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REVIEW

Integrated Strategies to Enhance Lipid Productivity in Microalgae for Sustainable Biodiesel Production

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ABSTRACT

Microalgae have emerged as a promising third-generation feedstock for sustainable biodiesel production due to their rapid growth, high photosynthetic efficiency, and ability to grow on non-arable land without competing with food resources. However, their relatively low natural lipid content remains a critical challenge for commercial-scale biodiesel viability. This review presents a comprehensive overview of integrated strategies to enhance lipid productivity in microalgal biomass, including optimization of cultivation media under nutrient stress, nanoparticle-mediated stimulation, and genetic engineering interventions. Recent advancements in cell wall disruption and lipid extraction techniques are also discussed, alongside the development of in situ transesterification to improve biodiesel conversion efficiency. Furthermore, a techno-economic analysis highlights the potential and limitations of these approaches in industrial applications. By integrating biological, chemical, and engineering innovations, this paper underscores the viability of microalgae as a renewable and scalable source for biodiesel, contributing toward global energy sustainability.

KEYWORDS Microalgae, Lipid Enhancement, Biodiesel Production, Genetic Engineering, Transesterification

1. Introduction

Microalgae, comprising a diverse array of uncomplicated photosynthetic and autotrophic microorganisms, thrive in both marine and freshwater environments. While predominantly consisting of single-celled prokaryotes like *Chloroxybacteria* (cyanobacteria), there are also numerous species of multicellular eukaryotes, such as *Bacillariophyta* (diatoms), *Chlorophyta* (green microalgae), and *Rhodophyta* (red microalgae) (Brennan and Owende 2010). Utilizing solar energy, microalgae play a critical role in carbon sequestration by absorbing significant amounts of CO_2 and transforming it into biomass. In contrast to typical vegetation, microalgal biomass boasts rapid reproduction, growth, efficient photosynthesis, high yield, and rich nutrient production. Adapted to survive under adverse conditions, microalgae require minimal resources such as fresh water and land, thereby alleviating competition for agricultural resources (Shakir and Ahmad 2025). In addition to their role in environmental management through wastewater treatment and CO_2 capture, microalgae are a rich source of valuable bio-compounds, including proteins, carbohydrates, lipids, and carotenoids. These attributes make microalgae a versatile solution to meet the increasing global demand for nutraceuticals, pharmaceuticals, cosmetics, and, importantly, sustainable biofuels. Their unique position at the intersection of environmental sustainability and biotechnological potential underscores the significance of microalgae in advancing marine biotechnology for renewable energy production. Furthermore, microalgae are increasingly recognized as a cornerstone of third-

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generation biofuel development, owing to their high productivity and low dependency on arable land and freshwater resources. As the global community seeks to reduce reliance on fossil fuels, microalgae-based biodiesel presents a compelling alternative due to its closed carbon cycle and lower greenhouse gas emissions (Tarigan 2023; Uyar and Hayber 2025). The use of microalgae for biodiesel aligns with the principles of sustainable development by promoting energy security, reducing environmental impact, and utilizing non-food biomass that does not compete with agricultural production (Tan et al. 2020; Liu et al. 2023; Binhweel et al. 2025).

Building upon their established role in sustainable biofuel production, the diverse biochemical composition of microalgae also supports a wide range of applications across industrial and commercial sectors. In addition to energy, microalgae biomass is increasingly utilized in nutraceuticals, pharmaceuticals, cosmetics, and functional food industries due to its rich content of bioactive compounds. Microalgae-derived carbohydrates serve as an alternative carbon source, replacing conventional materials such as sugar and lignocellulose (Li *et al.* 2025). Proteins, which can constitute up to 50–70% of certain microalgal species biomass, are valuable for both human nutrition and animal feed. Moreover, microalgae are a natural source of pigments – such as chlorophylls, carotenoids, astaxanthin, and phycobiliproteins which are widely used in health supplements and cosmeceuticals for their antioxidant, anti-aging, and photoprotective properties (Nobre *et al.* 2013; Cheng *et al.* 2016). Vitamins and trace elements extracted from microalgal biomass further enhance their value in dietary and therapeutic applications. Lipids, one of the most critical components for both biodiesel and health-related uses, are meticulously extracted through solvent and non-solvent methods and include n-3 polyunsaturated fatty acids known to reduce cardiovascular risks (Wang *et al.* 2015). As marine fish oil reserves decline, microalgae have emerged as a sustainable and renewable source of these essential fatty acids. This growing commercial relevance, coupled with their environmental advantages, positions microalgae as a cornerstone of the bioeconomy, supporting both green energy initiatives and global health priorities (Chew *et al.* 2017).

The integration of microalgae in the bioenergy sector is a burgeoning trend driven by their high biomass productivity and biochemical versatility, which make them suitable for producing a wide range of biofuels. Microalgae biomass can be converted into gaseous fuels (syngas, biogas), liquid fuels (bio-oil, bioethanol, biodiesel), and solid fuels (biochar), using techniques such as anaerobic digestion, pyrolysis, enzymatic fermentation, and catalytic transesterification (Shakir *et al.* 2024). Among these options, biodiesel derived from microalgal lipids has received the most attention due to its compatibility with existing diesel infrastructure and potential for carbon-neutral combustion. However, despite these promising attributes, large-scale commercialization remains limited by challenges such as suboptimal cultivation conditions, inefficient harvesting technologies, and most critically, the inherently low lipid content of many microalgal strains. Given that lipid content is the most decisive factor influencing biodiesel yield, enhancing lipid accumulation has become a central focus of research and development efforts. This calls for the implementation of integrated strategies that address the entire production chain from upstream biomass cultivation to downstream processing and conversion (Chew *et al.* 2017; Hossain *et al.* 2019; Binhweel *et al.* 2026).

Lipids are the primary component of interest in microalgae for biodiesel production, typically comprising 10% to 30% of the dry weight but potentially exceeding 80% under stress-optimized conditions (Wood 2021; Rawat *et al.* 2022). However, a well-documented inverse relationship exists between growth rate and lipid accumulation, where rapid cell proliferation often results in lower lipid yields (Arora *et al.* 2018). Additionally, unicellular microalgal species generally exhibit higher lipid content than multicellular types (Wood 2021). Recent research has focused on enhancing lipid synthesis through various strategies, including nutrient starvation like nitrogen depletion, nanoparticle-based stimulation, and genetic engineering approaches that modulate lipid biosynthetic pathways (Yaakob *et al.* 2021; Rawat *et al.* 2022). Despite these advances, a comprehensive review that consolidates these multidisciplinary strategies, particularly those that combine cultivation optimization, nanotechnology, genetic modification, and downstream

processing remains scarce in the current literature. Therefore, the contribution of the current review beyond prior researches is integrating the innovative approaches to enhance lipid productivity in microalgae feedstock for the purpose of biodiesel synthesis. Additionally, particular emphasis was made on methods of cell wall disruption, advanced lipid extraction, and in situ transesterification for direct biodiesel synthesis from microalgae feedstock. Moreover, a techno-economic analysis was presented to assess the feasibility and sustainability of these strategies, offering critical insights for future research and commercial implementation.

This narrative review is based on a comprehensive survey of up-to-date literature published in reputable peer-reviewed journals. Relevant studies were identified through systematic searches of major scientific databases covering publications related to microalgae lipid, lipid enhancement strategies, extraction techniques, microalgae-based biodiesel, and techno-economic analysis of biodiesel from microalgae were used. Studies were screened based on relevance, scientific quality, and contribution to the field, while non-peer-reviewed and unrelated works were excluded. The selected literature was critically analyzed and synthesized to provide an integrated perspective on lipid enhancement and biodiesel production from microalgae.

2. Lipid enhancement

The high productivity of lipid content is crucial for the utilization of microalgae in the biodiesel industry. However, conventional microalgae cultivations typically result in lipid content below 30% at best, necessitating the enhancement of lipid productivity to achieve economically feasible revenues in the biodiesel industry. To address this challenge, researchers have developed multiple techniques, including controlling cultivation medium, introducing nanoparticles, and modifying genes using genetic engineering. Figure 1 shows theses augmentation techniques for microalgae lipids followed by a comprehensive discussion.

Figure 1. Summary of contemporary approaches to boost lipid levels in microalgae.

