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Abstract

Biofuels have incrementally advanced as promising alternatives to fossil fuels, offering a sustainable solution to alleviate environmental degradation and energy security concerns. The exploration of novel feedstock resources has expanded the scope of biofuel production, diversifying raw material sources beyond conventional crops like corn and sugarcane which also serve for food purposes. Lignocellulosic residues, microalgae, and genetically engineered organisms represent promising alternatives for biofuel production that offer higher sustainability and zero competition with food production. The present chapter provides a detailed overview of recent advancements and trends in biofuel research, highlighting key breakthroughs and their implications for sustainable energy production. The development of biofuels with superior combustion characteristics and reduced emissions is crucial for the widespread adoption and integration of biofuels into existing transportation systems. It scrutinizes the relevance of biofuels in addressing the pressing concerns of green aviation and emphasizes the necessity of sustainable energy alternatives in the aviation sector. The chapter highlights key phases of biofuel life cycle assessment (LCA) for sustainable development and consumption. Moreover, an overview of global biofuel production trends is presented emphasizing the worldwide transition from fossil fuels to biofuels, along with its limitations and future directions.

Keywords (separated by '-'): Biofuels - Energy security - Microalgal biodiesel - Green aviation fuels - Biofuel LCA

Biofuels: Sustainable Alternatives for Green Aviation and Global Energy Transition

S. M. D. C. Bandara and R. R. Ratnayake

Abstract Biofuels have incrementally advanced as promising alternatives to fossil fuels, offering a sustainable solution to alleviate environmental degradation and energy security concerns. The exploration of novel feedstock resources has expanded the scope of biofuel production, diversifying raw material sources beyond conventional crops like corn and sugarcane which also serve for food purposes. Lignocellulosic residues, microalgae, and genetically engineered organisms represent promising alternatives for biofuel production that offer higher sustainability and zero competition with food production. The present chapter provides a detailed overview of recent advancements and trends in biofuel research, highlighting key breakthroughs and their implications for sustainable energy production. The development of biofuels with superior combustion characteristics and reduced emissions is crucial for the widespread adoption and integration of biofuels into existing transportation systems. It scrutinizes the relevance of biofuels in addressing the pressing concerns of green aviation and emphasizes the necessity of sustainable energy alternatives in the aviation sector. The chapter highlights key phases of biofuel life cycle assessment (LCA) for sustainable development and consumption. Moreover, an overview of global biofuel production trends is presented emphasizing the worldwide transition from fossil fuels to biofuels, along with its limitations and future directions.

Keywords Biofuels · Energy security · Microalgal biodiesel · Green aviation fuels · Biofuel LCA

1 Introduction

In the global pursuit of sustainable energy solutions, biofuels have progressively developed as a promising substitute for fossil fuels (Neag et al. 2023). The finite fossil fuel resources cause geopolitical instability and deleterious environmental impacts

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with the expanding human population and industrialization, increasing global energy demand. Biofuels derived from energy-enriched organic matter (comprehensively depicted as biomass), such as conventional crops, lignocellulosic residues, and microalgae, significantly decrease greenhouse gas emissions and global dependence on fossil fuel reserves that are non-renewable and depleting over time due to extensive consumption (Rodionova et al. 2017; Liu et al. 2018).

Biofuels can be categorized depending on several key characteristics, including feedstock type, conversion process, technical specification of the fuel and its applications (Jeswani et al. 2020). Biofuels represent a diverse array of fuel types, including bioethanol, biodiesel, biogas, and biobutanol, each with distinct production methods and applications. Among these, bioethanol and biodiesel are the most widely utilized biofuels worldwide. Bioethanol is primarily produced through the fermentation of sugars and starch found in conventional crops (e.g., sugarcane, sweet sorghum, sugar beet, corn, wheat, cassava), energy-rich crops (e.g., switchgrass, miscanthus, alfalfa, and bermudagrass), and lignocellulosic residues (e.g., sugarcane bagasse, corn stover, rice straw, sawdust, wood chips, and paper-pulp residues) while biodiesel is derived from oil-rich crops (e.g., soybeans, canola, sunflower, palm oil, and coconut), animal fats, recycled cooking oils, and algal lipids (Ambaye et al. 2021; Malode et al. 2021).

Mitigation of climate change by reducing net carbon dioxide (CO_2) emissions is one of the key advantages of biofuel consumption. Unlike fossil fuels, which release carbon stored deep within the Earth's crust, biofuels recycle CO_2 absorbed by plants during photosynthesis, effectively closing the carbon cycle (Liu et al. 2018). Moreover, biofuel production processes can be tailored to minimize environmental impact, such as utilizing marginal lands for feedstock cultivation and employing advanced technologies to increase efficiency and reduce waste (Liu et al. 2018). Biofuels offer a promising pathway toward energy security and rural development by diversifying energy sources and creating new markets for biomass resources (Jeswani et al. 2020). In many regions of the world, biofuel production has stimulated economic growth providing opportunities for farmers, entrepreneurs, and researchers alike.

Microalgal biodiesel is a promising solution over conventional biodiesel as microalgae are highly effective at converting sunlight, CO_2, and nutrients into their oleaginous biomass, where certain species can reach up to 90% under specific conditions (Harris et al. 2018). The incorporation of biofuels into the aviation sector is an exciting avenue, safeguarding greener air travel over fossil fuels. Governments, industrial stakeholders, researchers, and environmental advocates should come together to realize the full potential of green aviation powered by biofuels.

While biofuel production presents promising opportunities, it is not without its challenges and controversies across technological, political, socioeconomic and environmental aspects. Biofuel LCA is a detailed evaluation of the environmental impacts linked with each stage of biofuel production, from feedstock cultivation to fuel combustion. The results of a biofuel LCA help to guide policies and practices to optimize the sustainability of biofuels (Brandão et al. 2022). As societies strive to shift toward a greener energy future, biofuels are poised to play a pivotal role in the global energy landscape. Through continued research, innovation, and cross-sector collaboration, biofuels hold the promise of providing a cleaner, greener, and more resilient energy future for generations to come. This chapter explores the major generations of biofuels with a specific emphasis on microalgal biodiesel production, emerging biofuels in the aviation sector, fundamentals of biofuel LCA, global biofuel production trends following limitations and future trends in biofuel research.

2 Major Generations of Biofuels

Biofuels are being developed as a promising substitute for fossil fuels since they are carbon–neutral, sulfur-free, biodegradable, and derived from renewable sources. Biofuels are categorized into four major types as first-generation (1-G), second-generation (2-G), third-generation (3-G), and fourth-generation (4-G) biofuels based on the biomass feedstock utilized.

2.1 First-Generation (1-G) Biofuels

The 1-G biofuels are produced from conventional food crops that are rich in sugars and starch (e.g., sugarcane, sugar beet, sweet sorghum, corn, wheat, cassava), and oil-rich crops, such as sunflower, soybeans, canola, palm oil, and coconut. Although biofuels emerge as carbon–neutral fuels, 1-G biofuels have significant economic, societal, and environmental limitations (Mahmood et al. 2023). One of the primary concerns regarding 1-G biofuels is their impact on food security, resulting in the "food versus fuel" debate, and as production capacity develops, so does rivalry with land utilized for food production. This increased pressure on arable land can lead to severe food scarcity, especially in low- and middle-income countries where over 900 million people already suffer from starvation and malnutrition. Additionally, the intense usage of arable land with high applications of fertilizers and pesticides, along with significant water consumption, can cause serious environmental issues (Malode et al. 2021).

2.2 Second-Generation (2-G) Biofuels

The advent of 2-G biofuels is intended to produce fuels from non-edible feedstocks (also known as lignocelluloses), thus avoiding competition with food production. Lignocellulosic biomass typically consists of lignin (26–31%), hemicellulose (25–32%), and cellulose (41–46%) (Malode et al. 2021). Lignocellulosic biomass includes various agricultural residues (e.g., rice straw, corn straw, wheat straw, sugarcane bagasse), forestry residues (e.g., softwood, hardwood, sawdust, wood chips, and wood yard waste), sewage sludge, pulp and paper industry waste and herbaceous energy crops (e.g., switchgrass, alfalfa, miscanthus, and bermudagrass) (Vaid et al.

