The American Journal of Applied Sciences
ISSN 2689-0992 Volume 08 - 2026

Proceedings of Women in Academia, Research and Management for Work-life Initiatives for Sustainable Health & Empowering Safety (WARM-WISHES 2026)

Alternative Biofuels as Sustainable Replacements for Fossil Fuels: A Comprehensive Review

Saurabh Singh

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India

Srishti Singh

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India

Kritik Rai

Department of Zoology, Jagatpur P.G. College, Varanasi- 221302, UP, India

Garima Awasthi

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India

Received: 18 Apr 2026 | Received Revised Version: 30 Apr 2026 | Accepted: 11 May 2026 | Published: 13 May 2026

Volume 08 Issue 05 2026 | DOI: 10.37547/tajas/warm-07

Abstract

We are witnessing a paradigm shift in the world energy sector as the unsustainability of the fossil fuel era is becoming more evident. As the world's crude oil reserves are likely to become depleted between 10-20 years from now and the dire environmental impacts of greenhouse gas (GHG) emissions continue to grow, the need for sustainable and renewable energy sources is more critical than ever for environmental and economic sustainability. Biofuels, produced from a range of biological feedstocks, hold the key to a carbon-neutral bio-economy. Our review examines the development of biofuel technologies from first-generation feedstocks (food crops) to fourth-generation genetically engineered and carbon-negative biofuels. We find that although first-generation biofuels (i.e., ethanol from corn, biodiesel from rapeseed) are currently leading the market, they are limited by the "food versus fuel" conundrum and land-use footprint. Second-generation lignocellulosic biofuels and third-generation algal biofuels offer better sustainability but are limited by cost and technical challenges. Fourth-generation fuels, incorporating carbon-capture and metabolic engineering, are the pinnacle of sustainable fuel production but are still in the early stages of development. This report identifies that "drop-in" fuels compatible with existing technologies, waste-to-energy systems (e.g., waste cooking oil), and integrated biorefineries are critical. Notwithstanding progress in technology, global-scale biofuel integration is hampered by resource constraints, regulatory complexities and market dynamics. This report concludes that a sustainable energy future will be achieved through a complex approach including advanced conversion technologies (e.g., consolidated bioprocessing), enabling policy settings (e.g., carbon taxes), and localised production to promote local economic development and environmental sustainability.

Keywords: biofuel, biodiesel, bioeconomy, lignocellulosic, sustainable, environmental, GHG

Cite This Article: Saurabh Singh, Srishti Singh, Kritik Rai, & Garima Awasthi. (2026). Alternative Biofuels as Sustainable Replacements for Fossil Fuels: A Comprehensive Review. The American Journal of Applied Sciences, 64–74. Retrieved from https://theamericanjournals.com/index.php/tajas/article/view/7906

Graphical Abstract

1. Introduction

The fossil fuel empire, comprised of coal, oil and natural gas, currently meets 80% of the world's primary energy needs (Kumar & Verma, 2024; Maina et al., 2013; Moodley, 2021). But this dependency has resulted in a two-fold energy and environmental security crisis. Fossil fuels are non-renewable and highly concentrated geopolitically, which has resulted in geopolitical disputes and price fluctuations (Sharma et al., 2023; El-Araby, 2024). Recent forecasts estimate that low-hanging fruit crude oil reserves may reach "peak oil" within 10-20 years, resulting in a dire shortage of oil and the potential implosion of energy-intensive societies (Röttig et al., 2010).

Meanwhile, fossil fuel burning is the main source of anthropogenic global warming. The emission of carbon dioxide (CO_2) and other greenhouse gases (GHGs) has already caused devastating environmental consequences, including unprecedented global warming, extreme droughts and extreme weather patterns, such as Hurricane Sandy. The longevity of CO_2 in the atmosphere means that global warming will continue to accelerate for hundreds of years unless substantial decarbonization measures are taken (Latin, 2014).

In this regard, biofuels play a crucial role. Biofuels are produced from biological feedstocks, making them renewable, inexhaustible and theoretically carbon-neutral since CO_2 emissions generated from combustion are balanced by CO_2 absorption during the feedstock's growth (Sharma et al., 2023; Huang et al., 2021). They are "drop-in" fuels, meaning they can be used to replace conventional fuels (gasoline and diesel) to a greater or lesser degree in existing engine and infrastructure systems (Luján et al., 2009; Feser and Gupta, 2021). In this review, we examine the technological, environmental and economic aspects of biofuels as an alternative to fossil fuels.