2.1. Cultivation medium

The cultivation medium profoundly influences microalgal growth and composition, primarily through constituent nutrients and physical factors such as temperature, light, light-dark cycles, and chemical factors including pH, turbulence, salinity, and CO_2 concentrations (Zarrinmehr *et al.* 2020). Nitrogen and phosphorus, among the nutrients, play a crucial role in microalgae growth and protein synthesis when adequately supplied in the cultivation medium. However, lipid content typically does not thrive in microalgae cultivated in nutrient-replete conditions (Yodsuwan *et al.* 2017). Thus, limited concentrations of certain nutrients like nitrogen and phosphorus can enhance lipid productivity (Yaakob *et al.* 2021).

2.1.1. Nitrogen deprivation

Nitrogen stands as a key macronutrient in microalgal culture mediums, typically taken up by microalgae cells in various forms, including nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and

urea ($CO(NH_2)_2$). However, nitrate is commonly preferred due to its stability, pH compatibility, and lack of toxicity (Procházková *et al.* 2014). Nitrogen, a vital macronutrient, plays a crucial role in influencing both the growth and biochemical compositions of microalgae. While a nitrogen-rich medium stimulates growth rates and increases protein content within microalgae, a nitrogen-deficient medium boosts lipid and carbohydrate productivity (Zarrinmehr *et al.* 2020). Under nitrogen-deprived conditions, microalgal strains typically exhibit a twofold increase in lipid formation (Jia *et al.* 2015). In nitrogen-depleted conditions, the culture medium triggers a decrease in thylakoid cellular membranes, activates acyl hydrolase, and boosts phospholipid hydrolysis, generating intracellular fatty acyl-CoA (Chu *et al.* 2013). Additionally, microalgal strains cultivated in nitrogen-deficient mediums undergo metabolic shifts to accumulate greater amounts of triacylglycerides (TAGs) in cytoplasmic organelles, providing a source of carbon and energy (Yaakob *et al.* 2021). Table 1 illustrates a comparison between the impacts of nitrogen-rich and nitrogen-deficient media on microalgal growth and lipid content.

Table 1. Effects of nitrogen on the growth and lipid content in microalgae.

Cultural medium	Microalgal strain	Effect of nitrogen conditions on microalgal growth and lipid content	Reference
Nitrogen-replete culture medium	Scenedesmus obliquus	The highest microalgae growth, 1.7×10^7 cells mL^{-1}, occurred with the addition of 2 mg N L^{-1} of CH_4N_2O, while the highest lipid content, 30.6%, was observed with the addition of only 0.1 mg N L^{-1} of NH_4Cl.	(An et al. 2020)
	Phaeodactylum tricornutum	At the initial concentration of $NaNO_3$ which was 0 mgL^{-1}, the growth was 68.57 mgL-1 and lipid content was 53.04%. However, once the concentration of $NaNO_3$ increased 64.29 mgL^{-1}, the microalgae growth increased to 225.81 mgL^{-1} while lipid content was reduced into 2.79%.	(Yodsuwan et al. 2017)
	Chlorella zofingiensis	Under nitrogen-replete conditions, the growth of microalgae increased from 0.2 to 3.1 g L^{-1} by day 10. However, the lipid content decreased from 6% to 2.6% within the first two days, then increased to 8.6% by day 6 and remained constant thereafter.	(Zhu et al. 2014)
	Nannochloropsis sp.	Under sufficient nitrogen, algae productivity was 0.36 $gL^{-1}day^{-1}$ with 32% lipid content. In contrast, nitrogen deprivation reduced growth to 0.30 $gL^{-1}day^{-1}$ while increased lipid content to 60%.	(Rodolfi et al. 2009)
Nitrogen-deplete culture medium	Picocystis salinarum	Biomass decreased from 0.96 to 0.74 g/L as nitrogen levels decreased from 1 to 0.125 N. However, lipid content increased from 21.23% to 33.87% under the same nitrogen reduction.	(Delgado et al. 2021)
	Rhodomonas sp.	Under nitrogen-starvation conditions, the microalgal biomass decreased from 6.3×10^6 to 2.5×10^6 cells/mL after 192 hours. In contrast, lipid content increased significantly from 9.2% to 30.3% within 120 h.	(Latsos et al. 2020)
	Phaeodactylum tricornutum	Decrease N of $NaNO_3$ from 64.29 to 0 mgL^{-1} led to reduction in the microalgae growth from 225.81 to 68.57 mgL^{-1}, respectively, and an increase in lipid content from 2.79 to 53.04%, respectively.	(Yodsuwan et al. 2017)

Table 1. (continued)

Cultural medium	Microalgal strain	Effect of nitrogen conditions on microalgal growth and lipid content	Reference
	Chlorella zofingiensis	Under N-deplete conditions, the lipid content of microalgae increased rapidly from 6.2% to 24.5% based on dry weight.	(Zhu et al. 2014)
	Nannochloropsis sp.	Under nitrogen-deplete culture, biomass productivity was 0.30 g L^{-1} day^{-1}, and lipid content reached 60%, which is 93% higher compared to the lipid content under nitrogen-sufficient culture that reached 32%.	(Rodolfi et al. 2009)

2.1.2. Phosphorous depletion

While phosphorus makes up less than 1% of microalgae, it is crucial for their growth and sustainability, with optimal ratios ranging from 0.001 to 0.179 $g.L^{-1}$ in microalgae (Roopnarain *et al.* 2014) and 0.3 - 0.6 $g.L^{-1}$ in the culture medium (Hannon *et al.* 2010). Phosphoric nutrients, primarily in the form of polyphosphate or orthophosphate, serve dual roles in microalgal growth: polyphosphate acts as a protective agent against toxic metals like copper and cadmium, while orthophosphate transforms into adenosine triphosphate (ATP), vital for sustaining microalgal biomass processes and facilitating phosphorus uptake in adverse nutritional conditions (Solovchenko *et al.* 2020). Thus, phosphorus is vital for microalgae, influencing cellular composition and biomass structure. It is integral to synthesizing cellular DNA, RNA, ATP, and phospholipids, and contributes to biomass growth, lipid productivity, photosynthesis, and energy and signal transfer (Atiku *et al.* 2016). Under phosphorus-limiting conditions, microalgae demonstrate different responses in biomass growth and biochemical compositions. Nonetheless, lipid production in microalgae strains can be enhanced by cultivating them in phosphorus-depleted or restricted mediums. In phosphorus-deficient environments, carbon acquired through photosynthesis is redirected towards the synthesis of energy-rich macromolecules, predominantly lipids, which are stored within the microalgae (Yaakob *et al.* 2021). Table 2 examines the influence of phosphorus concentration on the growth of microalgae and the production of lipids.

Table 2. The relationship between phosphorus concentration and biomass/lipid content of microalgae.

Microalgal strain	Effect of phosphorous conditions on growth and lipids	Reference
Scenedesmus sp	Decreasing phosphorus concentration from 50 to 1 mg L^{-1} led to a reduction in microalgal biomass from 0.9 g L^{-1} to 0.6 g L^{-1} and an increase in lipid content from 22.3% to 42.5%, respectively.	(Yang et al. 2018)
Rhodosporidium kratochvilovae	At a constant N concentration of 0.1 g L^{-1}, 9.23 g L^{-1} of microalgal biomass and 59.69% lipid content were obtained at a phosphorus concentration of 1 g L^{-1}. However, when the phosphorus concentration decreased to 0.05 g L^{-1}, biomass reduced to 7.54 g L^{-1}, while lipid content showed a slight increase to 60.34%	(Patel et al. 2017)
Chlorella ellipsoidea	The highest biomass (1.56 g L^{-1}) was obtained at a phosphorus concentration of 1.5 g L^{-1}. In contrast, the highest lipid content (41.8%) was observed at a phosphorus concentration below 0.5 g L^{-1}.	(Satpati et al. 2016)
Chlorococcum infusionum	The highest biomass (2.17 g L^{-1}) was obtained at a phosphorus concentration of 1.5 g L^{-1}. In contrast, the highest lipid accumulation (31.3%) was observed at an almost zero phosphorus concentration.	

