Journal of Science Research International (JSRI)
ISSN: 2456-6365 (Online)

# PLANT-BASED BIOFUELS: A COMPREHENSIVE REVIEW OF SOURCES, TECHNOLOGIES, AND SUSTAINABILITY

**Suprita Rao*, Divya Khot, Nupur Pradhan and Vedant Patil**

*Department of Biotechnology,*

*Pillai College of Arts, Commerce & Science (Empowered Autonomous), New Panvel, India*

**Corresponding author E-mail:* supritarao@mes.ac.in

| Received: 21 January 2026 | Revised: 28 February 2026 | Accepted: 12 March 2026 | Published: 30 March 2026 |
|---|---|---|---|

***DOI:*** **https://doi.org/10.5281/zenodo.19465837**

**Abstract:**

*With the increasing preference for renewable and sustainable energy sources, research into plant-based biofuels as viable replacements for fossil fuels has been accelerated. This analysis focuses on the recent developments that have been made in using plant feedstock, such as Crambe abyssinica, Dovyalis caffra, and third-generation non-edible oils, to produce biodiesel. They emphasize biological and transesterification techniques, plant simulation-based approaches, and new methods of separation, including high-voltage glycerol extraction. Furthermore, the article covers their emissions profiles and environmental impact as well as their engine performance. The development of sustainable biofuels is constantly evolving, and a comparative analysis of feedstock types, process efficiencies, fuel characteristics, etc. The integration of biological and engineering principles can facilitate the development of biodiesel in order to tackle energy security and climate change. This examination combines recent research findings with potential future research directions to optimize plant-based biodiesel technologies for both commercial and environmental purposes.*

**Keywords:** *Plant-Based Biofuels, Biodiesel Production, Non-Edible Oil Feedstocks, Transesterification, Renewable Energy, Biofuel Sustainability, Combustion Performance.*

## 1. Introduction

With the growing global demand for energy and the negative environmental impact of fossil fuels, there has been a renewed interest in renewable and sustainable alternatives. The. Biofuels derived from plants have become increasingly popular as a potential solution for climate change, energy security, and the need for low-carbon technologies [1,2]. This is just one of many promising developments. The use of biofuels, particularly biodiesel and bioethanol, is not only a sustainable option but also enables significant reduction in greenhouse gas (GHG) emissions, unlike traditional petroleum-based fuels [3,4]. Throughout the wider biofuel structure, there has been significant interest in plant-derived biodiesel, which is compatible with existing diesel engines and can be

© 2026 The Author(s). This is an open access article distributed under the terms of the
Creative Commons Attribution (CC BY 4.0) License

produced from a variety of biological sources such as edible oils, non-edible oils (such as palm oil and barley), lignocellulosic biomass, or algal biomass [4].

The early biodiesel production was mainly based on the use of first-generation feedstocks, such as soybean, rapeseed, and palm oil [5]. The focus on second- and third-generation feedstocks has been replaced by the debate over food versus fuel and environmental concerns related to land use changes. Examples of this include non-edible oil seeds (*Crambe abyssinica* and *Dovyalis caffra*), agricultural waste such as potato peels and mango seeds, and algae, all of which offer promising yields without directly competing with food crops [3]. In addition to easing the stress on food supplies, these feedstocks allow for waste valorization and convert agricultural or industrial residue into energy-providing compounds [5].

Transesterification techniques have been a major breakthrough in the technological development of biodiesel. Alcohol is used to catalyze the conversion of triglycerides from plant oils into methyl esters (biodiesel) and glycerol through a chemical or biological reaction [6]. Efforts have been made in recent studies to optimize biodiesel yield and quality by optimizing reaction parameters, catalysts, and separation technologies. However, results are still lacking. Innovative techniques, such as glycerol separation using alternating current (AC) high voltage and the use of simulation models for plant design and process optimization, have enabled new paths to ensure efficient and cost-effective production [6].

Similarly, there is an increasing interest in biological pathways that involve microbial enzymes and fermentation techniques as both ecologically beneficial or efficient ways of handling complex plant-based materials [4]. Farm residues, such as straw, husks and wood waste, yield lignocellulosic biomass (which can be converted into fermentable sugars or lipids) in the form of organic molecules made up of cellulose/hemicellulose and a few trace elements called linoleic acid [6]. While the bioconversion of lignocellulosic feedstock (and thus other related products) still faces some limitations in terms of what is and is not available to store for enzymes, new breakthroughs in pre-treatments techniques and microbial engineering promise that this will change soon [7].

The development of third-generation biofuels, particularly from microalgae, has set a new standard in research due to their high photosynthetic efficiency, rapid growth rate, and ability to produce both lipids and carbohydrates under controlled environmental conditions [5]. Genetic engineering of algal strains and optimization of cultivation parameters, such as light intensity and CO2 supplementation, are among the most important strategies being used to increase biofuel yields [8]. The potential of algal biofuels is high, but the challenges of scaling up biomass harvesting, lipid extraction, and downstream processing have limited their commercial application [6].

Research priorities are influenced by the environmental impact of plant-based biofuels. Why is this? A number of studies have revealed that biodiesel combustion can reduce carbon monoxide (CO), unburned hydrocarbons (HC), and particulate matter (PM) while also having a positive impact on engine performance [9]. *Crambe abyssinica* has been found to enhance combustion characteristics and decrease emissions through the production of biodiesel. Nonetheless, lifecycle assessments are necessary to guarantee that the total GHG emissions from biodiesel production – including planting, harvesting, processing, and transportation — are lower than those from fossil fuels. This is due to the need for life cycles analyses [8,9].

Socially, economically and politically, the integration of plant-based biofuels into global energy systems is dependent on various factors such as public acceptance, government incentives, infrastructure readiness, environmental regulations, and other considerations [2]. In Europe, studies have shown varying levels of acceptance for genetically engineered algae biofuels, with different stakeholder opinions based on their perception of potential dangers and the benefits of sustainability [4]. The utilization of advanced biofuels necessitates open communication and inclusive policymaking, as highlighted [3].

Even with substantial advancements, there are still numerous hurdles to overcome in the development and commercialization of plant-based biofuels [5]. These include availability of feedstock, seasonal fluctuations, high production costs and technological limitations as well as the need for supportive policy frameworks [8]. Recent reviews have suggested that more sustainable and scalable biofuel production may require additional processes such as waste-to-energy conversion, biorefinery models or hybrid catalytic-biological systems [7].