2018). However, the transformation of the complex recalcitrant structure of lignocelluloses into fermentable sugars via pretreatment and hydrolysis requires expensive methods, implying that 2-G biofuels cannot be generated cheaply on a commercial scale (Saini et al. 2019).

2.3 Third-Generation (3-G) Biofuels

Microalgae have been identified as an ideal 3-G biofuel feedstock due to their rapid growth rate, CO_2 fixation ability, higher lipid production, ability to grow in diverse trophic modes of nutrition, zero competition with food and feed crops, and no need of arable land for cultivation (Mofijur et al. 2019; Yin et al. 2020). They can adapt and thrive in wastewater while remediating wastewater by removing pollutants. Wastewater-grown microalgal biomass can then be utilized to produce biofuels, resulting in a circular bioeconomy. Microalgae have a large bioenergy potential, making them suitable candidates for liquid transportation fuels such as biodiesel and bioethanol, that meet conventional fuel specifications. As a result, 3-G biofuels are identified as a promising energy resource capable of overcoming the major drawbacks of 1-G and 2-G biofuels. Microalgae can generate considerably higher oil levels for biodiesel production compared to conventional crops (Chowdhury and Loganathan 2019; Ziolkowska 2019). Microalgae have a shorter harvesting cycle than conventional crop plants, allowing numerous and continuous harvests with much higher yields (Alishah Aratboni et al. 2019; Ziolkowska 2019).

2.4 Fourth-Generation (4-G) Biofuels

The 4-G biofuels could be considered as the most advanced biofuel type evolved over time. The feedstock for the 4-G biofuel generation is either genetically engineered bacteria, fungi, cyanobacteria, or microalgae. Novel genetic engineering techniques may aid in the production of biofuels in a cost-effectively, boosting biomass productivity and lipid accumulation. However, there is still considerable concern about the negative environmental impacts exerted by the genetically modified microorganisms occupied in the 4-G biofuel production (Khan et al. 2021).

3 Microalgae-Based Biodiesel

Biodiesel production from microalgae is a sustainable alternative to petroleum-diesel due to its extensive capacity to rapidly accumulate lipids via year-round harvesting cycles, high lipid content, and high photosynthetic efficiency capturing CO_2, which aids in greenhouse gas (GHG) mitigation. Microalgae have been identified as one of

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the most efficient microorganisms capable of tolerating and adapting to various environmental conditions (Jiang et al. 2019). Under stress conditions, they can undergo physiological changes that alter cellular nutrition content and biomass yield (Jiang et al. 2019). This important trait could be achieved by changing the nutritional composition of the microalgal culture medium. Letting microalgae starve nitrogen in the medium is one such strategy to obtain higher biomass with a greater lipid content (Aslam et al. 2018).

After extracting lipids/fatty acids present in microalgal biomass, a transesterification reaction is done to convert microalgal fatty acids into their fatty acid methyl esters (FAMEs), which is known as biodiesel after separation and purification. Microalgal biodiesel should exhibit potential features similar to the standard diesel to be accepted as a potential alternative for the global energy transition. Fuel properties of biodiesel are mainly determined by the FAME composition (e.g., methyl stearate, methyl palmitate, methyl myristate, methyl oleate, and methyl linoleate), including lengths of carbon chains (e.g., C_1, C_2, and C_3), branching pattern, and saturation to unsaturation ratio (Knothe 2005; Zhang et al. 2014).

The biodiesel production from microalgae involves cultivation, harvesting, oil extraction, transesterification, separation and purification (Neag et al. 2023). The general consensus is that biodiesel is superior to fossil fuels due to its exceptional lubricity, viscosity, cetane number, renewability, lack of toxicity and sulfur content, etc. (Ananthi et al. 2021). Microalgal biodiesel should meet certain global standards such as the International Biodiesel Standard for Vehicles (EN14214) and the American Society for Testing and Materials (ASTM), on the physical and chemical properties for its acceptance as a suitable substitute for petroleum-diesel (Behera et al. 2015).

According to Karthikeyan et al. (2020), global algal biomass output averages 38 million liters per year. However, the commercialization of microalgae-based biodiesel faces significant barriers in terms of economic viability and biodiesel LCA (Rafa et al. 2021). Due to the increased production costs, this sustainable attempt is limited to the bench scale in most developing countries. To reduce overall production costs, several cost-cutting measures have been implemented, especially in cultivation, harvesting, dewatering, and pretreatment processes (Rafa et al. 2021).

3.1 Microalgal Lipids

Microalgae produce three main types of lipids: neutral lipids, glycolipids, and phospholipids. Neutral lipids include triacylglycerols (TAGs), which are the prime focus for biodiesel production due to their high energy storage potential. Glycolipids are predominantly found in the chloroplast membrane of microalgae. They are essential in photosynthesis and contribute to membrane fluidity and integrity. Glycolipids are rich in polyunsaturated fatty acids (PUFAs) like omega-3 and omega-6, a promising source of bioactive compounds beneficial for human nutrition (Alishah Aratboni et al. 2019; Ferella et al. 2022). Phospholipids form the structural backbone of cell

624487_1_En_7_Chapter ☑ TYPESET ☐ DISK ☐ LE ☑ CP Disp.:31/7/2025 Pages: 29 Layout: T1-Standard

membranes, playing a vital role in cellular function and signaling. Phospholipids from microalgae often contain essential fatty acids, such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), which play a crucial role in human health (Alishah Aratboni et al. 2019; Ferella et al. 2022). The microalgal lipid composition can be highly variable, depending on factors such as species, environmental conditions (i.e., light intensity, temperature, salinity), and nutrient availability. As mentioned earlier, nutrient deprivation often triggers lipid accumulation, especially TAGs (Aslam et al. 2018), while sufficient presence of nutrients favors the synthesis of membrane lipids (Table 1).

Table 1 Lipid content of some microalgae and cyanobacteria species

Microalgae/cyanobacteria species	Lipid content (% dry weight)	Reference
Chlorella sorokiniana	11–16	Menegazzo et al. (2022)
Chlorella pyrenoidosa	1.7–20	D'oca et al. (2011)
Chlorella vulgaris	1.8–58	Araujo et al. (2013)
Chlorella fusca LEB 111	15.5	Kuo et al. (2021)
Chlorella emersonii	23–63	Sajjadi et al. (2018)
Chlorella protothecoides	40–60	Sajjadi et al. (2018)
Chlorella minutissima	14–57	Sajjadi et al. (2018)
Nannochloropsis oculata	18–60	Levasseur et al. (2020)
Dunaliella salina	6–25	Sajjadi et al. (2018)
Isochrysis galbana	24–47	Levasseur et al. (2020)
Scenedesmus obliquus	35–50	Levasseur et al. (2020)
Botryococcus braunii	25–75	Levasseur et al. (2020)
Phaeodactylum tricornutum	32–41	Levasseur et al. (2020)
Spirulina platensis	4–11	Sajjadi et al. (2018)
Synechocystis sp.	11	Sajjadi et al. (2018)
Chlamydomonas rheinhardii	21	Sajjadi et al. (2018)
Neochloris oleoabundans	35–65	Sajjadi et al. (2018)
Nostoc commune	22	Sajjadi et al. (2018)
Oscillatoria sp.	32	Fuad Hossain et al. (2020)
Synecochoccus sp.	31	Fuad Hossain et al. (2020)
Croococcidiopsis sp.	23	Fuad Hossain et al. (2020)
Leptolyngbya sp.	21	Fuad Hossain et al. (2020)
Limnothrix sp.	21	Fuad Hossain et al. (2020)
Calothrix sp.	18	Fuad Hossain et al. (2020)
Cephalothrix komarekiana	14	Fuad Hossain et al. (2020)
Westiellopsis prolifica	13	Fuad Hossain et al. (2020)

624487_1_En_7_Chapter ☑ TYPESET ☐ DISK ☐ LE ☑ CP Disp.:31/7/2025 Pages: 29 Layout: T1-Standard

Table 2 Comparison between different microalgae cultivation methods

Cultivation mode	Energy source	Carbon source	Cell density	Lipid accumulation	Cultivation system
Photoautotrophic	Light	Inorganic CO_2	Low	Low	Open ponds/ photo-bioreactors
Heterotrophic	Organic carbon	Organic carbon	High	High	Fermenters
Mixotrophic	Light/ organic carbon	Inorganic/ organic carbon	Medium	High	Semi-closed system
Photoheterotrophic	Light	Organic carbon	Medium	Low	Photobioreactors

3.2 Microalgae Cultivation

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The cellular metabolism of microalgae is strongly influenced by their cultivation method. The biomass productivity and lipid content vary based on the given nutrient and energy conditions. They can alter their metabolic reactions under different environmental conditions while utilizing available nutrients and energy efficiently (Behera et al. 2015). The major microalgae cultivation modes are given in Table 2.