Table 2. (continued)

Microalgal strain	Effect of phosphorous conditions on growth and lipids	Reference
Isochrysis galbana	Microalgal growth increased from 0.1043 to 0.1293 cells μL^{-1} day^{-1} as phosphorus concentration decreased from 25% to 0%. The highest lipid accumulation (50%) was recorded at phosphorus concentrations between 0% and 12.5%.	(Roopnarain et al. 2014)
Chlorella sp.	Microalgal biomass increased with rising phosphorus concentrations from 16 to 80 µM. However, the highest lipid content, 23.60%, was observed at 32 µM.	(Liang et al. 2013)
Phormidium sp.	Microalgal growth increased from 0.097 to 0.207 g dry weight as the K_2HPO_4 concentration increased from 0% to 100%, respectively. The highest lipid accumulation (27.9%) was observed at a K_2HPO_4 concentration of 50%.	(Salem et al. 2013)
Chlorella vulgaris	Microalgal biomass growth increased from 0.0198 to 0.1582 g dry weight as the K_2HPO_4 concentration increased from 0% to 150%, respectively. In contrast, lipid content increased from 1.3% to 37.8% as the K_2HPO_4 concentration decreased from 250% to 0%, respectively.	
Chlorella zofingiensis	The highest specific microalgal growth rate (2.15 day^{-1}) was achieved at a $K_2HPO_4 \cdot 3H_2O$ concentration of 0.01 g L^{-1}, while the highest lipid content (44.7%) was attained under phosphorus-deficient conditions	(Feng et al. 2012)

2.2. Nanoparticles

Beyond cultivation-based nutrient optimization, emerging approaches such as nanoparticle application provide additional mechanisms to enhance lipid productivity. Nanoparticles have been integrated into microalgae for bioenergy production, capitalizing on their unique physical and chemical properties. These properties, including their large surface area and strong, persistent, elastic, and electrical characteristics, are leveraged to enhance microalgal growth, lipid production, harvesting efficiency, and catalytic biodiesel production. Specifically, nanoparticles facilitate increased gas-liquid mass transfer rates, thereby elevating CO_2 concentrations at the gas-liquid interface to promote both microalgal growth and lipid content (Zhu *et al.* 2010). Exposure to various nanoparticle composites significantly affected both the growth rate and lipid productivity of *Scenedesmus obliquus*. For example, a concentration of 5 $mg.L^{-1}$ of carbon nanotubes (CNT) led to a growth rate of 7.5 × 107 $cells.mL^{-1}$ and an 8.9% increase in lipid content, while the maximum lipid content of 39.6% was observed with a dosage of 5 $mg.L^{-1}$ of α-Fe_2O_3. Conversely, the growth rate declined when MgO nanoparticles were applied, but a concentration of 40 $mg.L^{-1}$ of MgO nanoparticles increased lipid content to 18.5% in S. obliquus (He *et al.* 2017). However, some composites of nanoparticles, such as gold, silver, palladium, selenium, zinc oxide, copper oxide, and ferrous oxide, exhibited toxic properties to various microalgae strains, particularly at high doses. Hence, reducing the doses of these nanoparticles would reduce toxicity and enhance content of lipids in microalgae (Rawat *et al.* 2022).

Excessive nanoparticle doses result in elevated levels of reactive oxygen species, leading to microalgae cell death, subsequently reducing growth rates and lipid productivity (He *et al.* 2017). Nanoparticles are employed not only to enhance algal growth and lipid productivity but also to efficiently harvest microalgal yield, thereby increasing lipid content, with silica-coated magnetic nanoparticles demonstrating the ability to separate over 95% of various freshwater and marine microalgal strains, such as *Chlamydomonas reinhardtii, Chlorella vulgaris, Phaeodactylum tricornutum,* and *Nannochloropsis salina*, through high flocculation facilitated by adsorption of micron-sized algal cells onto submicron-sized nanoparticles, depending on nanoparticle and medium characteristics (Cerff *et al.* 2012). Furthermore, the utilization of iron oxide nanoparticles in conjunction with the

cationic polyelectrolyte poly(dimethyldiallylammonium chloride); PDDA enabled the efficient harvesting of Chlorella sp. microalgae, resulting in a 99% removal efficiency (Lim *et al.* 2012). Moreover, under optimal conditions, Fe_3O_4 nanoparticles achieved a 98% efficient removal of *Botryococcus braunii* and *Chlorella ellipsoidea* (Xu *et al.* 2011).

Beside inducing growth rate, lipid productivity, and harvesting, nanoparticles have been utilized to catalyze transesterification reaction to produce biodiesel from microalgae lipids. Transesterification is a chemical reaction in which FA contained in lipid classes such as triglycerides, phospholipids or glycolipids are converted into esters with help of base and/or acid catalyst (Senusi *et al.* 2024). In this context, nanoparticle composites were used to catalyze the transesterification reaction. As an example, calcium oxide (CaO) was synthesized from eggshell waste and utilized as a catalyst for transesterifying microalgal *C. vulgaris* biomass, resulting in a 92.03% yield of biodiesel (Pandit and Fulekar 2019). This biodiesel yield is considered quite high compared to another study that utilized the same microalgal strain with an alkaline NaOH catalyst, which produced 77.6% biodiesel (Velasquez-Orta *et al.* 2012). This proves the significant contribution of nanoparticles in enhancing biodiesel yield synthesized from microalgae lipids. Table 3 shows the applications of various nanoparticles in enhancing lipid productivity, harvesting microalgal biomass, and catalyzing transesterification of microalgae biomass.

Table 3. Various applications of nanoparticles in microalgae biomass toward biodiesel production.

Microalgae strain	Result	References
Chlorella vulgaris	CaO was used to catalyze the transesterification of microalgae biomass for biodiesel production. The results showed 92.03% of biodiesel was yielded at optimum values.	(Pandit and Fulekar 2019)
Scenedesmus obliqus	A concentration of 5 mL^{-1} of carbon nanotubes (CNTs) enhanced microalgal growth to approximately 7.5 × 10^7 cells/mL and increased lipid content to 8.9%.	(He et al. 2017)
Scenedesmus obliqus	A concentration of 2 mg L^{-1} of α-Fe_2O_3 enhanced microalgal growth to approximately 5 × 10^7 cells/mL, while 5 mg L^{-1} of α-Fe_2O_3 increased lipid content to 39.6%.	
Scenedesmus obliqus	Microalgal growth was inhibited upon exposure to MgO. However, a concentration of 40 mg L^{-1} of MgO induced lipid accumulation to 18.5%.	
Freshwater and marine microalga	Silica-coated magnetic Fe_3O_4 nanoparticles were used to separate freshwater and marine microalgae. All microalgal strains were separated with an efficiency of more than 95%.	(Cerff et al. 2012)
Chlorella sp.	Iron oxide nanoparticles in presence of cationic polyelectrolyte (PDDA) was applied on the microalgae for harvesting. The result showed that microalgal biomass was harvested with an efficiency of 99%.	(Lim et al. 2012)
Botryococcus braunii and Chlorella ellipsoidea.	Fe_3O_4 was used for harvesting the microalgal strains. The maximum microalgal recovery reached 98% for both strains at optimal conditions.	(Xu et al. 2011)

2.3. Genetic engineering

In contrast to external methods of nutrient optimization and nanoparticle application, genetic engineering enables direct modification of intracellular metabolic pathways for increasing lipid accumulation. The significant advancements in genetic engineering over the last few decades have spurred researchers to employ genetic tools for enhancing lipid productivity in microalgae biomass. Genetic modifications in microalgae can be achieved through random mutagenesis, physical or chemical adaptive laboratory evolution, and genetic engineering, with the latter being the most

precise method due to its ability to accurately insert, substitute, or delete targeted genes and avoid undesired outcomes (Muñoz *et al.* 2021). With the progress in omics technology and genetic modification tools, various genetic engineering strategies have been devised to increase lipid content in microalgal biomass. These strategies encompass adjustments to fatty acid synthesis metabolism, the Kennedy pathway, polyunsaturated fatty acid metabolism, transcription factors, nicotinamide adenine dinucleotide phosphate (NADPH) generation, central carbon metabolism, lipid catabolism, and other related pathways (Muñoz *et al.* 2021). Fatty acid synthesis occurs in the chloroplast through a series of conversion processes from acetyl-CoA to malonyl-CoA, then malonyl-ACP, and finally to saturated fatty acids (SFAs), facilitated by various enzymatic catalyses. The pivotal step in this process is the formation of malonyl-CoA, which is catalyzed by acetyl-CoA carboxylase (ACC). Therefore, overexpressing the ACC gene plays a significant role in increasing lipid content in microalgae strains (Li-Beisson *et al.* 2019). Moreover, genes such as acetyl-CoA carboxylase 1 (ACC1), glycerol-3-phosphate dehydrogenase 1 (GPD1), glycerol kinase (GUT1), acetyl-CoA synthetase (ACS), and Chlamydomonas reinhardtii fatty acid desaturase 2 (CrFAB2) have been identified to significantly contribute to lipid enhancement through the manipulation of fatty acid synthesis metabolism, as detailed in Table 4. In the Kennedy pathway, glycerolipid synthesis begins with glycerol-3-phosphate, catalyzed by glycerol 3-phosphate acyltransferase (GPAT), and undergoes a series of conversion processes ultimately leading to the formation of triacylglycerols (TAGs), which are abundant lipids in microalgae with potential applications in the biofuel industry (Yu *et al.* 2011).