In this review article, we aimed to critically assess and contextualize the recent developments in plant-based biofuel production, emphasizing new feedstocks, process technologies, environmental impact, and future prospects [8].

## *1. Sources of plant-based biofuel production*

The selection of appropriate feedstocks plays a pivotal role in the efficiency, environmental footprint, and scalability of biofuel production. Over the years, research in bioenergy has expanded beyond conventional agricultural crops to include a wide variety of plant-based sources, from non-edible oilseeds to agro-industrial waste and microalgae. This diversification is primarily driven by the need to improve sustainability, reduce competition with food crops, and utilize underused biomass resources.

### *1.1 Classification of plant-based feedstocks*

Plant-based biofuel sources are commonly classified according to "generations," which reflect their feedstock origin, technological maturity, and sustainability characteristics.

- First-generation feedstocks primarily consist of edible oils and sugar/starch-rich crops, such as soybean, palm oil, sugarcane, and corn. While effective in fuel yield, these sources are increasingly criticized due to the food-vs-fuel conflict and their high land and water requirements.
- Second-generation feedstocks include non-edible oils, agricultural residues, and lignocellulosic biomass. These feedstocks do not interfere with food supply chains and can be cultivated on marginal land or derived from waste streams. Examples include Jatropha curcas, *Crambe abyssinica*, *Dovyalis caffra*, mango seed oil, potato peels, rice husks, and sawdust.
- Third-generation feedstocks focus primarily on microalgae and genetically modified photosynthetic organisms, offering high lipid productivity and carbon sequestration potential.

### *1.2 Non-edible oilseeds and tree-borne feedstocks*

In the move toward sustainable biofuel production, non-edible oil crops such as *Crambe abyssinica* and *Dovyalis caffra* have gained attention due to their high oil content, adaptability to semi-arid regions, and minimal input requirements. These species do not compete with staple food crops and are often cultivated on marginal or degraded lands, making them suitable candidates for large-scale biodiesel production.

- *Crambe abyssinica* produces oil with a high erucic acid content, suitable for industrial biodiesel. Recent research has demonstrated its potential to improve combustion efficiency and reduce engine emissions, highlighting its dual environmental and performance benefits.

- *Dovyalis caffra*, a lesser-known indigenous plant, offers considerable seed oil yield and favorable fatty acid profiles suitable for biodiesel synthesis. Its application also supports biodiversity conservation and the valorization of underutilized flora.

### *1.3 Agricultural waste and residue-based sources*

Agricultural and food processing industries generate vast amounts of organic waste, much of which remains underutilized. Valorizing this biomass into biofuels offers a circular economy approach, reduces environmental burden, and supports rural energy initiatives.

- Mango seed oil, derived from mango processing by-products, has been investigated for biodiesel production through optimized transesterification methods. Its high lipid content and low cost make it a feasible raw material, especially in tropical regions where mango production is abundant.
- Potato peels, a major waste product in food processing industries, have shown promise as a source of fermentable sugars and oils. Bioconversion of potato peels into biofuels represents a sustainable waste-to-energy strategy, reducing landfill usage and methane emissions.
- Lignocellulosic biomass, including crop residues, wood chips, bagasse, and rice straw, is abundant and rich in cellulose and hemicellulose. Despite its recalcitrant structure, advancements in pre-treatments methods and microbial fermentation technologies have enabled partial success in converting these residues into fermentable sugars, which can be further transformed into ethanol, butanol, or biodiesel.

### *1.4 Microalgae and third-generation feedstocks*

Microalgae are recognized as one of the most promising third-generation biofuel sources due to their exceptional photosynthetic efficiency, high growth rates, and ability to accumulate large quantities of lipids. Unlike terrestrial crops, algae can be cultivated on non-arable land using brackish, saline, or wastewater, significantly reducing freshwater and land-use footprints.

Species such as *Microcystis aeruginosa* have been studied under varying light conditions to optimize lipid and carbohydrate yields. These compounds serve as substrates for biodiesel and bioethanol production, respectively. Genetic engineering and biotechnological enhancements are being actively explored to further increase lipid content, modify metabolic pathways, and improve stress tolerance in algal strains.

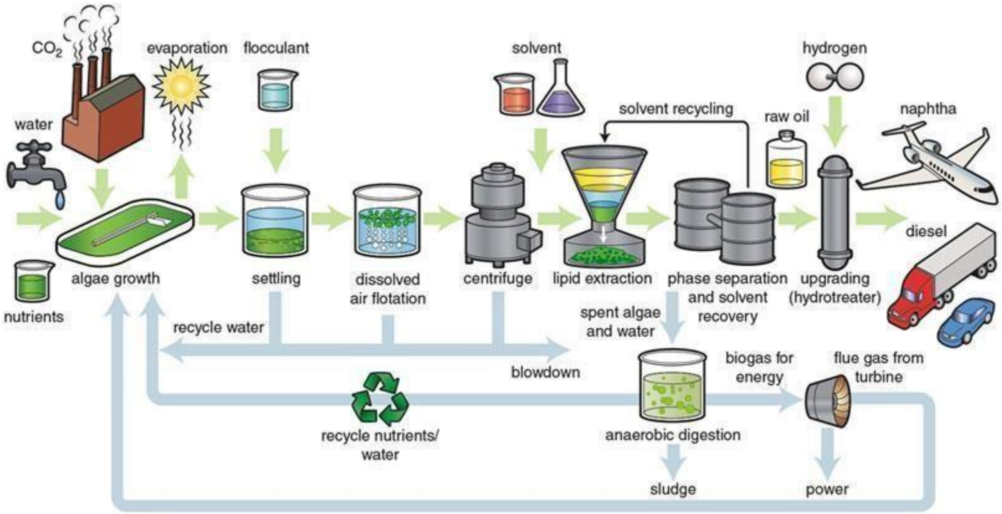
Despite their potential, algae-based biofuels face challenges in terms of high production costs, harvesting inefficiencies, and scalability issues. However, their integration into wastewater treatment systems and potential co-product generation (e.g., biofertilizers, pigments, animal feed) may enhance the overall economic feasibility.

### *1.5 Engineered and emerging biomass sources*

Advancements in biotechnology and synthetic biology are paving the way for genetically engineered feedstocks that exhibit enhanced biomass productivity, improved oil profiles, and resistance to abiotic stress. For instance, engineered algae strains have been developed with traits tailored for high lipid accumulation, minimal nutrient requirements, and faster growth cycles.