3.3 Microalgae Harvesting Methods

Efficient microalgae harvesting to recover entire oleaginous biomass is vital. This procedure involves separating algal biomass from the culture medium and thickening it with concentrated microalgae cells. Harvesting microalgae cells, which range in size from 1 to 20 µm, is a crucial step in biodiesel synthesis as it demands significant energy input. Harvesting costs can account for 20–30% of overall biodiesel production cost (Mofijur et al. 2019). There is no single method for harvesting that is both effective and inexpensive. The important characteristics of an ideal harvesting approach are species independence, maximum harvesting efficiency, minimal lipid loss, energy and chemical efficiency.

Choosing the appropriate harvesting technique depends on several parameters, including the type of microalgae species, their size, density, and target products, as well as harvesting efficiency and economical aspects. Harvesting techniques include chemical, biological, mechanical, and electrical processes, as well as combinations of two or more of these. A wide range of methods as centrifugation, gravity sedimentation, floatation, flocculation/coagulation, screening, and electrophoresis, have been studied for microalgae harvesting (Fazal et al. 2018).

Biomass drying is an important post-harvest technique that extends the shelf life of collected biomass before storage and prevents rotting. Sun drying is less expensive but takes a longer time. Dry biomass can be obtained from fresh microalgal biomass

624487_1_En_7_Chapter ☑ TYPESET ☐ DISK ☐ LE ☑ CP Disp.:31/7/2025 Pages: 29 Layout: T1-Standard

using efficient drying methods, such as spraying, drum drying, oven heating, freezing, and fluidized bed drying (Behera et al. 2015) (Fig. 1).

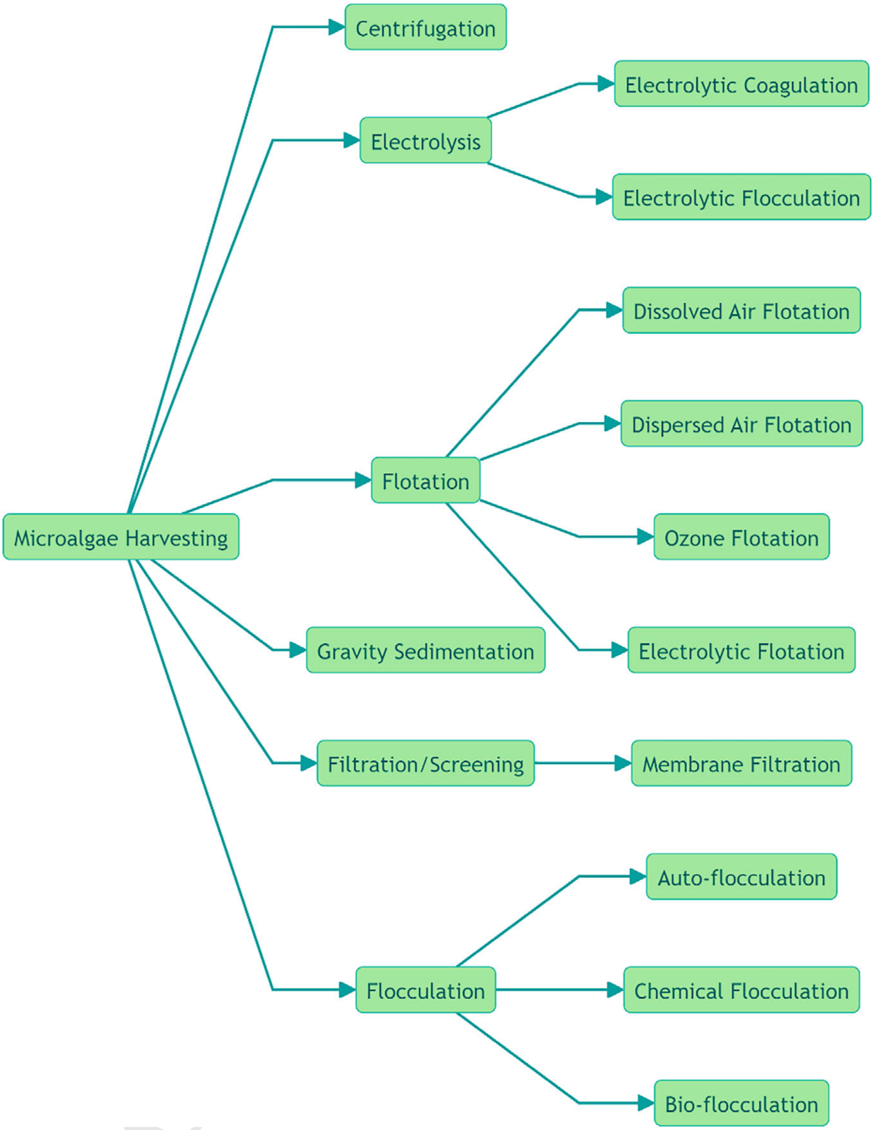

Fig. 1 Different microalgae harvesting methods

3.4 Lipid Extraction from Microalgal Biomass

The harvested microalgal biomass (either dry biomass or wet biomass) is occupied to extract lipids for biodiesel production (Sati et al. 2019). In both dry and wet routes, extraction solvents play a crucial role. The microalgal lipid extraction is solely solvent-based to date (Sati et al., 2019; Saini et al. 2021). The method used for lipid extraction should be lipid-specific, limit lipid loss, maintain cellular integrity, completely remove non-lipid content and be time-efficient (Fazal et al. 2018; Sati et al. 2019; Saini et al. 2021)). Choosing the best solvent or solvent system (e.g., hexane, methanol, ethanol, and chloroform) for lipid extraction is critical for increased lipid recovery while maintaining an economically feasible process.

Prior to solvent extraction, pretreatment of the algal biomass should be employed to disintegrate the robust oleaginous cell wall facilitating efficient solvent penetration into the cell to obtain higher lipid content (Sati et al. 2019; Saini et al. 2021). Various physical, mechanical, chemical, and biological pretreatment methods are available to achieve this goal. The mechanical and thermal disruption include bead beating, ultrasonication, grinding using mortar and pestle, high-pressure homogenization, pulse-electric field, microwave heating, autoclaving, etc. (Harris et al. 2018; Sati et al. 2019; Saini et al. 2021). Chemical pretreatment includes acid/base treatment, osmotic shock and the use of surfactants. Biological cell lysis is assisted with enzymes, such as cellulases, proteases, lipases, and pectinases (Sati et al. 2019; Saini et al. 2021).

The Bligh and Dyer, and Folch methods are two classic lipid extraction protocols that are acting as exemplars for lipid extraction. These two methods are widely used and involve a chloroform/methanol solvent mixture to extract lipids involving toxic solvents (Sati et al. 2019; Saini et al. 2021). Soxhlet extraction is a continuous process using solvents like hexane, methanol or ethanol. This is also considered as a standard method for lipid extraction and yields high lipid content but can be time-consuming. Supercritical CO_2 extraction is another lipid extraction method where CO_2 at high pressure acts as a solvent, selectively extracting lipids. This method is efficient and eco-friendly but requires high pressure (Sati et al. 2019; Saini et al. 2021).