Table 4. Genetic engineering strategies for lipid augmentation in microalgae strains

Genetic engineering strategy	Microalgae species	Gene	Gene expression	Outcomes	References
Fatty acids synthesis metabolism	Scenedesmus quadricauda	acetyl-CoA carboxylase 1 (ACC1), glycerol-3-phosphate dehydrogenase 1 (GPD1), glycerol kinase (GUT1)	Heterologous expression	ACC1 enhanced fatty acid by 1.6-fold. GPD1 and GUT1 enhanced glycerol-3-phosphate by 1.6- and 1.9-fold, respectively.	(Gomma et al. 2015)
	Chlamydomonas reinhardtii	Chlamydomonas reinhardtii fatty acid desaturase 2 (CrFAB2)	Overexpression	28% increment in fatty acid content	(Hwangbo et al. 2014)
	Schizochytrium sp	acetyl-CoA synthetase (ACS)	Heterologous expression	The fatty acid content and microalgal biomass were enhanced by 11.3 and 29.9%, respectively.	(Yan et al. 2013)

Table 4. (continued)

Genetic engineering strategy	Microalgae species	Gene	Gene expression	Outcomes	References
Kennedy pathway	Neochloris oleoabundans	Plastidial lysophosphatidic acid acyltransferase (NeoLPAAT1), endoplasmic reticulum-located diacylglycerol acyltransferase 2 (NeoDGAT2)	Co-overexpression	1.6-fold and 2.1-fold increments in total lipid content and TAGs, respectively.	(Chungjatupornchai and Fa-aroonsawat 2021)
	Phaeodactylum tricornutum	glycerol-3-phosphate acyltransferase 2 (GPAT2)	Overexpression	Increment in TAG* by 2.9-fold.	(Wang et al. 2020)
	Chlamydomonas reinhardtii	lysophosphatidic acyltransferase (c-lpaat), glycerol-3-phosphate dehydrogenase (c-gpd1)	Heterologous expression	44.5% and 67.5% lipid increment by c-lpaat and c-gpd1, respectively.	(Wang et al. 2018)
Polyunsaturated fatty acids metabolism	Dunaliella salina	Thalassiosira pseudonana delta 6 fatty acid desaturase (TpFADS6), D. salina delta 6 fatty acid desaturase (DsFADS6)	Overexpression & heterologous expression	21.3 mg/L increment in EPA, and further increase to 91.3 mg/L, 192.9 mg/L, and 554.3 mg/L when additional agents of myoinositol, CO_2 with glucose/KNO_3, and addition of perilla seed meal.	(Shi et al. 2018)
	Nannochloropsis oceanica	Δ12 fatty acid desaturases (Δ12 FAD), Δ5 fatty acid desaturases (Δ5 FAD)	Overexpression	25% increment in EPA.	(Poliner et al. 2018)
	Phaeodactylum tricornutum	Phaeodactylum tricornutum delta 5 desaturase (PtD5b)	Overexpression	Increments in MUFA, PUFA*, EPA*, and neutral lipids (nonpolar lipids) by 75%, 64%, 58%, and 65%, respectively.	(Peng et al. 2014)

Table 4. (continued)

Genetic engineering strategy	Microalgae species	Gene	Gene expression	Outcomes	References
Transcription factors	Nannochloropsis oceanica	putative APETALA2-like transcription factor gene (APETALA2-)	Knockout	40% increment in neutral lipids.	(Südfeld et al. 2021)
	Nannochloropsis salina	Nannochloropsis salina basic leucine zipper (NsbZIP1)	Overexpression	33% and 88% increments in neutral lipids, and 21% and 39% increments in FAME* under normal condition and N limitation condition, respectively for both.	(Kwon et al. 2018)
	Chlorella ellipsoidea	Glycine max DNA binding with one finger (GmDOF4),	Heterologous expression	52.9% was the highest increment in lipid content.	(Zhang et al. 2014)
Photosynthesis and NADPH generation	Nannochloropsis salina	Chlorophyllide a oxygenase from chlamydomonas (CrCAO)	Heterologous expression	Increment in growth rate and lipid productivity under medium light condition.	(Koh et al. 2019)
	Schizochytrium sp.	superoxide dismutase (SOD1)	Overexpression	1.37-fold and 1.18-fold increments in PUFA and SFA, respectively, compared to wild-type situation.	(Zhang et al. 2018)
	Chlamydomonas reinhardtii	plastid-encoded ferredoxin (PETF), Ferredoxin 5 (FDX5)	Overexpression	Substantial increment in lipids content for both genes under stress condition.	(Huang et al. 2015)
Central carbon metabolism	halassiosira pseudonana	6-phosphofructo-2-kinase/fructose 2,6 bisphosphatase (PFK2/F2BP)	Overexpression	Recorded increments in neutral lipids and FAME content.	(Abbriano et al. 2018)
	Fistulifera solaris	Glycerol kinase (GK),	Overexpression	12% increment in lipid productivity	(Muto et al. 2015)

Table 4. (continued)

Genetic engineering strategy	Microalgae species	Gene	Gene expression	Outcomes	References
	Phaeodactylum tricornutum	Glycerol-3-phosphate dehydrogenase (GPDH),	Overexpression	60% increment in neutral lipid reaching 39.7% w/w dry weight.	(Yao et al. 2014)
Lipid catabolism and other strategies	Phaeodactylum tricornutum	stramenopile-type lipid droplet protein (StLDP),	Overexpression	The neural lipids increased from 45.3% to 53.5 %	(Yoneda et al. 2018)
	Phaeodactylum tricornutum	Human Patatin-like phospholipase domain-containing protein 3 (HsPNPLA3),	Heterologous expression	52% and 64% increments in total fatty acid and TAG, respectively.	(Wang et al. 2018)
	Chlamydomonas reinhardtii	Synchronous phospholipid: diacylglycerol acyltransferase (ScPDAT)	Heterologous expression	22% and 32% increments in total fatty acid and TAG, respectively.	(Zhu et al. 2018)

*Acronyms: TAG: triacylglycerol, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, EPA: eicosapentaenoic Acid, and FAME: fatty acid methyl ester.

Table 4 presents examples of how transcription factors contribute to lipid enhancement in microalgae. Photosynthesis in microalgae is initiated by light energy, where electrons coupled with H+ from water (H_2O) transfer from stroma to lumen, facilitating ATP formation. Additionally, glyceraldehyde 3-phosphate (GAP) is synthesized from atmospheric CO_2, which is then converted into sugars and lipids through various biological and chemical processes within the microalgae structure. Consequently, modifying photosynthetic capacity and enhancing reducing equivalent supplies can lead to lipid augmentation (Subramanian *et al.* 2013). Overexpressing Ferredoxin 1 (PETF) and 5 (FDX5) genes has been demonstrated to enhance the light reactions involved in lipid synthesis in microalgae strains (Huang *et al.* 2015). Moreover, the synthesis of lipids necessitates nicotinamide adenine dinucleotide phosphate (NADPH). Consequently, augmenting these factors results in an increase in lipid formation (Cornish-Bowden 2014). Central carbon metabolism is crucial for directing carbon flux and regulating acetyl-CoA and G3P, which can be utilized for TAG synthesis or converted into dihydroxyacetone phosphate (DHAP), thereby influencing glycolysis or gluconeogenesis (Subramanian *et al.* 2013; Han *et al.* 2016). Ultimately, the lipid catabolism strategy relies on triacylglycerols (TAGs) stored within multifunctional cytosolic lipid droplets (LDs). While the formation of LDs remains somewhat obscure, they primarily consist of TAGs enclosed by phospholipids. Consequently, certain microalgae have been genetically engineered to augment lipid content using this approach (Muñoz *et al.* 2021).