Public acceptability of genetically modified organisms (GMOs), however, remains a limiting factor in some regions, especially in Europe, where ethical, ecological, and regulatory concerns play a significant role. Nonetheless, with adequate risk assessment and transparent communication, engineered biofuel crops could revolutionize the sustainability and yield of plant-based fuels.

## 2. Methodology



This methodology integrates experimental data, technological evaluation, biochemical pathways, and critical literature synthesis to provide a thorough, multidisciplinary approach to the analysis of plant-based biofuels. The objective is to offer a methodical framework for evaluating different methods of producing biofuel, their sustainability, and the underlying chemical and biological processes. The approach is based on a number of highly influential, peer-reviewed studies that examine the production of biodiesel and bioethanol from various plant-based and microbial sources.

For third-generation biofuel innovation, special attention is paid to the use of particular algal strains like *Coccomyxa* sp., *Parachlorella kessleri*, Microcystis aeruginosa, and Nannochloropsis, as well as the incorporation of sophisticated gene editing tools, particularly CRISPR/Cas9. This methodology's gene editing techniques go beyond traditional recombinant DNA techniques and concentrate on applying and delivering ribonucleoprotein complexes (Cas9 protein + gRNA) via electroporation. Public and regulatory concerns are lessened by this technique, which enables precise, DNA-free modification of algal genomes without leaving behind foreign genetic material.

For example, genome editing was done in *Coccomyxa* sp. strain KJ by introducing Cas9-guide RNA complexes targeting the FTSY gene, which encodes a signal recognition particle-docking protein, using a dual-pulse electroporation protocol. Researchers were able to look into lipid biosynthesis regulatory mechanisms and possibly increase photosynthetic efficiency in industrial settings by disrupting this gene. [1]

Using CRISPR/Cas9, three specific genes were made non-functional in the *Parachlorella kessleri* strain NIES-2152 DMAN1. These genes are DMAN1, which makes an enzyme called endo-1,4-β-mannanase, AATPL1, which is a protein similar to an ATP/ADP transporter found in plastids, and CDMT1, which codes for a protein involved in cell wall synthesis.

A protein that targets membranes and requires calcium. This finding indicates that high-value phenotypes can be unlocked through deliberate gene disruptions with negligible off-target effects. Other industrial algae like Nannochloropsis and Chlamydomonas reinhardtii are now using CRISPR technology. By modifying the acetyl-CoA carboxylase and fatty acid biosynthesis pathways, CRISPR/Cas9 was used to promote lipid accumulation in Nannochloropsis.

By increasing the yields of terpenoid and isobutanol in Chlamydomonas, metabolic engineering made it possible to produce advanced bio-alcohol fuels as well as biodiesel. Because of its well-characterized genome and receptiveness to CRISPR-based manipulation, Chlamydomonas reinhardtii is also acknowledged as a promising chassis for synthetic biology. Another significant algae is Microcystis aeruginosa, a cyanobacterium that showed notable control over lipid and genes involved in the biosynthesis of carbohydrates in different light levels. M. aeruginosa exhibited elevated expression of genes related to fatty acid synthesis, carbohydrate storage, and chlorophyll biosynthesis when exposed to red light. By maximizing biomass and lipid yield through environmental optimization, this physiological approach enhances gene-editing techniques. [2]

### *1. Biofuel production approaches*

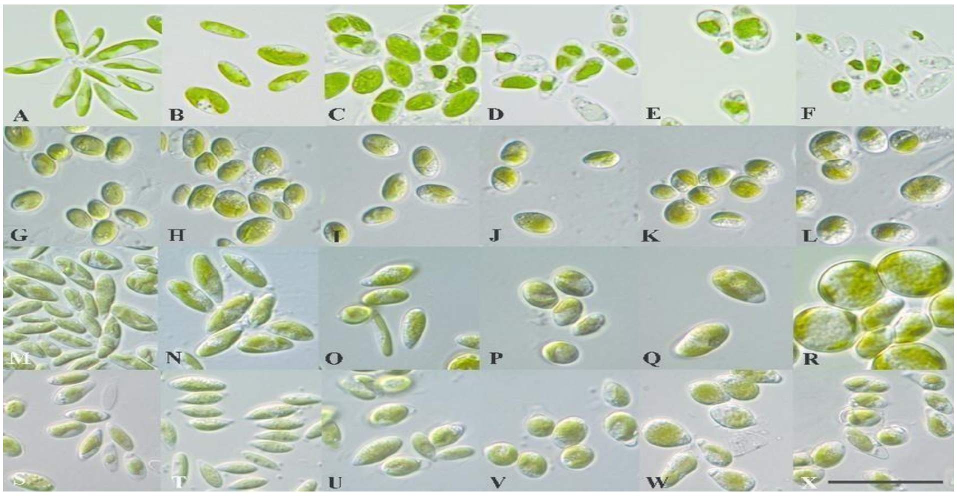
| **Biofuel Type** | **Source Material** | **Key Process** | **Technology Used** |
|---|---|---|---|
| Bioethanol | Banana stem, lignocellulosic biomass | Fermentation, enzymatic hydrolysis | Acid/alkaline pretreatment, bioreactor |
| Biodiesel | Jatropha, Crambe, *Dovyalis caffra* oils | Transesterification (acid/base) | Chemical and enzymatic catalysis |
| Microbial Oil | Oleaginous yeast, microalgae | Lipid accumulation and extraction | Fermentation, lipid extraction |

### *2. Gene editing applications in algae-based biofuels*

Gene editing, particularly CRISPR/Cas9, has revolutionized algal biofuel research by enabling precise genetic modifications to enhance productivity, tolerance to stress, and metabolic output. The CRISPR/Cas9 system utilizes a Cas9 protein complexed with a guide RNA (gRNA) that targets specific DNA sequences for editing. This system enables targeted gene knockouts, knock-ins, and base edits with high precision, minimal off-target effects, and no requirement for insertion of foreign DNA thus making it highly suitable for commercial biofuel applications.

