CHEMICAL ENGINEERING TRANSACTIONS
VOL. 122, 2025
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Bohong Wang, Guo Ren Mong, Petar S. Varbanov

ISBN 979-12-81206-23-6; **ISSN** 2283-9216
DOI: 10.3303/CET25122045

Fourth Generation Biodiesel: a Review of Technology Development, Challenges, and Future Prospects

Nur Atikah Mohidem*, Nabilla Afzan Abdul Aziz

Faculty of Energy Transition, i-CATS University College, Jalan Stampin Timur, 93350 Kuching, Sarawak
atikah.mohidem@icats.edu.my

The need to combat climate change has accelerated the global energy transition from fossil-based sources to renewable energy, such as biofuels. Among other biofuels, fourth-generation biodiesel that is derived from genetically modified microalgae (GMO) combined with carbon capture systems stands out as a promising approach. In contrast to carbon-neutral alternatives, this biodiesel is also regarded as achieving carbon-negative energy generation by capturing and storing carbon. Due to the extensive review papers available on first- to third-generation biodiesel, the present manuscript focuses on fourth-generation biodiesel which utilises GMOs and carbon capture for production. This manuscript provides broad information about the benefits, limitations, and characteristics of the fourth generation of biodiesel. It also reviews technological advancements, including the genetic modifications of microalgae and the incorporation of carbon capture for producing fourth-generation biodiesel, along with the associated challenges and future directions. It is suggested that modifying microalgae strains could increase lipid accumulation by two to three times, thereby increasing the production of biodiesel. The study also demonstrates the survival of microalgae in 8,000-L open raceway ponds fed with flue gas from coal-fired power plants.

1. Introduction

Awareness of the increase in greenhouse gas (GHG) emissions, climate change, and energy security drives the transition to renewable energy. In Malaysia, particularly Sarawak, through Sarawak's Post COVID-19 Development Strategy 2030 (PCDS 2030), there is a strong commitment and focus to transform into a sustainable and green ecosystem (Ashari and Farouk 2023). Therefore, the global energy transition demands fuels that are more sustainable and greener (Lim et al., 2020). Biodiesel is an alternative fuel that produces fewer CO_2 emissions and can be used in most of the engines without notable modification (Abed et al., 2019).
Biodiesel can be divided into four generations. It is recognised based on the feedstock utilised to manufacture biodiesel (Balwan and Kour 2021). Edible crops are used in the first generation; non-edible biomass is used in the second; algae are used in the third; and genetically modified organisms, such as microalgae, are the focus of the fourth generation (Panchal et al., 2025). This fourth generation of biodiesel intends to increase biofuel output by enhancing biomass yield, oil content, and CO_2 capture that leads to a circular bioeconomy (Cavelius et al., 2023). Figure 1 depicts the advantages and limitation of fourth generation biodiesel.
During the production of fourth-generation biodiesel, it is emphasizing on potential of carbon-negative ecosystem with using genetically modified microalgae and CO_2 capture. By integration with advanced bioprocess engineering, it can produce high-value co-product that support bioeconomy (Shiong et al., 2023). Through genetic modification, it will alter the genetic code of microalgae and further enhance its characteristics. Consequently, it will improve the quality of oil and biomass production. Table 1 shows the characteristics of the fourth generation of biodiesel.
Moreover, genetically engineered microalgae could have better photosynthesis and carbon dioxide assimilation, accelerating sustainability goals. Tailoring the fatty acid composition of microalgal lipids enables optimising biodiesel's oxidative stability as well as other desired characteristics (Grama et al., 2022).

Paper Received: 4 August 2025; Revised: 6 November 2025; Accepted: 7 December 2025
Please cite this article as: Mohidem N.A., Abdul Aziz N.A., 2025, Fourth Generation Biodiesel: A Review of Technology Development, Challenges, and Future Prospects, Chemical Engineering Transactions, 122, 265-270 DOI:10.3303/CET25122045

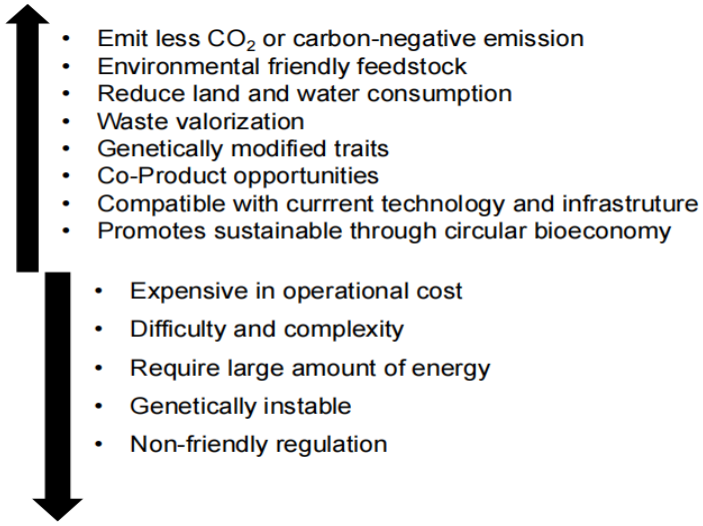

Figure 1: Advantages and limitations of fourth generation biodiesel (Aguilar-aguilar et al., 2025)