Overall, lipid enhancement methods in microalgae feedstock demonstrate a clear integration between biomass and lipid productivity. Nutrient deprivation methods, nitrogen and phosphorus limitation, are simple and less costly. However, they often result in reduced biomass yield, which may negatively affect the total lipid productivity since the produced feedstock is low. Conversely, nanoparticle application approaches offer multifunctional merits such as improved growth,

harvesting efficiency, and catalytic conversion. The toxicity, cost, and environmental sustainability remain a great concern to large-scale implementation of nanoparticle application. Genetic engineering provides the most targeted and potentially high-yield techniques, enabling precise manipulation of metabolic pathways, yet its industrial application is limited by regulatory challenges, genetic stability, and scalability issues. Importantly, most studies focus primarily on lipid accumulation without sufficiently evaluating how these upstream strategies influence downstream biodiesel conversion efficiency, fuel properties, and techno-economic feasibility. Therefore, future research should emphasize integrated approaches that balance lipid productivity, biomass yield, and downstream processing performance to ensure economically viable biodiesel production.

3. Lipids recovery

Following lipid enhancement approaches, efficient recovery of the produced lipids is an essential step in the overall biodiesel production. The effectiveness of lipid separation is highly dependent on both the quantity and composition of lipids generated during upstream processes, in addition to the extraction techniques that include cell disruption and lipid extraction methods.

3.1. Cell disruption

Recovering lipids from microalgae is vital for their use in biodiesel industry, but the thick cell wall surrounding microalgal cells represents a challenge in accessing and extracting the lipidic compounds. Therefore, breaking down the rigidity of the cell wall is necessary to maximize the extraction of microalgal lipid content. The process of lysing the cell wall precedes lipid extraction, although they are often carried out simultaneously as a single operation (Lee *et al.* 2021). Figure 2 depicts various mechanical, chemical, and physical approaches employed to disrupt the microalgal cell wall.

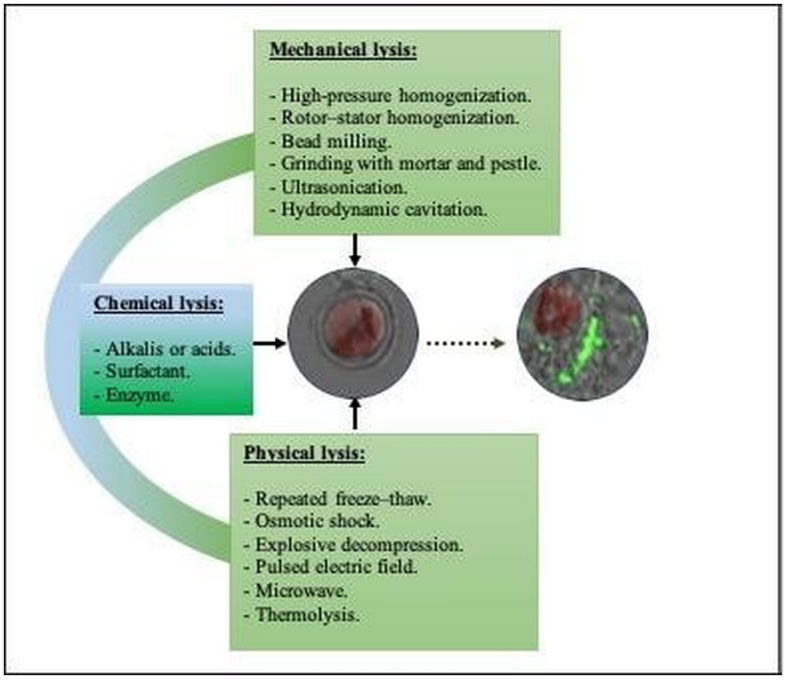

Figure 2. Approaches of microalgal cell disruption (Adapted from (Lee et al. 2021)).

Mechanical disruption involves applying external force to microalgae to rupture the cell wall. There are multiple mechanical techniques that can be used to disrupt the rigid microalgal cell wall. High-pressure homogenization technique forces microalgal cells to go through a high pressurized and narrow valve that leads to break the microalgal cell wall. In contrast, rotor–stator

homogenization breaks the rigid microalgal cell wall by the shearing action between a constant outer stator and a fast-spinning inner roto. Another technique depends on bead milling, in which agitating beads collide with the microalgae cells, leading to the crushing of the cell walls. Grinding with mortar and pestle uses two hard surfaces to crush the microalgal cell wall. Ultrasonication technique depends on high frequency waves of sound to make cavitation and rupture the cell wall of microalgae. Cavitation that ruptures the microalgal cell wall can be resulted from the rapid change in pressure, this technique is called hydrodynamic cavitation. The final mechanical disruption method for the microalgal cell wall is screw expeller pressing, in which dry microalgal cells are positioned in a cavity and compressed (Vasistha *et al.* 2021).

Chemical disruption utilizes acids, alkalis, enzymes, and other agents to lyse the cell wall without force. Alkalis or acids are used to solubilize the microalgal cell wall through saponification. The proteins contained within the microalgal cell membrane can also be solubilized by detergent or surfactant molecules. Lastly, the rigid microalgal cell wall can be digested chemically using enzymes that promote hydrolysis reaction (Lee *et al.* 2021; Vasistha *et al.* 2021).

Physical disruption methods such as thermolysis, osmosis, electrolysis, and radiation are also used to cleave the rigid walls of microalgal cells. The technique of repeated freeze-thaw disrupts microalgal cell wall through repeated freezing and thawing process. During osmotic shock technique, microalgal cell receives amounts of water causing internal pressure and bursting the cell. Moreover, the cell wall can be lysed by gas bubbles escaping from the cell as a result of a sudden release of pressure. Electroporation has the effect of cell disruption when it is applied in high electric fields which called pulsed electric field. In addition, the oscillating electric field initiated by microwaves results in frictional forces and heat, causing water to move rapidly inside the microalgal cell, leading to the rupture of the cell wall. Finally, heat is also considered a physical cell disruption technique, where the microalgal cell wall can be completely disrupted at 121°C for a duration of 30 minutes. This technique is called thermolysis (Lee *et al.* 2021).

3.2. Lipid extraction

Beside the effectiveness of prior cell disruption techniques, the yields of extraction of lipids from microalgae is influenced by factors such as the microalgae structure, lipid composition, pretreatment, and the efficiency of the extraction technique, with considerations for both polar and nonpolar lipids, which must be addressed alongside technical and economic considerations (Zhou *et al.* 2022). The lipid extraction techniques from microalgae, as illustrated in Figure 3, are broadly categorized into solvent-assisted extraction, enzyme-assisted extraction, electric field-assisted extraction, ionic liquids extraction, and instrument-assisted extraction. Table 5 resumes approaches of lipid extraction from various microalgal strains employing these techniques.

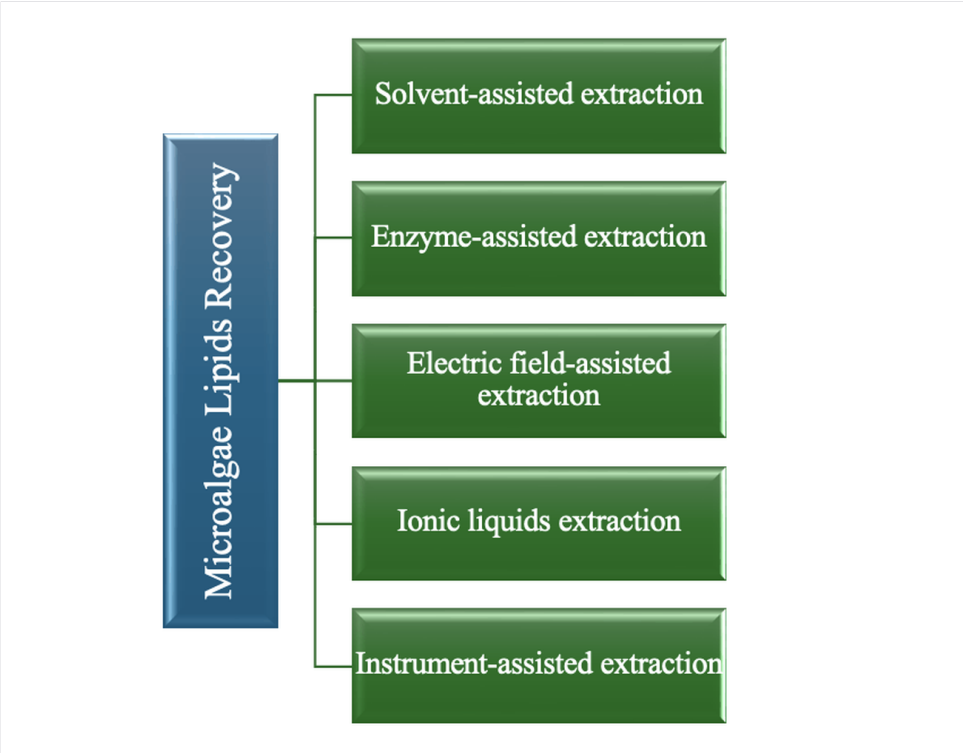

Figure 3. Methods of lipid recovery from microalgae feedstock.