### *Detailed algal examples and their genetic modifications*

#### 1. *Coccomyxa* sp. (Strain KJ)

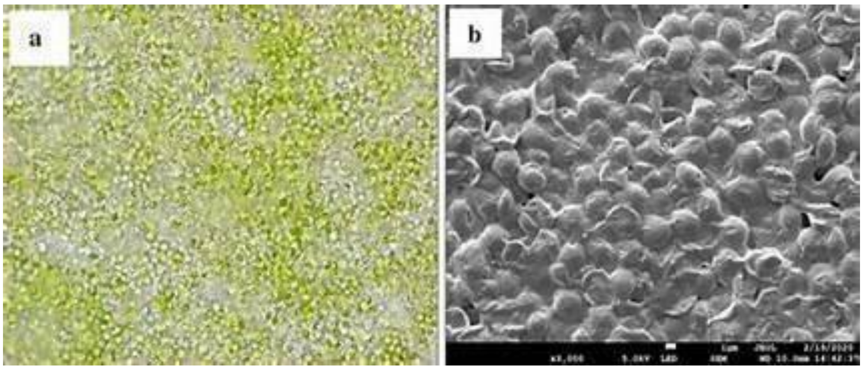


In *Coccomyxa* sp. (Strain KJ), targeted disruption of the FTSY gene, which encodes a chloroplast signal recognition particle docking protein, was achieved using CRISPR/Cas9 ribonucleoproteins (RNPs) delivered via dual-pulse electroporation. This DNA-free genome editing approach enabled precise and efficient gene knockout without introducing foreign genetic material, marking a significant advancement in algal biotechnology. The loss of *FTSY* function offered deeper insight into chloroplast protein import pathways and was also associated with altered lipid accumulation under stress conditions, suggesting a broader metabolic influence. This study not only enhances our understanding of chloroplast-related mechanisms but also demonstrates the high transformation efficiency and applicability of non-integrative genome editing techniques in green algae, supporting their potential in sustainable bioengineering and metabolic pathway research. [3]

### ***2. Parachlorella kessleri*** **(Strain NIES-2152)**

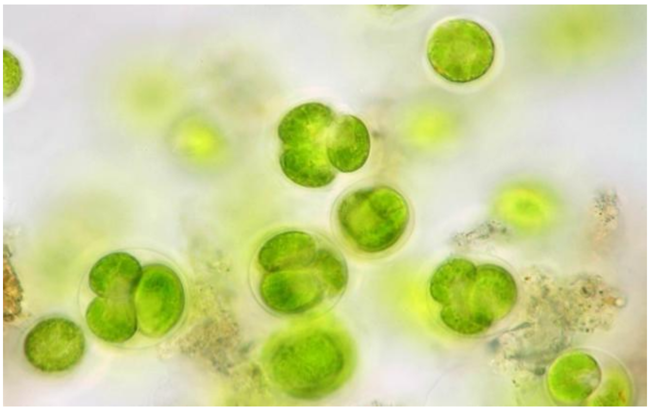
In *Parachlorella kessleri* (Strain NIES-2152), precise marker-free genome editing was achieved by targeting three nuclear genes CDMT1, DMAN1, and AATPL1 using Cas9-gRNA ribonucleoprotein complexes delivered via electroporation. These genes are associated with membrane signaling, carbohydrate metabolism, and plastid energy exchange, respectively. Among the edited strains, the AATPL1 knockout exhibited a notable increase of over 30% in lipid content compared to the wild-type, with no negative impact on cell growth or viability. This successful application of DNA-free CRISPR-based editing in *Parachlorella* marks the first report of its kind in this species, providing a powerful tool for metabolic engineering and reinforcing its potential in sustainable lipid production and algal biofuel development. [4]

### **Nannochloropsis sp**



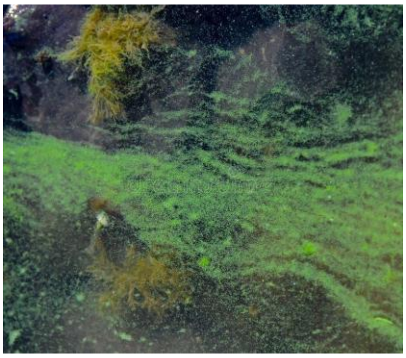
In *Nannochloropsis* sp. a plasmid-based CRISPR/Cas9 system driven by native promoters was employed to target key genes involved in fatty acid biosynthesis including acetyl-CoA carboxylase a crucial enzyme in the lipid synthesis pathway. The genome editing resulted in a significant increase in triacylglycerol (TAG) accumulation and more efficient carbon partitioning toward lipid pathways, without compromising overall cell function. This study highlights the potential of *Nannochloropsis* as a scalable marine algal platform for industrial biotechnology, particularly in biodiesel production, demonstrating both the feasibility and effectiveness of genetic engineering approaches for enhanced lipid yields in commercially important microalgae.

### 3. Chlamydomonas reinhardtii



*Chlamydomonas reinhardtii* has emerged as a model microalga for genetic engineering, owing to its well-annotated genome and a highly adaptable genetic toolbox that includes CRISPR/Cas9, TALENs, and homologous recombination. These tools have been utilized across a range of biotechnological applications, such as the production of bioalcohols (notably enhanced isobutanol yields), terpenoids, and the development of engineered chloroplast expression systems. Additionally, gene editing has enabled improvements in phototrophic growth under nutrient-limited conditions, contributing to more robust photosynthetic efficiency. As a result *C. reinhardtii* continues to play a central role in algal synthetic biology and metabolic engineering, offering valuable insights for both fundamental research and industrial-scale bioresource development.

### 1. Microcystis aeruginosa



*Microcystis aeruginosa*, a species of cyanobacteria, has shown promising responses to environmental modulation, despite the absence of direct genome editing in referenced studies. When cultured under varying light conditions particularly red light this organism exhibited enhanced expression of genes associated with chlorophyll biosynthesis, carbohydrate storage, and lipid production. These findings highlight the potential of non-genetic strategies, such as light-based environmental manipulation, to optimize metabolic pathways and improve biofuel-relevant traits. This approach underscores an alternative avenue for enhancing biomass and lipid yields, emphasizing that regulation of culture conditions can play a significant role in bioengineering efforts, even in the absence of genetic modification. [5]

## Enzymes and catalysts employed in biofuel production

The production of plant-based biofuels, especially biodiesel and bioethanol, involves several chemical and biochemical conversions, most of which are mediated by specific catalysts and enzymes. These agents are crucial for enhancing reaction efficiency, reducing energy demands, and enabling sustainable processing. Based on the reviewed literature, four main types of catalysts and bio-tools are prominently used in biofuel processing base catalysts, acid catalysts, enzymatic biocatalysts, and genetic tools.