2. Evolution of Biofuel Generations

Biofuels are categorized into four generations depending on the type of feedstock as well as the complexity of the production process (Moodley, 2021; Huang et al., 2021; Dutta et al., 2014). Figure 1 depicts the four generations of biofuel production, as well as the constraints of each generation. These problems have spurred the continued research and development of the next generation of biofuels in a bid to address the increased demand of sustainable biofuels.

2.1 First-Generation: Edible Feedstocks

First-generation (1G) biofuels are derived from food crops. They include bioethanol from corn starch (mainly in the US) and sugarcane (Brazil), and biodiesel from edible oils such as rapeseed, soybean and palm oil (Kumar and Verma, 2024; Solomon, 2010; Salim et al., 2018). Bioethanol typically obtained by fermentation of carbohydrate crops such as sugar-based feedstocks also include sugar beet and starch-based feedstocks include maize and wheat. The conversion of starch to sugars (glucose) and fermentation process is carried out using yeasts (*Saccharomyces cerevisiae*) and further purification and distillation is done to obtain ethanol fuel. Bioethanol is often mixed with petrol (e.g. E10, E20) to decrease greenhouse gas emissions and to enhance combustion. The biodiesel is made from edible vegetable oils such as soybean oil, rapeseed oil, palm oil and many more. The conversion of oil into biodiesel is done by transesterification reaction, in which the triglycerides react with alcohol (usually methanol) in the presence of a catalyst (acid, alkaline or lipase) and produce fatty acid methyl esters (FAME) and a byproduct, glycerol (Naik et al., 2010).

First-generation fuels are technologically well developed and commercially established but they are not sustainable. The food versus fuel problem is the key one, whereby land utilization in the production of biofuels can lead to increased food prices, and food insecurity (Moodley, 2021; Latin, 2014; Solomon, 2010). First generation (1G) biofuels are produced using edible biomass including traditional crops which contain sugars, starch and oils. They are the oldest type of biofuels and are the most widespread type of biofuel used in the world due to the maturity of the technology and infrastructure.

Advantages of First-Generation Biofuels

The first-generation biofuels were having few advantages such as the production technologies and supply chains for the first-generation biofuel was well-developed. They were used at industrial level all over the world. They reduce the dependence on fossil fuels and reduced emissions of carbon monoxide and particulate compared to other fuels.

Though 1G biofuels were having benefits, still it was having some limitations and sustainability concerns such as “Food vs Fuel Conflict”, means the competition with food was the major problem. Biofuels produced from food crops, means increased food prices, reduced food availability, socio-economic inequalities in developing nations, land use and deforestation. The land-use for biofuel crops can result in deforestation, people will start growing only fuel-based crops such as palm oil, loss of biodiversity, soil degradation, water and fertilizer demand will increase. This all will lead to increases environmental pollution and resource depletion. Biofuels are renewable but the total greenhouse gas emissions (from growing, harvesting, processing and transport) may limit their positive impact. Certain 1G biofuels (particularly corn ethanol) have low energy return on energy invested, implying that significant energy is required to produce biofuels (Kumar and Verma, 2024; Latin, 2014; Solomon, 2010).

2.2 Second-Generation: Lignocellulosic Biomass

Second-generation (2G) biofuels use non-food lignocellulosic feedstocks such as crop residues (wheat straw, corn stover, bagasse), forest residues and energy crops (switchgrass) (Solomon, 2010; Zinoviev et al., 2010; Pompelli et al., 2025). 2G fuels do not compete with food production as they use residues and wastes (Moodley, 2021; Ganguly et al., 2021). But the structure of lignocellulose (recalcitrance) means that it needs to be pre-treated and hydrolysed by enzymes to release sugars that can be fermented, which makes it currently more expensive to produce (Moodley, 2021; Pompelli et al., 2025; Adewumi and Agbaghare 2025).

2.3 Third-Generation: Microalgae and Aquatic Biomass

3G biofuels are produced from microalgae and aquatic biomass (Maliha and Abu-Hijleh, 2023; Bhagea et al., 2019). Algae are seen as an attractive feedstock given their high photosynthetic efficiency, ability to grow in a variety of places (including wastewater and seawater), and lack of land-use. Algae have the potential to provide more energy per unit land area than other plants. However, 3G technology is limited by the difficulties of scaling up production, efficient harvesting and the high cost of extraction (Sadatshojaei et al., 2020; Patouillard and Collet, 2016; Aitken and Antizar-Ladislao, 2013; Muthuraman & Kasianantham, 2023).