Table 5. Lipid extraction techniques for microalgal strains.

Microalgae strain	Extraction technique	Yield (%)	Reference
Scenedesmus sp	Enzyme pretreatment combined by solvent extraction	14.07%	(Zhang et al. 2022)
Botryococcus braunii	Soxhlet extraction	24%	(Boni et al. 2018)
Auxenochlorella protothecoides	PEF combined with Soxhlet	36%	(Silve et al. 2018)
Nannochloropsis oculata	SC–CO_2 with ethanol as a co-solvent	83%	(Obeid et al. 2018)
Chlorella vulgaris	SC–CO_2 with ethanol as a co-solvent	97%	(Obeid et al. 2018)
Chlorella protothecoides	SC–CO_2	21%	(Viguera et al. 2016)
Scenedesmus sp	Bligh-Dyre extraction	35.32%	(Chen et al. 2015)
Scenedesmus sp	ILs ($[HNEt_3][HSO_4]$)-assisted subcritical water	35.67%	
Chlorella saccharophila	High-pressure homogenization	89.91%	(Mulchandani et al. 2015)
Scenedesmus sp	Folch extraction following focused pulse	21%	(Lai et al. 2014)
Dunaliella tertiolecta	MAE with an involvement of chemical reagent	57.02%	(Qv et al. 2014)
Dunaliella tertiolecta	UAE with chemical reagent	45.94%	
Nannochloropsis oculata	Bligh-Dyre extraction	23.78%	(Dejoye Tanzi et al. 2013)
Nannochloropsis oculata	Soxhlet extraction	8.31%	
Dunaliella salina	Bligh-Dyre extraction	4.03%	
Dunaliella salina	Soxhlet extraction	1.90%	
Chlorella vulgaris	UAE combined with Bligh-Dyer	52.50%	(Araujo et al. 2013)
Nannochloropsis oculata	PLE with ethanol solvent	36%	(Pieber et al. 2012)

3.2.1. Solvent-assisted extraction

Solvent-assisted extraction, a conventional method for lipid extraction, relies on direct contact between the microalgal sample and organic solvents such as methanol, ethanol, chloroform, acetone, and diethyl ether (Lee *et al.* 2021). The Soxhlet apparatus, comprising an evaporation flask, extraction chamber, and condenser, is commonly employed for this purpose, with advanced variants like high pressure, automatic, and microwave Soxhlet developed for improved efficiency (Ahmad et al. 2024). During Soxhlet extraction, the hot solvent interacts with the sample over several cycles, dissolving lipids and changing the color of the solvent in the extraction chamber, indicating the end of the process. Following extraction, lipids are separated from the solvent using a rotary evaporator, and microalgal debris and impurities are removed through filtration techniques (Zhou *et al.* 2022; Binhweel *et al.* 2023).

3.2.2. Enzyme-assisted extraction

Utilizing enzymes for microalgal lipid extraction offers the advantage of simultaneously breaking down the cell wall and extracting lipids. It has been demonstrated that enzyme-assisted extraction enhances both the extraction rate and lipid fractionation (Zhou *et al.* 2022). Additionally, enzyme-assisted extraction can be carried out under normal temperature and pressure conditions, reducing energy consumption and overall costs. Various hydrolase enzymes, including cellulase, hemicellulase, papain, and pectinase, have been employed for this purpose, and selecting the appropriate enzyme and hydrolysis conditions is crucial for optimizing the enzymatic reaction. Combining two or more enzymes may further leverage their synergistic effects to enhance the extraction process (He *et al.* 2020). Combining enzyme-assisted extraction with other techniques can enhance lipid separation and extraction of bioactive compounds, as demonstrated by the synergistic effects observed when enzymatic hydrolysis was combined with the Bligh-Dyer extraction method, resulting in improved lipid extraction and protein separation from *C. reinhardtii* microalgae strain due to the effective degradation of the rigid cell wall by the enzymes (Sierra *et al.* 2017).

3.2.3. Electric field-assisted extraction

In addition to solvent and enzyme assistance, microalgal lipid extraction can be achieved using electric field-assisted extraction, with pulsed electric field (PEF) being a widely used technique. PEF operates on the principle of electroporation, applying high voltage for a short duration to create pores in the rigid microalgal cell wall, allowing for the extraction of lipids and other components while preserving their original composition (Gómez *et al.* 2019). Combining PEF with other extraction methods can boost lipid yield. For example, lipid extraction from the microalgal strain *Chlorella pyrenoidosa* using PEF and Bligh-Dyer solvent extraction increased FAME yield by 12%, attributed to improved permeability in the microalgal cell wall (Han *et al.* 2019). The electric field-assisted extraction method stands out for its ability to operate continuously on large quantities of microalgal biomass feedstock without generating pollutants (Zhou *et al.* 2022).

3.2.4. Ionic liquids extraction

Ionic liquids extraction (ILs) emerged as an innovative chemical technique for extracting lipids and other components from microalgae samples. ILs, defined as organic salts with melting points below 100 °C depending on constituent cations and anions, offer favorable characteristics such as lower viscosity for industrial applications, lower vapor pressure for safety, modification possibilities for tailored conditions, stability, conductivity, and solubility for various compounds, including microalgal cell wall components, facilitating cellular component extraction (Tan et al. 2020). However, toxic substances may be produced as by-products during ILs composite and production (Zhou *et al.* 2022). ILs such as alkylammonium, alkylnitride, and 1-butyl-3-methylimidazolium hydrogen sulfate can be combined with other extraction methods to enhance the extraction efficiency. The integration of [BMIM]HSO4 ILs with microwave/ultrasound techniques for three

microalgal species—Chlorella sorokiniana, Nannochloropsis salina, and Galdieria sulphuraria resulted in significantly higher lipid yields compared to conventional solvent extraction techniques (Pan *et al.* 2016).