### *1. Base catalysts*

Commonly used base catalysts like sodium hydroxide (NaOH) and potassium hydroxide (KOH) are industrial standards in biodiesel production. They facilitate the transesterification reaction, where triglycerides (from oils or fats) react with methanol or ethanol to produce fatty acid methyl esters (FAMEs) the chemical constituents of biodiesel and glycerol as a by-products. Base catalysts are preferred due to their fast reaction rates, low cost, and operational simplicity. However, they are sensitive to the presence of free fatty acids (FFA) in feedstocks, which can lead to soap formation and reduced yield.

### *2. Acid catalysts*

To address the limitations of base catalysts when dealing with high-FFA feedstocks (e.g., waste cooking oils, non-edible oils), acid catalysts such as sulfuric acid ($H_2SO_4$) are employed. Acid catalysts are effective in esterification reactions, where FFAs are converted into esters before proceeding to transesterification. Although acid-catalyzed processes are slower than basecatalyzed ones and may require higher temperatures, they are crucial for pretreating challenging feedstocks to improve final biodiesel quality.

### *3. Enzymes (lipases)*

In recent years, lipase enzymes particularly those derived from microbial sources such as *Candida antarctica* have gained attention for their ability to catalyze both esterification and transesterification under mild conditions (lower temperatures and pressures). Enzymatic biodiesel production offers several advantages it avoids soap formation, tolerates high FFA feedstocks, and is environmentally benign. enzyme cost and reuse potential remain challenges for large-scale deployment. Researchers have focused on immobilized lipase systems to enhance enzyme stability and economic feasibility.

### *4. Genetic tools (crispr/cas9)*

Beyond chemical catalysts, genetic engineering especially using CRISPR/Cas9 has emerged as a powerful tool in biofuel biotechnology. This approach enables precise genetic modifications in biofuel-producing organisms such as microalgae and yeasts, aiming to improve lipid biosynthesis, carbohydrate utilization, or stress tolerance. CRISPR has been applied to disrupt genes regulating competing metabolic pathways or enhance expression of lipid synthesis genes, leading to increased biofuel yield and process sustainability. [6]

## Analytical methods and tools in algae-based biofuel research

The development of algae-based biofuels demands a multidisciplinary framework that integrates chemical, biological, and computational methods to assess the quality of biofuels, optimize metabolic pathways, and monitor the performance of production systems. A broad array of analytical tools is used across each stage of algal biofuel research, from feedstock cultivation and lipid extraction to fuel characterization and genetic optimization. These tools not only validate process efficiency but also contribute to innovations in strain improvement and system sustainability. Among the most critical techniques is Gas Chromatography–Mass Spectrometry (GC–MS), widely employed to characterize the fatty acid methyl ester (FAME) composition of algal lipids.

This technique enables detailed profiling of biodiesel precursors, such as palmitic acid, oleic acid, and linoleic acid which influence fuel properties like oxidative stability, cold flow behavior, and energy content. For example, in studies involving *Coccomyxa sp.* and *Nannochloropsis*, GC–MS was utilized to confirm increased FAME accumulation after targeted gene editing. Fourier Transform Infrared Spectroscopy (FTIR) is often used alongside GC–MS to confirm the functional groups indicative of esterification, providing a rapid method to monitor biodiesel formation. To assess sugar metabolism and alcohol production in engineered strains capable of producing bioethanol or isobutanol, HighPerformance Liquid Chromatography (HPLC) is used. In *Chlamydomonas reinhardtii*, genetically modified to synthesize isobutanol, HPLC quantification of intermediates and end products played a pivotal role in verifying gene pathway function and conversion efficiency. These physicochemical methods are essential for establishing the viability and reproducibility of algal fuel synthesis, particularly in scaled-up processes. In addition to chemical characterization, statistical design of experiments (DoE) techniques such as the Plackett–Burman design and Response Surface Methodology (RSM) are applied to optimize cultivation and processing conditions. [7]

These approaches allow for the systematic analysis of factors like light intensity, nutrient concentration, pH, carbon dioxide levels, and salinity, which significantly influence lipid productivity. For instance, in *Microcystis aeruginosa*, light spectrum modulation specifically red-light exposure was found to significantly enhance the expression of genes related to photosynthesis, carbon fixation, and lipid biosynthesis without genetic modification, demonstrating the value of controlled environmental interventions. To simulate and assess the combustion behavior of algal biodiesel, MATLAB/Simulink platforms are used to model engine performance parameters such as brake thermal efficiency, torque, specific fuel consumption, and emission characteristics. In comparative simulations of B10 and B20 biodiesel blends from *Crambe abyssinica* (an algallike oilseed with high erucic acid content), these tools demonstrated the operational feasibility of algae-derived fuels under real-world engine conditions. These computational models are vital in transitioning laboratory-developed fuels to market-ready energy solutions.

**CRISPR/Cas9 genome editing has become a powerful tool in algal biotechnology**

By enabling precise modifications in target genes without foreign DNA insertion, this method allows the enhancement of lipid accumulation, stress resilience, and carbon flux in microalgae. In *Parachlorella kessleri*, the knockout of the AATPL1 gene, responsible for plastidic ATP/ADP exchange, led to a marked increase in intracellular lipid content. Similarly, *Coccomyxa sp.* was edited at the FTSY locus, influencing chloroplast protein import and enhancing lipid storage under stress. These edits were delivered using ribonucleoprotein complexes (RNPs) via electroporation, offering a DNA-free, markerless editing strategy with minimal off-target effects. Insights into the cellular impact of genetic or environmental modifications are gained through transcriptomic analyses and quantitative PCR (qPCR). For example, red-light culture of *Microcystis aeruginosa* led to the upregulation of genes involved in carbon assimilation and lipid synthesis, highlighting the utility of transcript-level data in understanding and manipulating metabolic pathways for biofuel improvement.

### Comparative critical analysis of algae-based biofuel technologies

The development of algae-based biofuels has been extensively studied in the last two decades. A comprehensive review of the literature reveals both significant agreements and disagreements among researchers regarding the technological, economic, and environmental dimensions of this field. While consensus exists on several foundational methods and benefits of algal systems, divergence in opinion is noted concerning the scalability, public acceptance, and economic viability of genetically modified algae for commercial fuel production.

### Agreements among Studies

Across multiple studies, there is strong consensus that transesterification either chemical (acid or base-catalyzed) or enzymatic remains the primary method for converting algal lipids into biodiesel. This process has been applied successfully to several strains, including *Coccomyxa sp.*, *Nannochloropsis*, and *Chlamydomonas reinhardtii*, using both traditional catalysts and biocatalysts such as lipases. For instance, enzymatic transesterification in *Chlorella vulgaris* has been shown to yield high-quality biodiesel with minimal soap formation and lower energy input, making it more environmentally sustainable than conventional chemical approaches.