Shokravi et al. (2022) reported that the modification of genes such as ACCase could improve Scenedesmus quadricauda's fatty acid by 1.6-fold. In addition, manipulating the ACCase gene in Dunaleilla salina and Schizochytrium sp. results in a 140 % increase and an 11.3 % increase, respectively, thereby enhancing lipid accumulation and fatty acid content. Ouellet and Abdel-Mawgoud (2024) investigated the genetic modification of oleaginous yeast Yarrowia lipolytica to produce enhanced biodiesel efficiency. The genetic manipulations resulted in enhanced lipid accumulations by two to three times. This approach demonstrates a significant improvement in biodiesel production by utilising genetically modified organisms (GMOs) as feedstock.

Table 1: Characteristics of the fourth generation of biodiesel

Characteristic	Description	References
Potential for Carbon-Negative Output	Reduce more CO_2 by integrating with a carbon capture approach to absorb more CO_2.	Haider et al., 2025
Genetically Modified Feedstocks	GMOs together with the photosynthesis system can increase lipid properties and CO_2 fixation.	Abdullah et al., 2019
Use of Non-Agricultural Lands	The cultivation of GMO microalgae does not interfere with food production, as the land is non-arable.	Gaurav et al., 2024
Increased Lipid Production	Engineered strains have much higher oil yield per hectare than traditional oil crops.	Zhang et al., 2025
Sustainable Farming Inputs	Leveraging wastewater and flue gases as cost-effective and sustainable feedstock.	Wang et al., 2024
Biorefining Systems	Can produce co-product other than biodiesel such as bioplastic and nutraceuticals.	Chalermthai et al.,, 2023
Bioprocess system with the latest technology	Use advanced technology such as photobioreactor, open ponds, automation and AI.	Razzak et al., 2024
Low Freshwater Requirement for Cultivation	Can produce co-product other than biodiesel such as bioplastic and nutraceuticals.	Beckstrom et al., 2020
Small Land Area Needed to Produce Lots of Biomass	Less land area is needed compared to palm or soybean fuel, making it suited for biodiesel production in urban or industrial areas.	Chhandama et al., 2023
Waste valorisation and circular bioeconomy	By-products of biomass can supply fertilisers, animal feed, or biochar, which closes the resource loop.	Ghosh et al., 2024

Due to substantial reviews on first- through third-generation biofuels, the present manuscript focuses on fourth-generation biodiesel, which is genetically modified organism- and carbon capture-based biodiesel. Furthermore,

the existing literature reviews overlook at how techno-economically feasible, scalable, or environmentally friendly something is. The purpose of this review is to look at fourth-generation biodiesel from a variety of viewpoints and find the technological, regulatory, and environmental issues it confronts. The present manuscript discusses the advantages, drawbacks, and characteristics of fourth-generation biodiesel that uses GMOs as a feedstock to produce and enhance biodiesel efficiency. It is also a review about the strategies of genetic modification of microalgae and the integration with carbon capture. The challenges and future direction are also discussed.

2. Technological developments in fourth generation biodiesel

2.1 Genetic modification of microalgae for biodiesel production

Biodiesel generated from microalgae becomes more efficient and economical when their genes are genetically modified. Through genetic modification, certain pathways can be targeted to increase the lipid content constituting triacylglycerols of microalgae, which boost biodiesel production. These changes could also enhance growth rates, biomass production, or tolerance to environmental stresses like high salinity levels and extreme temperatures, thus improving reliability in cultivation (Khoo et al., 2023). Figure 2 shows the strategies to improve lipid accumulation in microalgae by using genetic engineering.