2.4 Fourth-Generation: Genetically Engineered Systems

Fourth-generation (4G) biofuels are distinguished using genetically engineered systems to directly produce sustainable fuels from various carbon sources such as CO_2, with improved efficiency and lower environmental footprint. Fourth-generation (4G) biofuels aim to improve the efficiency of feedstock conversion and efficiency using advanced genetic engineering and synthetic biology techniques (Huang et al., 2021; Dutta et al., 2014). This includes creating "designer" microbial strains with improved characteristics, such as higher lipid content and direct secretion of biofuels, which simplifies recovery. Advancements in synthetic biology and metabolic engineering have facilitated the fine-tuning of microbial pathways, resulting in improved synthesis of lipids, alcohols and hydrocarbon fuels. This all adds up to more sustainable and cost-effective biofuel production (Röttig et al., 2010; John et al., 2025). Genetically engineered *Escherichia coli* and *Saccharomyces cerevisiae* strains have been developed to enhance the production of ethanol, butanol and precursors for biodiesel via pathway engineering and gene overexpression. The use of CRISPR-Cas9 has expedited genome engineering to insert new biosynthetic pathways and knock out competing pathways. Additionally, 4G fuels are often coupled with carbon capture and sequestration (CCS) to achieve a carbon-negative system, in which fuel synthesis consumes CO_2 (Moodley, 2021; Huang et al., 2021; John et al., 2025). Photosynthetic organisms like *Chlamydomonas reinhardtii* and cyanobacteria are modified to use sunlight and CO_2 directly for biofuel production, helping achieve carbon-neutral energy production. Efforts also aim to improve carbon fixation and re-channel metabolic fluxes for biofuel production. Synthetic gene circuits enable dynamic control of metabolic processes in response to environmental changes. However, issues including low yield, scalability and genetic stability remain. Biosafety and regulatory issues need to be considered for commercialization. In conclusion, fourth-generation biofuels are an innovative approach that combines genetic engineering and renewable energy technologies for sustainable fuel production (Liao et al., 2016; Ducat et al., 2011).

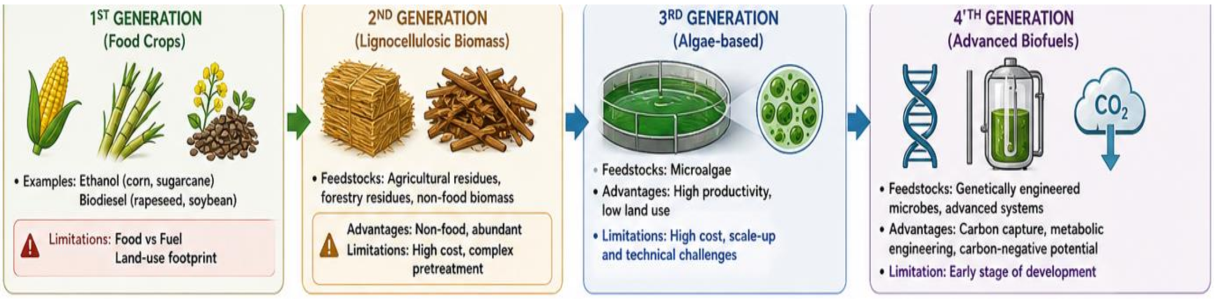

Figure 1: Evolution of Biofuel Technologies

3. Feedstocks and Resource Assessment

Resource assessment and sustainability of biofuel production are inexorably linked to the feedstock employed. Figure 2 illustrate the various feedstock, conversion technologies and outcome impact during the biofuel production.

3.1 Agricultural Residues and Waste

Crop residues, like corn stover, wheat straw and rice bran, are a vast, underutilised energy source for biofuel production (Moodley, 2021; Sajid et al., 2025; Laguado-Ramírez et al., 2024). This approach offers the opportunity to generate renewable energy and waste management (Dubey and Avinashe, 2024). Studies have demonstrated the use of residues can result in improved Energy Return on Investment (EROI) over 1G feedstocks (Salim et al., 2018).