Another point of consensus is the recognition of microalgae and lignocellulosic biomass as promising feedstocks due to their non-food-based origin, rapid growth rates, high lipid content, and the potential for $CO_2$ mitigation. Unlike traditional oil crops, microalgae can be cultivated in brackish or wastewater, reducing land use pressure and avoiding food vs. fuel conflicts. Research on strains like *Parachlorella kessleri* and *Coccomyxa sp.* demonstrates that with proper optimization, these organisms can accumulate lipids up to 50% of their dry cell weight under stress conditions, significantly outperforming conventional crops such as soybean or rapeseed in lipid productivity per hectare. Studies generally agree on the potential role of gene editing, particularly CRISPR/Cas9, in enhancing algal productivity. Precision genome modifications have enabled the upregulation of lipid synthesis pathways, suppression of competing metabolic routes, and improvement in stress tolerance.

The use of ribonucleoprotein (RNP) delivery systems has also mitigated biosafety concerns by avoiding integration of foreign DNA, which is often a point of regulatory scrutiny.

### Disagreements and controversies

There are several points of disagreement in the field most notably regarding the economic feasibility and social acceptance of algal biofuels. A major debate concerns the cost-effectiveness of large-scale algal cultivation, harvesting, and downstream processing. Some researchers argue that current systems, especially open raceway ponds and closed photobioreactors, suffer from high operational and energy costs, which reduce the energy return on investment (EROI) and make commercialization economically unviable without significant subsidies or technological breakthroughs. Others contend that integrated biorefinery approaches, where multiple valuable products (e.g., proteins, pigments, biofertilizers) are extracted alongside biodiesel, can offset production costs and improve overall sustainability. There is also lack of consensus on the use of genetically modified algae (GM algae) While several studies demonstrate the benefits of CRISPR-edited strains in improving lipid yields, public acceptance remains low, particularly in Europe and parts of Asia. Regulatory frameworks surrounding genetically modified microalgae are still evolving, and concerns about environmental release, horizontal gene transfer, and ecosystem impact persist. In contrast, non-GMO approaches, such as selective breeding or environmental stress induction (e.g., nitrogen starvation or light manipulation), are perceived as more publicly acceptable, though often less efficient than gene-edited systems. While many studies praise algae as a carbon-neutral or even carbon-negative biofuel source, others challenge this assertion by pointing to the high life-cycle emissions associated with energy-intensive harvesting (e.g., centrifugation), drying, and lipid extraction steps. Life-cycle assessments (LCAs) conducted under real-world conditions have revealed that unless renewable energy is used throughout the production process, the carbon savings from algae biodiesel may be lower than previously assumed.

### Sustainability perspective of biodiesel production

The transition toward sustainable energy systems has become increasingly critical due to depleting fossil fuel reserves and the escalating threat of climate change. Biodiesel, a renewable, biodegradable, and cleaner-burning alternative to fossil diesel, offers promising potential to support global sustainability goals. Its production, especially when sourced from non-edible oils, waste resources, and agro-industrial byproducts, embodies the core principles of economic, environmental, and social sustainability.[3]

### *1. Environmental sustainability*

Biodiesel presents significant environmental advantages throughout its life cycle: Reduced Greenhouse Gas Emissions: Biodiesel combustion releases significantly fewer GHGs due to its biogenic $CO_2$ origin. Biodiesel releases less carbon monoxide, sulfur compounds, and harmful particles into the air than regular diesel, making it a cleaner and healthier option for the environment and for us.[7] Waste Utilization: Waste Cooking Oil (WCO): Prevents improper disposal that pollutes water and soil while reducing landfill burden. Its reuse contributes to a circular bioeconomy and prevents environmental hazards.[7] Potato Peel Waste (PPW): Valorization of this food industry byproduct avoids waste accumulation and utilizes its energy-rich content for second-generation biofuel production.[21] Cleaner Processes: Technologies like glycerol separation via high-voltage fields and hydrodynamic cavitation improve process efficiency and reduce chemical usage, making biodiesel processing more environmentally friendly.[9] Biorefinery Approach: Integration of biodiesel plants with biogas and organic fertilizer production (e.g., from PPW) enhances environmental gains by recovering maximum value from biomass. Life Cycle Assessment (LCA): LCA helps track environmental impacts across all biodiesel production stages. It reveals hotspots like transesterification and ensures transparency in sustainability evaluations.[1]

### *2. Economic sustainability*

Despite the ecological benefits, biodiesel production faces economic challenges—mainly due to high feedstock costs and energy-intensive processes. However, several strategies help improve economic sustainability:

- Low-Cost Feedstocks: Non-edible oils (e.g., Pongamia pinnata, Jatropha curcas, and Calophyllum inophyllum) and waste streams like WCO or PPW reduce raw material expenses and do not compete with food markets.[5][6]
- Process Optimization: Use of simulation tools (e.g., Aspen Plus) helps in validating plant designs, minimizing fluid losses, reducing heat energy waste, and maximizing output. For instance, a designed plant achieved an 88% biodiesel yield, indicating high commercial viability.
- Co-product Valorization: Biodiesel production yields glycerol as a valuable byproduct, and PPW bioconversion generates ethanol, biogas, and organic fertilizer, diversifying revenue streams.[10]
- Infrastructure Efficiency: Choosing corrosion-resistant materials, energyefficient pumps, and minimal heat loss systems significantly reduces long-term operational costs.

### *3. Social sustainability*

Biodiesel production supports inclusive and community-based sustainability through:

- Rural Employment: Cultivation of non-edible oil crops like jatropha and pongamia on marginal lands promotes income generation in rural areas, especially in semi-arid and economically weaker regions.[4]
- Local Resource Utilization: Community participation in WCO collection or oilseed farming strengthens local economies and encourages cleaner urban environments.
- Food Security Assurance: By relying on inedible feedstocks and waste materials, biodiesel avoids the food vs. fuel conflict, preserving agricultural produce for consumption and not energy production.[4][19]

### *4. Alignment with Sustainable Development Goals (SDGs)*

Biodiesel production intersects with multiple UN SDGs, including:

- SDG 12: One way we can do this is by turning waste materials like used cooking oil (WCO) and fruit peels (PPW) into useful products, instead of letting them go to waste.
- SDG 13: Supports climate action through significant GHG emission reduction.
- SDG 11, 14, 15: Reduces urban pollution and protects aquatic and terrestrial ecosystem.[19][20]

### *5. Challenges and future directions*

While promising, biodiesel sustainability is not without challenges:

WCO Pretreatment: Energy and chemical inputs required for feedstock preparation may offset some environmental benefits. Many life cycle assessment (LCA) studies often leave out emissions from things like soap, catalysts, or how solid waste is disposed of. As a result, they might not fully capture the true environmental impact.