Green solvent-assisted extraction is another breakthrough for lipid extraction from microalgae (Sati et al. 2019; Saini et al. 2021). One such is ionic liquids; these solvents are eco-friendly alternatives to organic solvents and can effectively extract lipids without toxic residue. Switchable solvents are another green approach with low energy consumption that can directly extract lipids from wet oleaginous biomass (Harris et al. 2018; Sati et al. 2019).

3.5 Transesterification

In this process, the extracted lipids (TAGs) are chemically reacted with an alcohol to produce their FAMEs, which are known as biodiesel and glycerol as the by-product. In this reaction, one molecule of TAG reacts with three molecules of methanol to

624487_1_En_7_Chapter ☑ TYPESET ☐ DISK ☐ LE ☑ CP Disp.:31/7/2025 Pages: 29 Layout: T1-Standard

produce three molecules of FAMEs/biodiesel and one molecule of glycerol. Due to its higher density, glycerol settles down at the bottom, while FAMEs remain on the top layer. Major transesterification types include acidic, alkaline, enzymatic and direct transesterification. Since the transesterification reaction is reversible, an excess amount of alcohol is used to drive the reaction forward (Fazal et al. 2018). Several factors influence biodiesel production, including reaction time, reaction temperature, alcohol concentration, and type and concentration of catalyst. Typically, either chemical catalysts (acids or bases) or biological catalysts (enzymes) are employed in transesterification reactions. Alkaline catalysts, such as sodium hydroxide, potassium hydroxide, aluminum oxide, sodium methoxide, and sodium ethoxide, are commonly used for biodiesel production. Acid catalysts include sulfuric acid, sulfonic acid, and hydrochloric acid, while lipase can serve as an enzymatic biocatalyst (Fazal et al. 2018).

4 Genetic Engineering of Microorganisms for Enhanced Biofuel Production

Microalgae hold immense promise as a sustainable feedstock for biofuel production due to their promising biochemical properties, rapid growth rates, and ability to thrive in diverse environments. Recently, gene modification techniques have been applied to microalgae to enhance lipid productivity (particularly TAG biosynthesis), improve lipid composition, reduce sensitivity to oxygen saturation, increase photosynthesis and enhance carbon fixation, and boost resistance against harsh operating conditions. These advancements enhance the overall efficiency of 4-G biodiesel production ((Dickinson et al. 2017); Mallick et al. 2016).

Before setting up metabolic engineering pathways, it is critical to understand microalgal lipid biosynthetic pathways in order to obtain optimal biodiesel yields. Before attempting to apply any transgenic technique to microalgal strains, it is vital to have an extensive understanding of lipid metabolism and how it is regulated by enzymes and genes. In microalgal lipid metabolism, free fatty acids (glycolipids) are generated in the chloroplast, whereas TAGs are synthesized in the endoplasmic reticulum. These two different routes come together in the cytosol to produce the TAG-lipid body. The enzyme acetyl-CoA carboxylase (ACCase) converts acetyl-coenzyme A (acetyl-CoA) into malonyl-CoA as the first step in the fatty acid biosynthesis pathway (Mallick et al. 2016). Despite numerous efforts that have been undertaken to enhance lipid metabolism by overexpressing ACCase, total success has yet to be achieved (Mallick et al. 2016).

Genetic manipulation of metabolic pathways in microalgae enables the enhancement of lipid biosynthesis. Strategies include overexpressing key enzymes involved in lipid synthesis (e.g., acetyl-CoA carboxylase and acyltransferases) and redirecting carbon flux toward lipid accumulation by manipulating carbon fixation and storage pathways. (Dickinson et al. 2017). Various microorganisms, including bacteria, yeast, and algae, are used as hosts for biofuel production due to their diverse metabolic flexibility, which allows them to utilize a broad range of substrates. For instance, genes from *Clostridia* sp. that are responsible for butanol production have been incorporated into strains of *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas putida* (Cavelius et al. 2023). Genetically modified algae demonstrate improved yields and efficiencies compared to their wild-type counterparts. Techniques, such as CRISPR/Cas9, enable precise genetic modifications, enhancing traits like photosynthetic efficiency and increased yields. Engineered cyanobacteria have been successful in producing ethanol, butanol, isobutanol, and modified fatty acids for biodiesel production (Cavelius et al. 2023).

Random mutagenesis on microorganisms, also known as accelerated evolution, can be achieved through various approaches, such as ultraviolet irradiation, alkylating agents, acridine dyes, transposons, and DNA shuffling. This technique has been applied to organisms like *Cutaneotrichosporon oleaginosus*, resulting in increased lipid production, making it a suitable candidate for biodiesel production. Biodiesel produced from notable yeast strains, including *Yarrowia lipolytica*, *Cutaneotrichosporon oleaginosus*, *Rhodosporidium toruloides*, and *Lipomyces starkii*, meets international biodiesel standards, such as US ASTM D6751 and EU EN 14,214 (Varela Villarreal et al. 2020; Cavelius et al. 2023).

Systems biology approaches can offer valuable insights for effectively optimizing native biofuel candidates. Access to complete genome sequences of suitable candidates is crucial, as it enables the gene annotation to their respective functions and the reformation of intrinsic metabolic pathways, which can then be altered. Recent advancements in genome sequencing have significantly accelerated and streamlined data collection processes. Omics technologies, such as transcriptomics, facilitate the analysis of gene expression patterns across various growth environments, while proteomics enables the identification of protein products (Cavelius et al. 2023). Moreover, nanotechnology can be employed in biofuel production to enhance biomass production efficiency and reduce various production costs, making it a cost-competitive addition to the biofuel market (Ziolkowska 2019). Overall, 4-G biofuels represent a frontier in sustainable energy transition, integrating advanced genetic engineering techniques with diverse production strategies to meet growing global energy demands.

4.1 Considerations and Challenges

4.1.1 Regulatory Compliance

Wider adoption of genetically engineered biofuels hinges on political acceptance and support, amid ongoing debates about genetic engineering's implications mainly in agriculture and medicine (Cavelius et al. 2023). Genetically engineered organisms (GMOs) for biofuel production must adhere to regulatory frameworks, ensuring compliance with safety, environmental, and ethical standards which are essential for the commercialization and deployment of engineered microbial strains.

4.1.2 Environmental Impacts

The release of GMOs into natural ecosystems raises concerns regarding potential ecological impacts, such as gene flow, competition with native species, and ecosystem disruption. Comprehensive risk assessments and environmental monitoring are necessary to evaluate and mitigate potential risks associated with engineered organisms.

By adopting proper containment strategies and choosing the right locations, the related risks can be greatly reduced. Consequently, it is expected that secure closed production systems will be developed (Awad et al. 2020). Extra biocontainment approaches might include genetic alterations in production cells, like auxotrophies (auxotrophic mutants) or kill switches, which significantly lessen the chances of GMOs escaping into the environment from their controlled production environment (Awad et al. 2020; Cavelius et al. 2023).

4.1.3 Scalability and Commercialization

Scaling up GMO cultivation to industrial levels presents technical and economic challenges. Optimizing cultivation systems, downstream processing, and biorefinery integration is critical for achieving cost-effective and sustainable biofuel production (Muñoz et al. 2021).

5 Green Aviation: Biofuels in Aviation

Green aviation, a rapidly growing concept in the modern world, strives to mitigate the environmental impact of air travel. One of the key strategies for achieving this goal is the adoption of biofuels as an alternative to traditional aviation fuels derived from fossil resources (Cabrera and Melo de Sousa 2022; Detsios et al. 2023).