3.2 Waste Cooking Oil (WCO)

Waste cooking oil (WCO) is a growing feedstock for biodiesel. It solves two issues: it eliminates environmental pollution caused by improper disposal, and offers a cheap, non-edible feedstock. WCO biodiesel has been reported to have lower GHG emissions and improved lubricity compared to diesel (Salim et al., 2018; Dubey and Avinashe, 2024).

3.3 Energy cane and sugarcane

Sugarcane is widely used for biofuel production in Brazil. Juice is used for 1G ethanol while bagasse and straw are gaining popularity for 2G ethanol and bioelectricity co-generation (Pompelli et al., 2025; de Paula and de Araújo, 2009). "Energy cane", a specific type of sugarcane with high biomass and fibre content is currently gaining importance as a key bridging crop with potential yields of up to 180 tons of biomass per hectare (Pompelli et al., 2025).

4. Advanced Conversion and Production Technologies

Advanced conversion and production technologies are playing a major role in improving the efficiency and sustainability of biofuel generation. Modern approaches such as enzymatic conversion, thermochemical processing, and microbial engineering help achieve higher fuel yields with lower energy consumption. These technologies also support the utilization of diverse waste materials and non-food biomass as feedstocks. As a result, biofuel production is becoming more cost-effective, environmentally friendly, and suitable for large-scale industrial applications. Biomass conversion efficiency is crucial for cost-effectiveness.

4.1 Biochemical and Thermochemical Processes

Biochemical (fermentation, anaerobic digestion) and thermochemical (pyrolysis, gasification, hydrodeoxygenation) are the two primary pathways used for biofuel production (Aitken and Antizar-Ladislao, 2013; El-Desouky et al., 2022). Biochemical pathways are widely used in the production of ethanol and biogas, but thermochemical pathways (such as hydrodeoxygenation) are emerging to produce "renewable diesel" that is chemically identical to fossil diesel (Coumans, 2017; Luna, 2019).

4.2 Advanced Pretreatment and Fermentation

To improve the accessibility of the recalcitrant 2G biomass, innovative pretreatment technologies such as organosolv fractionation, surfactant-assisted hydrolysis, mechano catalysis are under development (Pompelli et al., 2025; Adewumi and Agbaghare, 2025). In fermentation, the trend is towards integrated processes such as Simultaneous Saccharification and Fermentation (SSF) and Consolidated Bioprocessing (CBP) to minimize processing steps and costs. Simultaneous Saccharification and Co-Fermentation (SSCF) enable the co-fermentation of hexose and pentose sugars (Adewumi and Agbaghare, 2025).

4.3 Bioinformatics to design high yielding strain

The various researchers are utilizing bioinformatics tool and techniques to develop and design genetically engineered microorganisms that would be promising source to enhance the biofuel yield ultimately (Awasthi, 2026). The algae have gained significant attention as a 3rd generation renewable source of biodiesel, leading to efforts to enhance its oil production efficiency. A hybrid open reading frame (ORF) in this study was composed of the conserved regions of six lipid-related algal genes which belong to various superfamilies. The stability and accuracy of the designed protein was validated by structural modeling and validation, and the pathway analysis supported the role of the designed protein on lipid biosynthesis. Such results suggest that the designed ORF has the potential of enhancing the production of oil in algae. Also, the further *in silico* analysis assists in the experimental designing and validation (Nigam et al., 2020).

4.4 AI, IoT and Nanotechnology.

Recent developments in the biofuel industry are more and more based on nanotechnology, artificial intelligence (AI), machine learning (ML), and the Internet of Things (IoT) to increase the efficiency and yield of the processes. The biofuel industry is one of the latest industries to have the latest technologies applied to enhance efficiency. Nanotechnology is used to design superior transesterification catalysts as well as to augment microbial bioengineering. The catalysts based on nanotechnology, including metal oxide nanoparticles (e.g., ZnO, TiO2, and CaO) are able to offer higher surface area and catalysis, creating higher efficiencies in the transesterification process, and enabling biodiesel yields of over 9098% under optimal conditions. Nanomaterials are also used to enhance stability of enzymes and their rate of conversion with the substrates in microbial bioengineering (El-Desouky et al., 2022; Vijaya et al., 2024). Artificial Intelligence (AI), Machine Learning (ML) and Internet-of-Things (IoT) are being combined in order to track and optimise the process parameters as well as to maximise yields and resource efficiency. Complex bioprocesses are being modeled using AI and ML, and parameters of reaction optimization as well as yield outcomes are being predicted with great accuracy, with it often reducing the time and cost of an experiment by up to 3040. The IoT-enabled sensors will enable real-time monitoring of the changes of variables like temperature, pH, and composition of feedstock, making it possible to have automated control mechanisms that will improve the stability of processes and resource utilization (Huang et al., 2021). Together, these technologies are transforming biofuel production into a more efficient, data-driven, and sustainable process.