Technology Gaps: Adoption of green technologies like renewable energy integration, ecofriendly catalysts, and sensitive modeling tools (e.g., Monte Carlo analysis) is essential to bridge current shortcomings.[26]

Data Uncertainty: Improving the quality and availability of real-time data is critical to making accurate environmental and economic assessments.

## Future prospectives

### *1. Sustainable feedstock utilization*

The future of biofuels lies in using non-food, waste-based feedstocks to address the "food vs. fuel" conflict. Utilizing agricultural residues, waste cooking oil (WCO), municipal solid waste, lignocellulosic biomass, and plastic pyrolysis waste (PPW) ensures that biofuel production does not compete with food security. These second- and third-generation feedstocks not only minimize pressure on arable land but also contribute to circular economy principles, where waste is transformed into valuable energy.[6]

For instance, converting PPW into biodiesel via pyrolysis technology offers a dual advantage—managing plastic pollution and creating fuel. Similarly, WCO-based biodiesel reduces environmental hazards caused by improper oil disposal and provides a cheaper raw material alternative.[22]

### *2. Technological advancements and process improvements*

Modern biofuel production is rapidly evolving due to biotechnological innovations, enzyme-based conversion processes, and improved transesterification methods. Advanced bio-refineries now focus on integrated processing, where multiple value-added products (like bioethanol, biogas, and biofertilizers) are generated simultaneously.[11]

The development of genetically engineered microorganisms, improved catalysts, and energy-efficient reactors are key trends that enhance yield and reduce operational costs. In the long term, carbon-negative processes, such as algae-based $CO_2$ capture and bio-oil production, could revolutionize how we think about energy production and emissions control.[22]

### 3. Environmental and climate benefits

Biofuels offer significant environmental benefits. They are biodegradable, have lower sulfur content, and produce fewer particulate emissions than conventional diesel. Research shows that biodiesel can reduce carbon monoxide, unburnt hydrocarbons, and particulate matter, which directly impacts air quality and public health.[1]

Moreover, replacing fossil fuels with biofuels in the transport and agricultural sectors can contribute meaningfully to climate goals under the Paris Agreement. Life Cycle Assessments (LCA) have shown that when waste-based feedstocks are used, the net carbon footprint of biofuels can be significantly reduced compared to fossil fuels.[3]

### 4. Policy support and global trends

The government is actively supporting the growth of biofuels through helpful policies and incentives, making it easier and more attractive for people and companies to invest in this cleaner energy source. National bioenergy missions, mandatory blending targets (e.g., 20% ethanol blending by 2025 in India), and subsidies for waste-to-energy projects have created a conducive ecosystem for investment and innovation.[24] Globally, countries like the USA, Brazil, and members of the European Union are aggressively promoting bioenergy transitions. These actions are encouraging the private sector to invest in scalable biofuel production technologies and public-private partnerships for rural energy generation and job creation.

### 5. Socio-economic and rural development

Biofuel production can generate employment in rural areas, enhance energy security, and create local value chains. The use of locally available biomass reduces import dependence and provides farmers with an additional income stream by selling agricultural waste. Moreover, setting up decentralized biodiesel plants in villages can empower communities, encourage sustainable agriculture, and provide reliable energy access for domestic and small industrial use.

## Conclusion

As we stand at a crossroads in the global energy journey, the need to shift away from fossil fuels has never been more urgent. Plant-based biofuels offer not just an alternative, but a hopeful pathway forward—one that combines science, sustainability, and social impact.

This comprehensive review brings to light how non-edible oils, agricultural waste, and even tiny algae are being transformed into powerful sources of clean energy. These aren't just theoretical ideas; real breakthroughs—like transesterification techniques, enzyme-based conversions, and gene editing with CRISPR—are already reshaping how we produce fuel. For example, crops like *Crambe abyssinica* and *Dovyalis caffra*, or wastes like potato peels and mango seeds, are now recognized not as leftovers, but as valuable resources.

Algae-based fuels, in particular, are leading the way in innovation. These micro-organisms grow quickly, don’t need farmland, and can thrive even in wastewater. With the help of precise genetic tools, scientists are learning how to make algae produce more oil, tolerate stress, and grow better—all without inserting foreign DNA, which makes the process more publicly acceptable and ethically sound.

On the environmental front, these fuels significantly cut down on carbon emissions, reduce harmful pollutants, and even make use of waste that would otherwise pollute landfills or waterways. This directly supports major global goals like climate action (SDG 13) and responsible consumption (SDG 12).

Economically, while high production costs and technological challenges remain, there's real potential for cost-saving through co-product generation (like glycerol and bio-fertilizers) and smart plant designs using simulation tools. Socially, biofuel initiatives can create jobs in rural areas, reduce dependency on imported fuels, and avoid the "food vs. fuel" problem by using non-edible or waste feedstock.

Still, there are challenges ahead. Public hesitation around genetically modified organisms, inconsistent life cycle data, and scalability issues all require careful handling. More transparent policies, improved technology, and inclusive discussions with communities will be essential to move from lab success to global impact.

In short, plant-based biofuels aren't just about energy. They represent a bigger vision for a sustainable, inclusive, and cleaner world—where waste is turned into opportunity, where rural economies thrive, and where innovation fuels progress without compromising food, health, or nature.