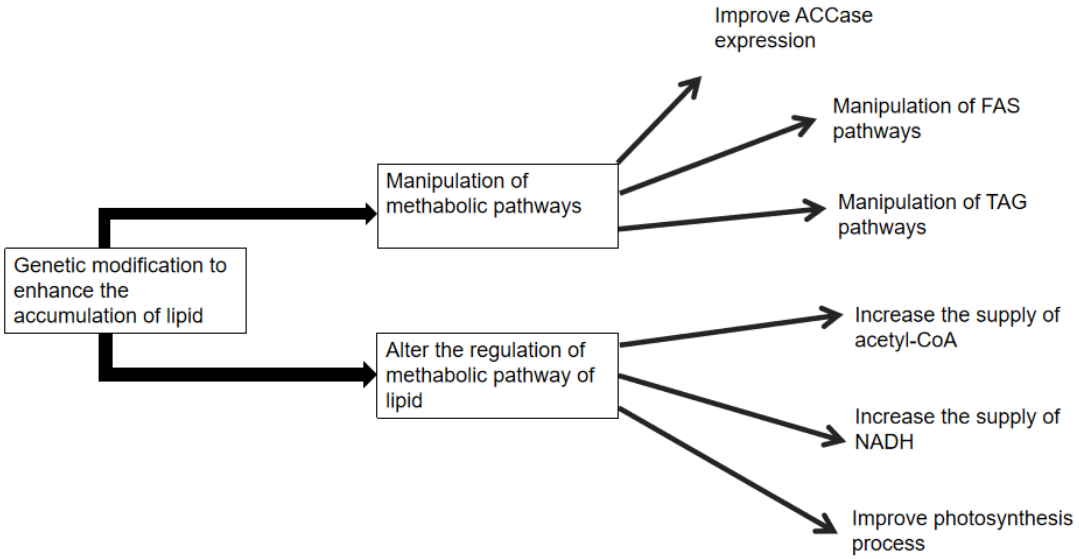

Figure 2: Strategies to improve lipid accumulation in microalgae (Shokravi et al., 2022)

The economic value of biodiesels made from microalgae increases because these genetic changes create strains that can produce other products at the same time as the fuel in biorefineries.
Factors including the selection of a microalgal strain, cultivation system design, nutrients, energy demand, and CO_2 source and concentration might affect the integration of carbon capture in the production of biodiesel (Onyeaka et al., 2021). Even though flue gas that contains pollutants can stop cell growth, high-purity and concentrated CO_2 could improve microalgal growth and lipid accumulation.
Genetic engineering can improve the efficiency and practical relevance of microalgae-based biodiesel by developing high-performing strains with increased lipid accumulation. Genetically modifying the lipid biosynthesis pathway in microalgae could improve the economic sustainability of biodiesel production and commercialization (Zhang et al., 2025). Genetic modification of microalgae could enhance lipid accumulations and increase efficiency of fourth generation biodiesel, thus supporting bioeconomy policies. By modifying and manipulating pathways of triacylglyceride and fatty acid synthesis could increase the lipid accumulation by up to 45 times (Shokravi et al., 2022).
Fourth-generation biodiesel derived from genetically engineered algal biomass, for instance, has shown potential increases in biomass production rates of up to 40 % under optimal conditions, depending on strain and environmental condition. These integrated techniques call out the importance of combining precise genetic

engineering with specialised process design to produce sustainable and economically feasible biodiesel production systems (Shokravi et al., 2021).
Microalgae such as Chlorella vulgaris have great photosynthetic efficiency, fixing up to 1.5 g CO_2/L/day and eliminating more than 90 % of nitrogen and phosphorus from wastewater. Under optimal mixotrophic conditions, C. vulgaris achieves biomass concentrations of 4.0 g/L and lipid content of 20-40 %, indicating realistic biodiesel output. These properties make it especially useful for integrated environmental remediation and biofuel production (Alsalal et al., 2025).

2.2 Integration with carbon capture

The efficiency of fourth-generation biodiesel production with the integration of carbon capture technology is mainly determined by several factors, such as the source and concentration of CO_2, the types of microalgae, and the overall cultivation system. The choice of microalgae needs to be tolerant of the rise of CO_2 concentration and other external factors such as extremely high temperature and pH value (Ighalo et al., 2022).
Lipids extracted from microalgae can be used to produce biodiesel. This microalgae can be cultured with CO_2 as the main resource for the photosynthesis process. The CO_2 from flue gases produced from industrial activities can be captured and utilised as a cheap and sustainable carbon source. This integration of using GMOs and CO_2 captured from industrial activities can help to reduce greenhouse gas emissions while producing biodiesel. Zhu et al. (2014) reported that three Nannochloropsis strains (4-38, KA2, and 75B1) survived and blossomed in 8000-L open raceway ponds fed with flue gas from coal-fired power plants. The resulting biomass and lipid productivities can be decreased by lowering the temperature and decreasing sun irradiation. Among Nannochloropsis strains, Strain 4-38 resulted in enhanced productivities as a result of higher concentrations of triacylglycerols and fatty acids.
On the other hand, Aslam et al. (2018) investigated the fuel characteristic of biodiesel produced from oleaginous microalgae feedstock compared to conventional biodiesel. Fatty acid methyl ester (FAME) concentrations in mixed microalgae consortia under coal-fired flue gas exposure were examined in this study. The findings indicated that the lipid content was 14.03 µg/mL/D and the FAME content was 280.3 µg/mL at 1 % CO_2 concentration. The high levels of saturated and unsaturated fatty acids contained in mixed consortia with phosphate buffer and flue gases. The resulting biodiesel that was produced with 50 mM phosphate buffer and 5.5% CO_2 had greater quality. This work aims to develop a method that can enhance biodiesel efficiency by using GMO and CO_2 source of flue gas. The utilizing CO_2 as carbon source from industrial flue gas could reducing green house emission, thus providing sustainable and environmentally friendly of biodiesel production.