Currently, aviation fuels are predominantly kerosene-based, commonly referred to as jet fuels (e.g., Jet A or Jet A-1). The heavy reliance on jet fuels in aviation has significantly drained petroleum reserves. Additionally, the high consumption of jet fuels is a major contributor to greenhouse gas (GHG) emissions, making up 3% of the total global GHG emissions (Detsios et al. 2023). The International Air Transport Association (IATA) and the Air Transport Action Group (ATAG) aim to cut CO_2 emissions by 50% by 2050, compared to levels in 2005 (Detsios et al. 2023; (Su-Ungkavatin et al. 2023).

Biokerosene is a type of non-fossil aviation fuel derived from biological feedstocks and falls under "Sustainable Aviation Fuels (SAFs)." There are currently six certified methods for producing aviation biofuels: the Fischer–Tropsch (FT) process, hydroprocessed esters and fatty acids (HEFA), direct sugar to hydrocarbon (DSHC), alcohol-to-jet (ATJ, using isobutanol or ethanol intermediates), and catalytic hydrothermolysis jet (CHJ). Some methods, like FT, can include added aromatic compounds. Biofuels produced by these methods must be certified by the American Society for Testing and Materials International (ASTM) (ASTM D7566—Standard Specification for Aviation Turbine Fuel Containing Synthesized Hydrocarbons) or equivalent standards. These biofuels must still be blended with traditional fossil-based kerosene fuels to execute (Su-Ungkavatin et al. 2023).

5.1 *Aviation Biofuels*

5.1.1 Hydroprocessed Esters and Fatty Acids (HEFA)

Hydroprocessed renewable jet fuels (HRJs or HEFA) are derived from hydrogenating fatty acids sourced primarily from oily plants (sunflower oil, soybean oil, palm oil, and canola oil), recycled cooking oils, animal fats (tallow, pork fat, chicken fat), waste grease, or microalgal oil. These fuels have rich energy content, making them suitable for use in conventional aircraft engines without modifications. Many airlines have experimented with HEFA on passenger flights, but the high cost of the raw materials poses challenges related to competition with food production and changes in land use (Cabrera and Melo de Sousa 2022; Detsios et al. 2023). While biodiesel is produced by esterifying fatty acids, it is not suitable for aviation because of its low energy density and high freezing point.

5.1.2 Fischer–Tropsch Fuels (FT Fuels)

FT fuels are synthetic fuels produced through the Fischer–Tropsch process, a chemical reaction that converts a mixture of carbon monoxide and hydrogen (syngas) into liquid hydrocarbons. This syngas can be derived from various feedstocks, including biomass, allowing FT fuels to be a versatile alternative to conventional fossil fuels. They are known for their high energy density, and low in sulfur and aromatic compounds, which leads to lower emissions (Cabrera and Melo de Sousa 2022; Detsios et al. 2023).

5.1.3 Alcohol-to-Jet (AtJ)

Sugar- and starch-rich crops and lignocelluloses can be hydrolyzed and fermented to obtain alcohols to convert into jet fuel via dehydration, oligomer synthesis, hydrogenation, isomerization and finally, the distillation (Cabrera and Melo de Sousa 2022;

Detsios et al. 2023). The AtJ pathways provide the versatility to use different alcohols like ethanol and isobutanol sourced from a variety of origins.

5.1.4 Direct Sugars to Hydrocarbons (DSHC)

This type of biofuel employs genetically engineered microorganisms, like algae, bacteria, or yeast, to transform sugars into hydrocarbons or lipids. Presently, many biofuel methods rely heavily on traditional sugar sources. However, the challenge lies in the stubborn nature and inefficient conversion of lignocellulosic sugars into fuels via DSHC, leading to significant processing expenses and energy consumption. Consequently, DSHC stands out as the costliest alternative route for aviation fuel (Cabrera and Melo de Sousa 2022; Detsios et al. 2023).

5.1.5 Catalytic Hydrothermolysis Jet (CHJ)

In the CHJ method, also referred to as hydrothermal liquefaction, refined free FFAs derived from waste or energy oil processing are mixed with preheated feed water and directed into the catalytic reactor (Cabrera and Melo de Sousa 2022; Detsios et al. 2023). Then it is subjected to high temperature and pressure; then the oil undergoes conversion into a blend of hydrocarbons, encompassing n-paraffins, iso-paraffins, cycloparaffins, and aromatic compounds. Following subsequent hydrotreatment and product fractionation, the end result containing kerosene is achieved (Cabrera and Melo de Sousa 2022; Detsios et al. 2023).

5.1.6 Hydroprocessed Hydrocarbons, Esters, and Fatty Acids (HC-HEFA)

HC-HEFA operates similarly to the HEFA process, undergoing hydrodeoxygenation, cracking, and isomerization. HC-HEFA undergoes hydrotreatment of biobased hydrocarbons sourced from oils present in a microalga, *Botryococcus braunii* (Cabrera and Melo de Sousa 2022; Detsios et al. 2023). This specific alga serves as the exclusive feedstock for this conversion procedure. Notably, HC-HEFA is limited to a blending ratio of up to 10% due to being the initial pathway to receive certification via the ASTM D4054 "fast-track" process (Cabrera and Melo de Sousa 2022).

5.2 Key Issues Related to Aviation Biofuels

Even though these alternative aviation fuel options represent progress in sustainable aviation fuel development, ongoing research and innovations are necessary to overcome limitations and enable widespread adoption addressing the complex

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array of issues related to feedstock selection, technological viability, environmental impacts, and socioeconomic considerations. Collaborative efforts involving governments, industrial stakeholders, researchers, and environmental advocates are essential for overcoming these challenges and realizing the full potential of green aviation powered by biofuels.

5.2.1 Feedstock Selection

A major concern regarding aviation biofuels revolves around selecting feedstocks that are cost-effective, minimize water, land, and fertilizer usage, and avoid impacting food crop production, thus mitigating food versus fuel conflicts. Feedstock options like algae, waste oils, and non-food crops are often viewed as more sustainable (Kandaramath Hari et al. 2015). The production of substantial fuel quantities is necessary even for aircraft testing, requiring significant feedstock volumes. Cultivating biofuel feedstocks can contribute to land use changes, deforestation, habitat loss and competition with food crops, prompting concerns about the overall environmental benefits of aviation biofuels.

5.2.2 Technological Challenges

Despite progress in biofuel production technologies, notable hurdles persist. Challenges, such as scalability, cost-efficiency, and compatibility with current infrastructure and aircraft engines, impede broad implementation. Moreover, ensuring the sustainability and dependability of biofuel supply chains presents further technical obstacles (Neuling and Kaltschmitt 2018).

5.2.3 Indirect Land Use Change (ILUC)

Biofuel production can indirectly influence land use patterns and ecosystems, leading to unintended consequences such as deforestation, habitat loss, and biodiversity decline. ILUC refers to the displacement of existing agricultural activities or natural ecosystems by biofuel feedstock cultivation, which can offset the anticipated environmental benefits and exacerbate ecological pressures (Kandaramath Hari et al. 2015).

5.2.4 Food Security and Social Implications

The use of food crops for aviation biofuel production has raised attention, particularly in regions where food insecurity is already prevalent. Competition between fuel and food production can affect food prices, agricultural practices, and rural livelihoods, potentially worsening social inequalities and food shortages.

5.2.5 Distribution Problems

In the supply chain, achieving product quality and appropriate blending for efficient aircraft engine performance poses challenges (Kandaramath Hari et al. 2015). Compared to fossilized jet fuels, the biofuel production cost is considerably higher. Addressing tasks such as fuel infrastructure, marketing, and the storage of feedstock and fuel, as well as complying with regulations and certifications for byproduct marketing, are significant endeavors. The current high production costs of biofuels need to be reduced to ensure feasibility; otherwise, rising prices will impact production investments.

5.2.6 Compatibility with Conventional Fuel

Ensuring compatibility with conventional jet fuels is crucial. Renewable jet fuels must seamlessly integrate into commercial aviation service and overcome hurdles in certification, engine testing, and quality assurance. The developed biofuels should demonstrate high performance, safety assurance, and resilience across various operational conditions. Thermal and storage stability are critical considerations for aviation biofuels. Properties like low-temperature performance, combustion characteristics, impurities and micronutrient presence are also important factors to consider (Kandaramath Hari et al. 2015).

