:331–49. https://doi.org/10.1080/17597269.2014.913925.
171. Kumar S. GM Algae for biofuel production: biosafety and risk assessment. Collect Biosaf Rev. 2015;9:52–75.
172. Henley WJ, Litaker RW, Novoveská L, Duke CS, Quemada HD, Sayre RT. Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. Algal Res. 2013;2(1):66–77. https://doi.org/10.1016/j.algal.2012.11.001.
173. Orejuela-Escobar L, Gualle A, Ochoa-Herrera V, Philippidis GP. Prospects of microalgae for biomaterial production and environmental applications at biorefineries. Sustainability. 2021;13(6):3063. https://doi.org/10.3390/SU13063063.
174. Shokravi H, Shokravi Z, Heidarrezaei M, Ong HC, Rahimian Koloor SS, Petrů M, Lau WJ, Ismail AF. Fourth generation biofuel from genetically modified algal biomass: challenges and future directions. Chemosphere. 2021;285: 131535. https://doi.org/10.1016/j.chemosphere.2021.131535.
175. Glass DJ. Pathways to obtain regulatory approvals for the use of genetically modified algae in biofuel or biobased chemical production. Ind Biotechol. 2015;11:71–83. https://doi.org/10.1089/ind.2015.1503.