2.3 Challenges

Several challenges such as the complexity of the cultivation process, the high cost of production, and the potential of health risks associated with fourth-generation biodiesel, need to be tackled in order to fully utilise its potential. The cultivation instruments such as open ponds and photobioreactors are considered expensive. The cost associated with these instruments is often a barrier for many companies looking to invest in algae cultivation.
The process of harvesting, drying and lipid extraction contributes to high operational costs. The complexity of synthetic biology and genetic engineering is one of the main challenges. It is still difficult to engineer microalgae or photosynthetic organisms to produce lipids or hydrocarbons by modifying complex metabolic pathways. It is also unstable since it is exposed to contamination and climate change.
GMOs have gained public awareness and raised concern about the potential health and environmental risks. Thus far, there is still no security and guarantee that the GMO is genetically stable and biosafety for the long run. The implication of GMOs for public health is the major concern and is still being debated. Any potential risk to health and the environment will impact the sustainability and reliability of biofuel in the future.
Furthermore, the scalability could be one of the major obstacles since it requires a larger land area, an extensive amount of water and a continuous supply of CO_2 and nutrients. Land is expensive and needs substantial financial support. The reliance on GMOs and their associated risks could also lead to strict regulation and cause a hamper in larger production and scaling up (Ganev et al., 2022). There is limited research on projects that involve pilot-scale production, indicating that current technology needs further development before it can be transferred to industry and attract investments. The lack of effort in policy frameworks associated with fourth-generation biodiesel could delay the commercialisation investment (Potrč et al., 2021).

2.4 Future prospects

Despite the aforementioned challenges, fourth-generation biodiesel has a promising approach towards sustainable and carbon-negative goals. By utilising GMO to convert CO_2 that is produced from industrial activities into biodiesel, it is possible to produce a carbon-negative environment. Fourth-generation biodiesel

depends on GMOs. The manipulation of metabolic pathways could enhance lipid accumulation and enhance photosynthesis efficiency, thus improving overall biodiesel production (Apaza et al., 2022).
The integration with advanced photobioreactors, computational chemistry, and automation could facilitate biodiesel production. The microalgae's strain can be optimised using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), which produce more accurate and precise genes. The process involves editing and modifying genomes in order to get enhanced traits and genes more rapidly (Jeong et al., 2023).
The utilisation of carbon from wastewater and industrial activities to produce fourth-generation biodiesel can push circular bioeconomy and carbon-negative fuel to another level. The byproduct from microalgae can be extracted and reused to produce biochar, fertiliser, bioplastic, and animal feed. It can also potentially be utilised in the pharmaceutical, cosmetic, and food industries (Pirozzi et al., 2015).
The utilisation of GMO could enhance lipid accumulation and enhance the photosynthesis process, which leads to cost reduction. Co-products from lipid accumulation, such as residual biomass and other compounds (e.g., antioxidants, proteins), can be commercialised to increase revenue (Chowdhury et al., 2025).

3. Conclusions

This method has given several advantages such as high lipid extraction, reducing freshwater usage, minimal land required, and waste valorization using advanced bioprocess engineering. It is come along with challenges such as complexity for scaling up, economic constraints, regulatory frameworks and public response towards biodiesel production using GMOs.
Further study should be carried out to carefully investigate the method to enhance genetic modification methods and identify operational conditions and parameters that affect the optimisation of GMO using CO_2 to produce high-efficiency biodiesel and enhancing genetic modification methods. Public awareness and acceptance should be considered to assure the adoption of fourth-generation biodiesel in industries.

Acknowledgments

Many thanks and gratitude to Institute of Sustainable Green Energy and Technology (i-SGET), i-CATS University College for support this research.

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