5.2.7 Policy and Regulatory Frameworks

Developing effective policy frameworks and regulatory mechanisms plays a vital role in advancing sustainable biofuel production and encouraging investment in eco-friendly aviation initiatives. Policymakers encounter the challenge of balancing environmental goals with economic factors, industrial interests, and geopolitical dynamics when crafting biofuel strategies and mandates. Policies should serve as catalysts, motivating companies to gradually transition from fossil fuels to SAFs. Financial support mechanisms, such as tax exemptions recently approved in the United States or direct project funding, are typically the most crucial, in influencing a project's financial viability (Cabrera and Melo de Sousa 2022). Legislation, like the European Commission's ReFuelEU Aviation, is another avenue to promote biofuel adoption in aviation. ReFuelEU mandates specific targets for SAF integration in EU airports, starting from 2% in 2025 and aiming for 63% by 2050 for aviation biofuels (Cabrera and Melo de Sousa 2022; Arias et al. 2024).

6 Biofuel Life Cycle Assessment (LCA)

As per the ISO (International Standardization Organization) in the ISO 14040:2006 series standard, Life Cycle Assessment (LCA) is defined as the "gathering and assessment of the resources used, emissions generated, and the possible environmental effects of a product system over its entire life cycle" (ISO 14040:2006). LCA can also be defined as an internationally recognized procedure for evaluation of the global environmental performance of a product, process or pathway along its partial or whole life cycle, considering the environmental impacts generated from "cradle to grave" (Gnansounou et al. 2009; Brandão et al. 2022).

Biofuel environmental impact assessment requires a comprehensive understanding of their complete life cycle, from feedstock cultivation to fuel production, distribution, and combustion. LCA helps to evaluate the net GHG emissions, energy efficiency, and other environmental impacts associated with biofuels. Variability in feedstock sources, production processes, land-use change, modeling approach and GHG metrics significantly influence LCA analyses resulting in extremely variable outcomes in the estimation of the carbon footprint of biofuels (Brandão et al. 2022).

6.1 Major Phases of Biofuel LCA

According to the ISO 14040:2006 series standard, an LCA has four major phases as presented in Fig. 2.

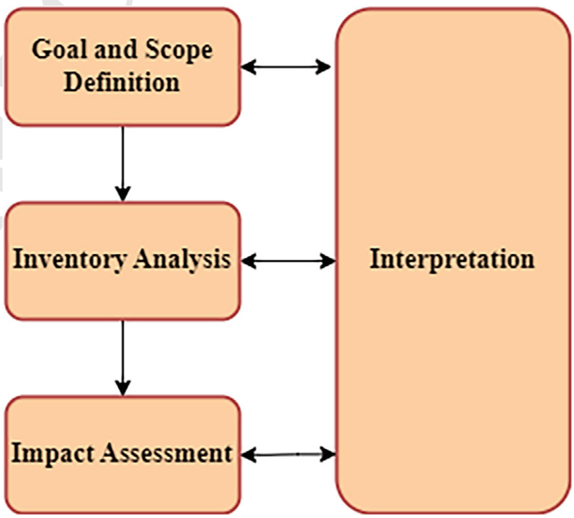

Fig. 2 Four major phases of LCA of biofuels

(a) **Goal and Scope Definition**: This phase involves defining the purpose, system boundaries, functional unit, and assumptions for the LCA study of the biofuel production system.
(b) **Life Cycle Inventory (LCI)**: During this stage, information is gathered on various inputs (such as raw materials, energy, and water) and outputs (like GHG emissions and waste), spanning from feedstock cultivation to production, utilization, and disposal.
(c) **Life Cycle Impact Assessment (LCIA)**: This phase evaluates the potential environmental impacts associated with the inventory data, such as GHG emissions, resource depletion, and toxicity, using standardized impact categories and characterization methods.
(d) **Interpretation**: The final phase involves analyzing the overall results, identifying areas for improvement, evaluating trade-offs, and making recommendations based on the LCA findings to boost the environmental performance of biofuel production pathways.

6.2 Scope and Functional Unit

An effective LCA system necessitates a clearly defined scope that aligns with the primary objective of the study (Rathore et al. 2013). The primary goal of performing a biofuel LCA is to evaluate the environmental impacts of the analyzed system and measure the ecological benefits gained by replacing the reference system, which is usually conventional fossil fuels. Furthermore, it can be an essential resource for policymakers and consumers to identify the most sustainable fuel options (Cherubini and Strømman 2011).

The functional unit in biofuel LCA refers to the reference unit used to quantify the inputs and outputs of the biofuel production system under study. It serves as a basis for comparing different biofuel production pathways or technologies. Common examples of functional units in biofuel LCAs include 1 MJ or 1 GJ of biofuel produced, 1 km driven by a vehicle using the biofuel, or annual biofuel production for a specific facility or region. Establishing the correct functional unit is pivotal during the first phase of an LCA, enabling consistent quantification and comparison of environmental impacts across different biofuel production scenarios (Cherubini and Strømman 2011; Gheewala 2023). The selection of the appropriate functional unit is crucial for making accurate and adequate comparisons of products (Rathore et al. 2013).

6.3 LCA Modeling Approaches

6.3.1 Attributional LCA (ALCA)

This approach focuses on the direct environmental impacts of biofuel production, such as energy consumption, water usage, and current or average GHG emissions. It is often used for evaluating the environmental performance of specific biofuel production pathways, technologies, and management practices. It provides a snapshot of the environmental performance of biofuels under present conditions. This approach is useful for identifying opportunities for emissions reduction and improving the environmental sustainability of biofuel production systems (Gheewala 2023; Osman et al. 2024).

6.3.2 Consequential LCA (CLCA)

This approach considers the indirect environmental impacts of biofuel production, including land use pattern changes, market dynamics, and changes in the other goods and services. It is often used for evaluating the broader environmental implications of biofuel production and consumption resulting from shifts in agricultural practices, energy markets, and trade patterns induced by biofuel production. Consequential LCA provides insights into the full lifecycle emissions and environmental implications of biofuel policies and investment decisions, helping to inform more sustainable energy strategies (Osman et al. 2024).

6.4 System Boundary

The goal and scope of the LCA help to determine the preliminary system boundaries. Various system boundaries are employed in LCA to estimate the performance of biofuels via the life cycle and to pinpoint areas where enhancements can be implemented to minimize their environmental impacts. The selection of the system boundary leads to significant variations in LCA estimates, as they differ not only in terms of starting and ending points but also across space and time, profoundly affecting energy and greenhouse gas balances (Cherubini and Strømman 2011; Rathore et al. 2013).

6.4.1 Well-to-Tank (WtT)

This approach focuses on the production and transportation of biofuel from the feedstock to the fuel storage tank. It includes processes like fermentation, distillation, and refining, as well as the energy consumption and GHG emissions associated with these processes.

6.4.2 Tank-to-Wheel (TtW)

TTW assesses the usage of biofuels in vehicles, including combustion emissions and tailpipe pollutants. It considers factors, such as fuel combustion efficiency, vehicle type, driving patterns, and emissions control technologies. The objective is to understand the environmental benefits or drawbacks of using biofuels compared to conventional fossil fuels during vehicle operation.

6.4.3 Well-to-Wheel (WtW)

This approach combines both WtT and TtW assessments to provide a comprehensive analysis of the complete life cycle of biofuels, from factory to vehicle use. It considers all stages of fuel production, distribution, and consumption, including energy inputs, emissions, and other environmental impacts. WtW analysis provides a holistic view of the environmental performance of biofuels compared to fossil fuels, considering both upstream and downstream effects.