References

1. Biernat, K., Matuszewska, A., Samson-Bręk, I., & Owczuk, M. (2021). Biological methods in biodiesel production and their environmental impact. *Applied Sciences, 11*, 10946. https://doi.org/10.3390/app112210946
2. Uyaroğlu, A., Kocakulak, T., & Aydoğan, B. (2020). Investigation of the effects of biodiesel produced from *Crambe abyssinica* using KOH and NaOH catalyst on combustion, engine performance and exhaust emissions. https://doi.org/10.21203/rs.3.rs-127212/v1
3. Hossain, N., Razali, A. N., Mahlia, T. M. I., Chowdhury, T., Chowdhury, H., Ong, H. C., Shamsuddin, A. H., & Silitonga, A. S. (2019). Experimental investigation, techno-economic analysis and environmental impact of bioethanol production from banana stem. *Energies, 12*, 3947.
4. Haq, A., Laiq Ur Rehman, M., Rana, Q. A., Khan, A., Sajjad, W., Khan, H., Khan, S., Shah, A. A., Hasan, F., Ahmed, S., Islam, A., Badshah, M., Shah, T. A., Dawoud, T. M., & Bourhia, M. (2023). Production, optimization, and physicochemical characterization of biodiesel from seed oil of indigenously grown *Jatropha curcas*. *Frontiers in Energy Research, 11*, 1225988. https://doi.org/10.3389/fenrg.2023.1225988
5. Verma, S., Sahu, D., & Almutairi, B. O. (2024). Production and characterization of biodiesel fuel produced from third-generation feedstock. *Frontiers in Materials, 11*, 1454120. https://doi.org/10.3389/fmats.2024.1454120
6. Ambat, I., Srivastava, V., & Sillanpää, M. (2020). Recent advancement in biodiesel production methodologies using various feedstock: A review. *Renewable and Sustainable Energy Reviews, 90*, 356–369. https://doi.org/10.1016/j.rser.2018.03.069
7. Hatsa, T. M., Kelile, A. W., Beyene, E. G., *et al.* (2025). Production and comprehensive analysis of biodiesel from *Dovyalis caffra* seed oil. *Sustainable Energy Research, 12*, 17. https://doi.org/10.1186/s40807-025-00161-3
8. Almady, S. S., Moussa, A. I., Deef, M. M., Zayed, M. F., Al-Sager, S. M., & Aboukarima, A. M. (2024). Biodiesel production through the transesterification of non-edible plant oils using glycerol separation technique with AC high voltage. *Sustainability, 16*, 2896. https://doi.org/10.3390/su16072896
9. Peiter, A. S., Lins, P. V., Meili, L., Soletti, J. I., Carvalho, S. H., Pimentel, W. R., & Meneghetti, S. M. P. (2020). Stirring and mixing in ethylic biodiesel production. *Journal of King Saud University–Science, 32*, 54–59.
10. Kasai, Y., Takagi, S., Ota, S., Ishii, K., Takeshita, T., Kawano, S., & Harayama, S. (2024).
11. Chen, W., Xu, S., Zou, S., Liu, Z., Liu, Y., Xu, H., Wang, J., Ma, J., Chen, R., & Zuo, Z. (2025). https://doi.org/10.1186/s13068-025-02615-8
12. Constantino, A., Rodrigues, B., Leon, R., Barros, R., & Raposo, S. https://doi.org/10.1016/j.algal.2021.102329
13. Muthuraman, V. S., & Kasianantham, N. https://doi.org/10.1016/j.psep.2023.04.043
14. Yoshimitsu, Y. *et al.* https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-018-1327-1
15. Villarreal, J. V., Burgués, C., & Rösch, C. (2020).
16. Méndez-Líter, J. A., de Eugenio, L. I., Prieto, A., & Martínez, M. J.
17. Jeya, M., Joo, A. R., Lee, K. M., Kumar, M. T., Lee, K. M., Kim, S. H., & Lee, J. K.
18. Ramani, G., Meera, B., Vanitha, C., Rajendhran, J., & Gunasekaran, P.

19. Barampouti, E. M., Christofi, A., Malamis, D., & Mai, S. (2021). A sustainable approach to valorize potato peel waste towards biofuel production.
20. Jie, Y. G., Khalid, A., Rahman, A. A. A., Sulaiman, S., Mohamed, N., Zulkifli, A. F. H., Leman, A. M., & Jacob, D. W. (2021). Biodiesel pilot plant system and conversion technology of multiple feedstocks.
21. Mathew, G. M., Raina, D., Narisetty, V., Kumar, V., Saran, S., Pugazhendi, A., Sindhu, R., Pandey, A., & Binod, P. (2021). Recent advances in biodiesel production: Challenges and solutions. https://doi.org/10.1016/j.2021
22. Borges, A. V., Fuess, L. T., Alves, I., Takeda, P. Y., & Damianovic, M. H. R. Z. (2021). Co-digesting sugarcane vinasse and distilled glycerol to enhance bioenergy generation in biofuel-producing plants. *Energy Conversion and Management*. https://doi.org/10.1016/j.enconman.2021.114897
23. Hosseinzadeh-Bandbafha, H., Nizami, A.-S., Kalogirou, S. A., Gupta, V. K., Park, Y.-K., Fallahi, A., Sulaiman, A., Ranjbari, M., Rahnama, H., Aghbashlo, M., Peng, W., & Tabatabaei, M. (2022). Environmental life cycle assessment of biodiesel production from waste cooking oil: A systematic review. *Renewable and Sustainable Energy Reviews*. https://doi.org/10.1016/j.rser.2022.112411
24. Pan, S., Zabed, H. M., Wei, Y., & Qi, X. (2022). Technoeconomic and environmental perspectives of biofuel production from sugarcane bagasse: Current status, challenges and future outlook. *Industrial Crops and Products*. https://doi.org/10.1016/j.indcrop.2022.115684
25. Uthandi, S., Kaliyaperumal, A., Srinivasan, N., Thangavelu, K., Muniraj, I. K., Zhan, X., Gathergood, N., & Gupta, V. K. (2021). Microbial biodiesel production from lignocellulosic biomass: New insights and future challenges. https://doi.org/10.1080/10643389.2021.1877045
26. Ohile, S. O., Aboje, A. A., Uthman, H., Usman, R. A., & Ighodaro, O. O. (2023). Optimization and characterization of biodiesel production from mango seed oil (*Mangifera indica*) via transesterification reaction. https://doi.org/10.5281/zenodo.8321396
27. Al-Mawali, K., Osman, A. I., Al-Muhtaseb, A. H., Mehta, N., Jamil, F., Mjalli, F., Vakili-Nezhaad, G. R., & Rooney, D. W. (2021). Life cycle assessment of biodiesel production utilising waste date seed oil and a novel magnetic catalyst: A circular bioeconomy approach. *Renewable Energy, 170*, 832–846. https://doi.org/10.1016/j.renene.2021.02.027
28. Saman, S., Balouch, A., Talpur, F. N., Memon, A. A., Mousavi, B. M., & Verpoort, F. (2021). Green synthesis of MgO nanocatalyst by using *Ziziphus mauritiana* leaves and seeds for biodiesel production. https://doi.org/10.1002/aoc.6199