3.2.5. Instrument-assisted extraction

Instrument-assisted extraction methods, such as mechanical pressing, pressurized liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, and supercritical fluid extraction, vary in complexity and efficacy for lipid extraction from microalgae; while mechanical pressing is straightforward, its limitations include suboptimal efficiency, heat generation, and potential distortion of extracted compounds, particularly in research applications (Zhou *et al.* 2022). Pressurized liquid extraction (PLE) employs pressure and temperature to extract lipids and bioactive compounds from microalgae, often utilizing water under subcritical conditions as a green solvent, although other organic solvents like dimethyl ether, n-butane, propane, and limonene derived from citrus peels can also be employed, with a limonene:ethanol ratio of 1:1 yielding the highest lipid yield from Spirulina and the highest omega-3 from Stigeoclonium among various microalgal strains (Golmakani *et al.* 2014). Microwave-assisted extraction (MAE), utilizing radiant electromagnetic waves, rapidly generates heat and pressure to disrupt microalgal cell walls, releasing cellular components such as lipids and bioactive compounds; employing this technique, 57.02% of lipids were extracted from the green microalgae strain *Dunaliella tertiolecta* under optimal conditions of 490 W microwave power, 160 s extraction time, and a liquid/sample ratio of 100 $g.mL^{-1}$ (Qv *et al.* 2014). While the MAE technique can be combined with solvent-based techniques and ILs, its limitations include the toxicity of organic solvents and the sensitivity of bioactive compounds to microwave heat (Lee *et al.* 2021; Zhou *et al.* 2022). Similarly, ultrasound-assisted extraction (UAE) demonstrated effectiveness in disrupting the microalgal cell wall and extracting lipids and other beneficial components. Ultrasounds, mechanical waves with frequencies above 20 KHz, propagate through the sample media. During UAE, solvent bubbles are generated due to the ultrasound waves, which burst upon contact with microalgal cells, creating shock waves that rupture the cell wall and facilitate the extraction of lipids and bioactive compounds. For instance, using an ultrasonic power of 370 W, a liquid/sample ratio of 125 $g.mL^{-1}$, and a duration of 5 min, 45.94% of lipids were extracted from *Dunaliella tertiolecta* (Qv *et al.* 2014). Furthermore, combining UAE with Bligh-Dyer extraction resulted in an increased lipid extraction rate from the microalgal strain Chlorella vulgaris, reaching up to 52.50% (Araujo *et al.* 2013). Generally, the extraction efficiency of UAE is influenced by the frequency of ultrasonic waves and the solvent medium. However, challenges such as solvent toxicity and high-frequency power costs hinder UAE application on an industrial scale (Lee *et al.* 2021; Zhou *et al.* 2022). Supercritical fluid extraction (SFE) is hailed as an advanced and environmentally friendly method for lipid extraction, utilizing compounds above their critical states as solvents to extract lipids and bioactive compounds from microalgae strains. Carbon dioxide, known as supercritical carbon dioxide (SC-CO_2), is the preferred solvent due to its lower critical temperature and pressure points, easy separation from extracted lipids, non-toxicity, and cost-effectiveness (Binhweel et al. 2024; Shalfoh et al. 2024; Binhweel, Mardiana and Shakir 2025). Under the operational conditions of 70 °C temperature, 30 MPa pressure, and a CO_2 flow rate of 72 kg/h per kg of sample, 21% of highly pure oil was extracted from *C. protothecoides* using SC-CO2 (Viguera *et al.* 2016). Despite the advancements in SC-CO2, the high cost of machinery and its non-polar characteristics may constrain its use in microalgal lipid extraction. Consequently, polar co-solvents are often employed to ensure the extraction of both polar and nonpolar lipids efficiently, as evidenced by a study conducted by (Obeid *et al.* 2018) on *Chlorella vulgaris* that found using SC-CO_2 alone extracted 12.5% of lipids. However, when 10% ethanol (v/v) was added as a co-solvent, the extraction increased significantly to 40.3%, with 97% as neutral lipids. The same study found that 83% of neutral lipids was extracted from *Nannochloropsis oculate* using SC-CO_2. Raising the pressure from 250 bar to 450 bar also boosted the overall lipid extraction rate from 15% to 20%.

In general, the recovery of microalgae lipid involves an essential balance between extraction efficiency, energy consumption, environmental impact, and economic feasibility. Mechanical and physical disruption techniques are generally effective but often energy-intensive, which may constrain their scalability. Despite its efficiency, chemical approaches raise concerns regarding solvent toxicity and downstream purification requirements. Advanced techniques like supercritical fluid and ionic liquid extraction offer environmentally friendly alternatives with high selectivity. However, the high capital and operational costs remain significant barriers to industrial adoption for the advanced extraction techniques. The effectiveness of lipid recovery is strongly influenced by upstream factors such as cell wall composition and lipid distribution, which are determined by cultivation conditions and genetic modifications. Thus, integrating upstream lipid enhancement strategies with optimized recovery processes is essential to maximize the viability of biodiesel yield.

4. Synthesis of microalgae-based biodiesel

After lipid enhancement and recovery, the final step is the conversion of lipids into biodiesel. Synthesizing microalgae-based biodiesel relies significantly on the lipid content of the microalgae feedstock. Consequently, efforts have focused on increasing lipid productivity to enhance microalgae-based biodiesel production. Fortunately, research has demonstrated a marked increase in microalgal lipids when cells undergo genetic modification or are exposed to stress conditions, which alter biosynthetic pathways and result in an accumulation of neutral lipids as an adaptation for survival. Traditionally, microalgae-based biodiesel is synthesized in two steps: lipid extraction followed by catalytic transesterification. However, this method faced reconsideration due to technical challenges related to high moisture content and economic challenges with the costs of pre- and post-treatments. As a solution, a one-step biodiesel synthesis method, known as in situ transesterification, was developed, allowing simultaneous lipid extraction and transesterification into FAME (Liu *et al.* 2024). Hence, a discussion was provided on these two approaches of synthesizing microalgae-based biodiesel.

4.1. Conventional transesterification

Initially, conventional transesterification for processing microalgae as a biodiesel feedstock involves several steps including collection, dehydration, lipid extraction, and transesterification. The process may include pretreatment or esterification depending on the free fatty acids (FFA) contained in the sample of microalgae oil. During transesterification reaction, ester group is exchanged with an alcohol group (generally methanol) to form FAMEs. This process is promoted by either acid or base catalyst. In addition to biodiesel, glycerol is a by-product resulting from the conventional transesterification. However, the high moisture content in some microalgae biomass, which could be as high as 90%, leads to additional expenses for thorough dehydration. Moreover, the lipid extraction process often results in the loss of valuable microalgal oil components, complicating traditional transesterification processes (Pandey *et al.* 2024).

4.2. In situ transesterification

In response to the challenges associated with the conventional transesterification, in situ transesterification (IST) emerged as a solution, allowing for the direct utilization of freshly harvested microalgae for biodiesel production in a single step. Generally, IST process refers to the direct procedures of converting raw microalgae into FAME. Since the first attempts at IST were in the 2000s, this approach was considered a modern and recent technique for biodiesel production from microalgae feedstock. The IST was classified into wet IST and dry IST. The wet IST is the process of utilizing freshly harvested microalgae for biodiesel production without dehydration and lipid extraction. Although wet IST shows promise in reducing energy consumption and costs by eliminating the need for dehydration and lipid extraction, challenges such as high-water content, the presence of microalgal proteins, and FFAs hindering catalyst activity have led to low efficiency in

early experiments. This prompted the exploration of techniques like dry IST, which involves dehydrating the microalgae feedstock before subjecting it to the IST process. Despite advancements and promising outcomes from both wet and dry IST approaches, further efforts are needed to improve efficiency and reduce costs in biodiesel production to meet industry standards (Kim *et al.* 2019). Table 6 explores part of previous studies that implemented both wet IST and dry IST to synthesize biodiesel from microalgae feedstock. In addition, Figure 4 illustrates the integrated tracks of conventional transesterification, wet IST, and dry IST for the production microalgal biodiesel.

Table 6. Biodiesel production from microalgae feedstock through dry and wet IST.

IST	Microalgae strain	Experiment conditions	Yield (%)	Reference
Wet IST	Chlorella pyrenoidosa	Reactants: oil/alcohol 0.1g/12ml. Catalyst: sulfuric acid 0.5 M. Temperature: 120 °C. Time:3 h.	92.5	(Cao et al. 2013)
	Chlorella vulgaris	Reactants: oil to methanol molar ratio 9.94. Catalyst: immobilized biocatalyst ratio 3369 U g^{-1}. Temperature: 40–45 °C. Time: 48 h.	95.74	(Tran et al. 2013)
	Chlorella vulgaris	Reactants: oil/methanol 1g/4ml. Temperature: 175 °C. Time: 4 h.	89.71	(Tsigie et al. 2012)
Dry IST	Chlorella sorokiniana	Reactants: oil/methanol ratio 1g/4 ml. Catalyst: KOH 0.3%. Temperature: 90 °C. Time: 10 min.	94.87	(Dong et al. 2013)
	Chlorella pyrenoidosa	Reactants: oil/alcohol 1g/10ml. Catalyst: sulfuric acid 0.5 M. Temperature: 90 °C. Time: 2 h.	95	(Li et al. 2011)
	Nannochloropsis oculata	Reactants: oil/methanol. Catalyst: hydrochloric acid. Temperature: 80 °C. Time: 2 h.	23.07	(Carvalho Júnior et al. 2011)