6.4.4 Cradle-to-Grave (CtG)

This methodology encompasses the complete life cycle of the biofuel, starting from the cultivation of the biomass feedstock to the ultimate disposal of the biofuel and its by-products. It includes all stages of production, processing, transportation, usage, and disposal, offering a thorough evaluation of the overall impact of the biofuel. In the context of biofuels, this cradle-to-grave (CtG) assessment encompasses not only Well-to-Wheel (WtW) impacts but also additional factors such as land use changes, water consumption, and potential effects on ecosystems and biodiversity. CtG analysis offers a more nuanced comprehension of the environmental implications associated with biofuel production and utilization, aiding policymakers and stakeholders in making well-informed decisions (Gheewala 2023; Osman et al. 2024).

For biofuel LCA studies, adopting a cradle-to-grave approach is essential, with the functional unit being unit energy utilization, given the substantial variation in conversion efficiency based on the production process (Rathore et al. 2013). Failure to select an appropriate system boundary can result in erroneous LCA outcomes that do not accurately reflect reality, leading to flawed interpretations and comparisons (Rathore et al. 2013).

7 Overview of Global Biofuel Production Trends

The adoption and production of biofuels exhibit significant disparity worldwide, with some countries employing advanced technologies while others are only beginning to explore the potential of biofuels. Globally, the blending concentrations of biofuels with petroleum fuels also vary. For biodiesel, blending concentrations of 2%, 5%, 20%, and 100% are typically available in the market, denoted as B2, B5, B20, and B100, respectively. Conversely, bioethanol is blended with petrol at concentrations of 10%, 15%, 25%, 85%, and 100%, marketed as E10, E15, E25, E85, and E100, respectively. E85 is compatible with dual-fuel automobiles (Puri et al. 2012).

The USA, Brazil, Germany, Indonesia, Sweden, and France are recognized as global leaders in biofuel development and consumption. Moreover, Indonesia, China, Russia, and India are actively promoting biofuel production, citing reasons such as environmental sustainability, energy security, and rural economic development. Together, the USA and Brazil produce over 75% of the world's bioethanol, derived from crops like corn, sugarcane, and lignocellulosic residues (Puri et al. 2012). In Brazil, annual bioethanol production reaches 27 billion liters, primarily from various sugarcane varieties and agricultural technologies, with domestic transportation relying on E25 (Puri et al. 2012).

Germany holds a prominent position in biodiesel production in Europe, boosting leading biodiesel plants nationwide. Tax exemptions for pure and blended biodiesel have enhanced the competitiveness of biofuels against fossil fuels in Germany. Asian nations like China and India are also emerging as significant players in biofuel production. China utilizes a substantial amount of agricultural residues for biofuel production, with bioethanol accounting for 20% of total petroleum consumption in the country. India is also recognized as a rising country stepping toward the incorporation of biofuels into their energy sector (Puri et al. 2012). Overall, these examples illustrate the global transition toward the development and adoption of biofuels as a renewable energy source, driven by factors such as energy security, environmental concerns, and socioeconomic opportunities.

Figures 3 and 4 show global bioethanol and biodiesel production since 2002 projecting toward 2030. In 2020, global biofuel production dropped significantly to around 1808 thousand barrels of oil equivalent per day (or 142 billion liters), cutting down 11.6% compared to 2019 levels due to the COVID-19 pandemic. Production rebounded in 2021, increasing by 5.8% producing 1914 thousand barrels per day. However, this was still below pre-pandemic levels and the USA, Brazil, and the European Union remain as the world's largest biofuel producers.

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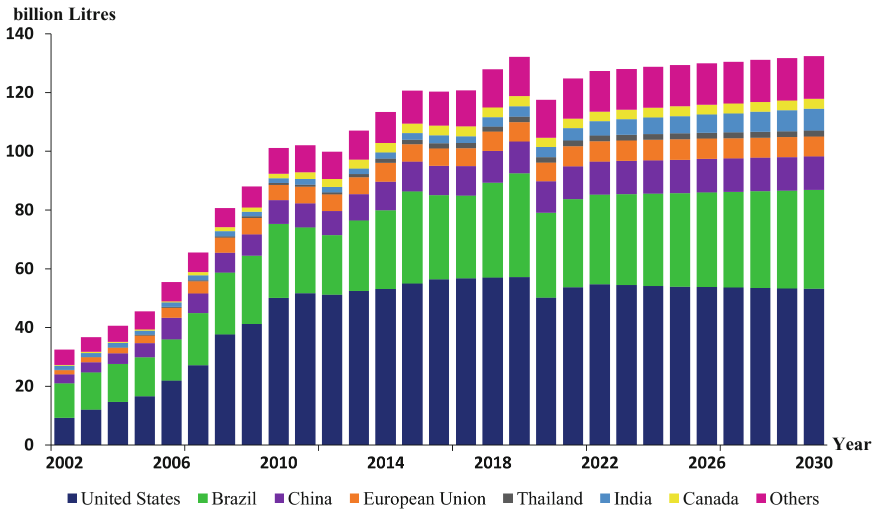

Fig. 3 Development of world bioethanol production (OECD/FAO 2021)

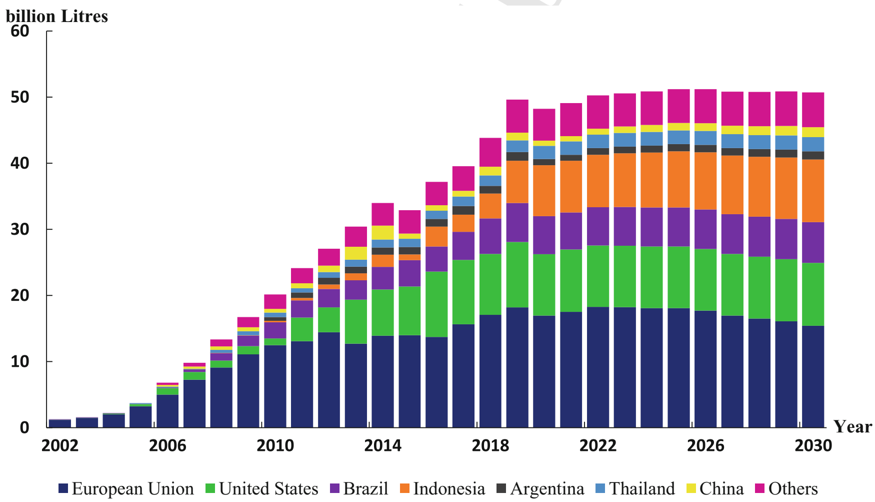

Fig. 4 Development of world biodiesel production (OECD/FAO 2021)

8 Limitations and Future Directions in Biofuel Research

The food versus fuel debate is the key drawback caused by the 1-G biofuels produced using food crops. This may scarce food for human consumption impacting food security, while creating competition for arable land and water resources (Hoekman 2009).

In the meantime, the expansion of biofuel crop cultivation may lead to deforestation and/or displacement of other agricultural activities due to the finite availability of land suitable for crop production while destroying natural habitats and threatening biodiversity. Although biofuels are generally considered cleaner than fossil fuels, their production can still contribute to greenhouse gas emissions, particularly if indirect land-use changes like deforestation are involved (Luque et al. 2008; Hoekman 2009). Clearing land for biofuel production can also release CO_2 stored in plants and soil, undermining the carbon-saving benefits of biofuels (Balan 2014). Current biofuel production cannot fully replace fossil fuels without causing severe land-use changes and ecosystem disruptions.

Processing challenges associated with 2-G biofuels that are derived from lignocellulosic residues that are non-food sources require complex and energy-intensive conversion processes due to the biomass recalcitrance of lignocelluloses, reducing the energy efficiency in the production process (Puricelli et al. 2021). Moreover, some biofuel feedstocks are region-specific, making it difficult to ensure a consistent global supply to address global energy demand. Advanced biofuels (2-G and 3-G) made from non-food sources like lignocellulosic biomass, waste oils and microalgae have fewer drawbacks than 1-G biofuels, but they are still in the early stages of commercialization. The technologies needed to produce these biofuels are expensive, requiring economically viable strategies to apply on a large scale (Hoekman 2009). Since microalgae are photosynthetic organisms, they require sunlight when growing in open ponds, which may be only available to most of tropical countries throughout the year. Contamination avoidance from other organisms is another crucial concern for large-scale cultivation of microalgae.

The biomass conversion processes like pretreatment, hydrolysis, fermentation, and separation are still being optimized in 2-G biofuel production. Many existing technologies require significant energy inputs and may not be efficient enough to compete with fossil fuels without further advancements. Biofuels, particularly biodiesel and ethanol, can pose challenges in terms of storage and distribution. They are often more prone to water contamination and degradation over time than petroleum fuels. Fuel compatibility is another limitation of biofuel production. Many biofuels, particularly ethanol and biodiesel, are not fully compatible with existing fuel infrastructure, requiring modifications to pipelines, refineries, and vehicles (Luque et al. 2008). There are limits to how much biofuel can be blended with traditional fuels. For instance, in many vehicles, ethanol blends above 10–15% v/v can cause engine damage unless specific adaptations are made in spark-ignition engines. The blending percentage of bioethanol with petrol for conventional vehicles in Europe is limited to 10% v/v (Puricelli et al. 2021). The vehicle engines have to be modified to boost the percentage of blending to optimize the operating parameters like injection timing, compression ratio and injection pressure (Puricelli et al. 2021).

The economic feasibility of biofuels depends on several factors, including government subsidies, oil prices, and the cost of feedstocks. Biofuels often require substantial government support to remain competitive with fossil fuels, and their profitability can fluctuate with changes in global energy markets. Public perception also plays a crucial role in the acceptance and integration of biofuels into existing energy systems

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(Luque et al. 2008). These limitations highlight the complexities involved in transitioning to biofuels, indicating that novel are needed to address these challenges effectively, requiring innovations in feedstock development, conversion technologies, and policy frameworks while minimizing negative environmental and social impacts (Luque et al. 2008; Hoekman 2009).

A successful commercial biofuel industry requires a combination of technological innovations in production processes, along with practical economic feasibility safeguarding environmental resilience. Since commercial-scale biofuel production remains a challenge, the exploration of new strains with commercial viability is still necessary (Malik et al. 2024). The integration of various genetic engineering approaches to optimize biofuel production is expected to be highly beneficial. With advancements in bioengineering, efforts to develop microalgae strains with high carbohydrate and lipid content are increasing. The rapid progress in genetics has made various transformation techniques possible, and experiments show that genetic tools can be applied in various processes (i.e., lipid production, pretreatment, hydrolysis, and fermentation) creating candidates superior for biofuel production (Olabi et al. 2022).

Novel approaches are emerging with the coupling of two or more industries (including petroleum biorefineries), facilitating a circular bioeconomy. Industrial wastewater can be remediated using microalgae, while biofuel production from wastewater-grown microalgal biomass can be attained (Fazal et al. 2021; Fal et al. 2022). Industries releasing higher amounts of CO_2 can be integrated with microalgae cultivation ponds to enhance their growth and yield (Hariz et al. 2019; Molazadeh et al. 2019). The maximum utilization of biofuel by-products also makes this attempt more cost-effective (e.g., the formation of glycerol in biodiesel production as a by-product can be converted into a wide range of high value-added chemicals; bioplastic production from forestry residues via biotransformation) (Luque et al. 2008). Global collaboration is crucial for successfully incorporating biofuels into the existing energy system. Such efforts will also contribute to advancements in cultivation methods and technological innovations in biofuel production, as knowledge gained through practice can be shared (Malik et al. 2024).

9 Conclusion

From traditional biofuel sources to cutting-edge advancements, such as microalgal biodiesel and genetically engineered biofuels, the landscape of biofuel production is continually evolving to address environmental resilience and enhance energy security. Microalgal biodiesel emerges as a particularly exciting prospect, offering high efficiency and minimal environmental impact. By harnessing the power of genetic engineering, researchers are pushing the boundaries of biofuel production enhancing yields and tailoring biofuels to meet specific industrial needs. Moreover, the integration of biofuels into aviation presents a promising avenue for reducing carbon emissions and fostering greener air travel. However, as we navigate the transition toward

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a biofuel-driven future, it is essential to assess the political, social and economic implications of biofuel production. Biofuel LCAs provide invaluable insights into the sustainability of biofuel production processes, guiding decision-makers toward more environmentally friendly practices. Furthermore, a closer examination of global biofuel production trends reveals a shifting landscape, with increasing emphasis on renewable substitutes. This global transition highlights the growing recognition of biofuels as integral components of a sustainable energy portfolio and it is evident that biofuels hold immense promise in addressing the dual challenges of climate change and energy security. At present, most of the developed countries like the USA, Brazil, and the European Union benefit from the biofuels in transportation sector including aviation. Governments and policy-makers should support and implement rules and regulations encouraging researchers and stakeholders to innovate and collaborate across various disciplines to unlock the full potential of biofuels overcoming the key hurdles in biofuel production and paving the way toward a cleaner, greener, and more sustainable energy future.

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Chapter 7

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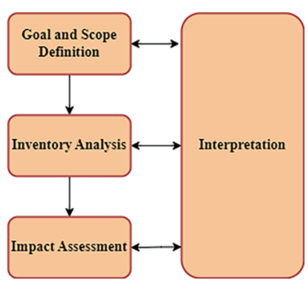
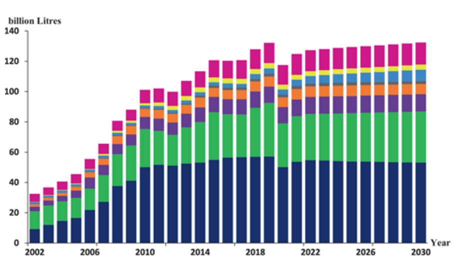
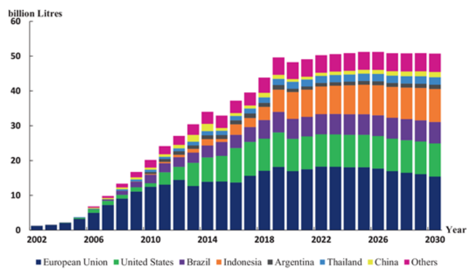
Alternative Texts for Your Images, Please Check and Correct them if Required

Page no	Fig/Photo	Thumbnail	Alt-text Description
8	Fig1		Flow chart illustrating methods of microalgae harvesting. The central node is "Microalgae Harvesting," branching into five main methods: Centrifugation, Electrolysis, Flotation, Gravity Sedimentation, and Filtration/Screening. Electrolysis leads to Electrolytic Coagulation and Electrolytic Flocculation. Flotation branches into Dissolved Air Flotation, Dispersed Air Flotation, Ozone Flotation, and Electrolytic Flotation. Filtration/Screening connects to Membrane Filtration. Flocculation branches into Auto-flocculation, Chemical Flocculation, and Bio-flocculation.
17	Fig2		Flow chart illustrating a process with three main steps: "Goal and Scope Definition," "Inventory Analysis," and "Impact Assessment," arranged vertically. Each step is connected by arrows, indicating sequence. A larger box labeled "Interpretation" is connected to each step with bidirectional arrows, suggesting ongoing analysis and feedback. The chart is designed to represent a systematic approach to a process.
22	Fig3		Stacked bar chart showing ethanol production from 2002 to 2030 in billion liters. The chart includes data for the United States, Brazil, China, European Union, Thailand, India, Canada, and others. The United States and Brazil are the largest contributors, with production increasing over

Page no	Fig/Photo	Thumbnail	Alt-text Description
			time. The x-axis represents years, and the y-axis represents production in billion liters.
22	Fig4		Stacked bar chart showing biodiesel production from 2002 to 2030 in billion liters. The chart includes data for the European Union, United States, Brazil, Indonesia, Argentina, Thailand, China, and others. Production increases steadily from 2002, peaking around 2022, with projections remaining high through 2030. Each region is represented by a different color, with the European Union and United States having significant contributions.