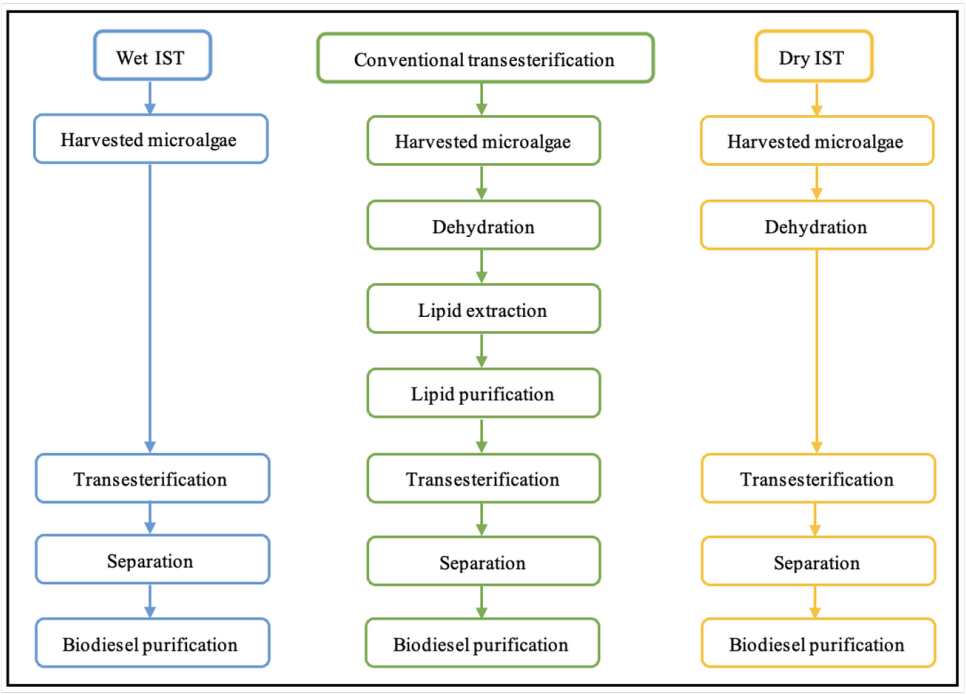

Figure 4. Integrated tracks of conventional transesterification, wet IST, and dry IST for microalgae feedstock toward biodiesel production.

The synthesis of microalgae biodiesel highlights a transition from conventional multi-step processes toward more integrated and efficient method of in situ transesterification. While conventional transesterification remains well-established and reliable, its dependence on energy-intensive drying and extraction steps constrains its economic feasibility. conversely, in situ transesterification simplifies the process by compiling extraction and conversion, thereby decreasing processing time, solvent usage, and costs. However, challenges related to moisture content, catalyst inhibition, and process optimization still hinder its large-scale implementation. Importantly, the success of both approaches is strongly dependent on upstream factors of lipid content, composition, and extraction. Hence, optimizing biodiesel yield needs a holistic integration of lipid enhancement, recovery efficiency, and conversion processes to achieve maximum economic outcomes.

5. Techno-economic perspective

Access to sustainable energy sources is crucial for economic growth and community development (Morozova *et al.* 2025; Razak *et al.* 2026). Biodiesel, as an environmentally friendly fuel, offers a viable alternative to traditional fossil fuels in compression engines with minimal modifications. However, sourcing suitable feedstock poses a significant challenge in the industrialization of biodiesel production. Given that raw feedstock materials account for 70-80% of biodiesel production costs, identifying affordable feedstock options is essential for investment viability in the biodiesel industry (Binhweel *et al.* 2022; Alsaadi *et al.* 2025). Initially, biodiesel production relied on the first generation of edible oils, but ethical concerns emerged due to competition with food resources and high raw material costs. While the second generation of inedible oils mitigated direct competition with food resources, they still competed for natural resources like arable lands, water, and fertilizers. Consequently, the third generation of algae oils emerged as a promising source for sustainable biodiesel production, provided the oil content exceeded 5%. Recent technological advancements have made it possible to significantly enhance lipid productivity in microalgae, rendering microalgal feedstock a promising and economically viable option for biodiesel production. Evaluating the overall costs associated with lipid augmentation and enhancement in microalgae biomass is crucial for the techno-economic analysis of commercial microalgae-derived biodiesel production. Despite

successful experiments demonstrating remarkable improvements in microalgae lipid productivity, scaling up production to an industrial level remains a significant challenge. Additionally, other factors such as microalgae cultivation, harvesting, and pretreatment costs must be considered for financial feasibility (Ahmad *et al.* 2023; Binhweel *et al.* 2023).

The estimated cost of closed cultivation ranges from $0.11 to $0.65 per kilogram of biomass, with energy consumption estimated between 0.1 and 0.7 kWh per kilogram of microalgal biomass. Harvesting costs typically account for 20% to 30% of the total production cost. Interestingly, 50% to 60% of production costs are attributed to lipid extraction from microalgal feedstock. Consequently, the combined costs of harvesting and lipid extraction represent approximately 90% of the overall production costs and energy consumption (Vasistha *et al.* 2021). The transesterification process used for biodiesel synthesis contributes to 10% to 15% of the total biodiesel production cost (Shalfoh *et al.* 2024). Based on these estimations, the cost of microalgal biodiesel production is calculated as $2.167/kg (cultivation + extraction + synthesis). This cost closely aligns with the estimate provided by (Sun *et al.* 2019), which is $2.29/kg.

Establishing a biodiesel production plant using microalgae over an area of 1.11 km^2, with an annual production capacity of 2.5 million kg, would require an estimated capital investment of $55.6 million, covering site preparation, construction, and equipment. The estimated annual operational costs, including labor, raw material consumption, and other operational expenses amount to $2 million (Sun *et al.* 2019). Based on the given prices and production capacity, the annual revenue from biodiesel sales is estimated at $5.4 million (production capacity * product cost). Additionally, the facility is expected to generate added value from by-products such as animal feed and glycerol. The residual microalgae biomass, which can be used as animal feed, is expected to generate an annual revenue of $2.28 million (Sun *et al.* 2019). Furthermore, glycerol, a by-product of transesterification that is commonly used in the detergent industry, is valued at $0.30/kg, contributing an estimated annual revenue of $0.30 million (Shalfoh *et al.* 2024). Thus, the facility is expected to generate a total annual revenue of approximately $7.98 million (biodiesel revenue + animal feed revenue + glycerol revenue). After deducting operational costs, the net annual profit is estimated at around $6 million. However, the current price of microalgal biodiesel remains significantly higher than that of conventional diesel and commercial biodiesel. Therefore, enhancing lipid productivity in microalgal feedstock, coupled with the adoption of streamlined approaches such as in situ transesterification, offers promising potential for the commercialization of cost-effective microalgal biodiesel (Sun *et al.* 2019).

Despite these promising estimates, the reported production cost of approximately $2.16–2.29/kg is still above current market fuel price which constitute a real challenge required technological and policy support. Furthermore, the assumption of high lipid productivity and efficient recovery at large scale may not fully reflect real industrial conditions, where operational inefficiencies and energy losses are common. Notably, lipid extraction and harvesting dominate the overall cost structure, highlighting these stages as critical bottlenecks. Therefore, future techno-economic improvements should prioritize reducing energy consumption, simplifying in situ transesterification, and enhancing lipid yield. However, these revenue estimations are greatly dependent on stable market prices for biodiesel and fuel that fluctuate significantly, and the achievable high recovery by-products like animal feed and glycerol that potentially affect overall profitability.

6. Conclusions

Microalgae represent a promising and sustainable feedstock for biodiesel production because of their fast growth, high biomass productivity, and their capability to grow on non-arable land. However, low intrinsic lipid content and high processing costs may limit their commercialization in biodiesel industry. This review critically examined integrated strategies, including nutrient optimization, nanoparticle application, and genetic engineering to enhance lipid productivity in microalgae feedstock for the purpose of biodiesel production. Prior research proved that application of these

strategies led to significant enhancement in lipid accumulation within the microalgae feedstock. From a techno-economic perspective, harvesting microalgae, lipid extraction, and conventional conversion methods remain the dominant cost contributors, representing key bottlenecks that must be addressed. Hence, advanced extraction techniques such as supercritical fluid and in situ transesterification have the potential to improve efficiency and economic feasibility. The future of biodiesel production derived from microalgae feedstock lies in the integration of upstream and downstream processes to optimize overall production process rather than individual steps. Developing low-energy, scalable, and cost-effective technologies that simultaneously enhance lipid productivity and simplify conversion should be prioritized and emphasized. Such integrated approaches can make microalgae-derived biodiesel a commercially viable and competitive alternative in the international renewable energy market.

Declarations

Author contributions. Fozy Binhweel: Formal analysis, investigation, and first-draft writing. **Abdalah Makaranga**: Investigation and, review, and editing. **Mardiana Idayu Ahmad**: Conceptualization, writing review and editing, supervision, and funding acquisition. **Pannaga Pavan Jutur**: Conceptualization, validation, and supervision. **Nor Hawani Salikin**: Data curation and investigation. **Mohammad Aliff Shakir**: Investigation and writing review and editing.

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