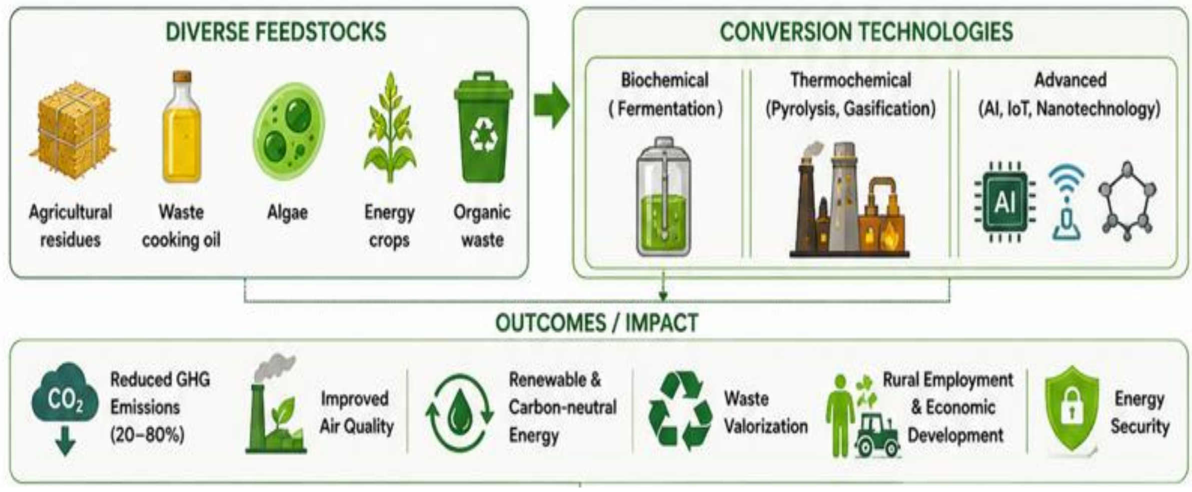

Figure 2: Interaction of feedstocks, conversion technologies and its outcome impact

5. Environmental and Sustainability Analysis

Environmental benefits are the main rationale for biofuels. Figure 3 illustrate the challenges and the pathways to an environmental and sustainable energy for the coming up future.

5.1 Greenhouse Gas Emissions and Air Quality

Biofuels can significantly reduce GHG emissions, with many studies showing 20% to 80% reduction over petroleum diesel (El-Araby, 2024; Rouhany and Montgomery, 2018). Soybean biodiesel emissions can be as low as 136 g CO_2/bhp-h compared to 633 g CO_2/bhp-h for petroleum diesel (de Paula and de Araújo, 2009). Biofuels may also reduce local air pollution; for instance biodiesel blends greatly lower emissions of sulfur dioxide (SO_2) and particulate matter (PM10) from diesel engines (Maina et al., 2013; Oloyede et al., 2024).

5.2 Life Cycle Assessment (LCA) and EROI

Sustainability needs to be assessed through a Life Cycle Assessment (LCA) from "well-to-wheel" (Patouillard and Collet, 2016; Niculescu et al., 2019; Nanaki and Koroneos, 2012). Energy Return on Investment (EROI) is an important factor as studies suggest higher EROI values when utilising by-products or waste compared to 1G feedstocks (Salim et al., 2018). Multidimensional approaches such as "Biodiesel-TBL+" offer hierarchical sustainability analyses in environmental, economic and social aspects (Bautista et al., 2016).

5.3 Land Use and Biodiversity

Large-scale biofuel production can have adverse effects such as deforestation and loss of biodiversity, if not properly managed (Latin, 2014). Sustainable land-use practices and the use of non-productive land (in the case of algal biofuel) are important to avoid adverse impacts on ecosystems (El-Araby, 2024; Solomon, 2010).

6. Policy and Economic Considerations

Biofuels are no less an economic than technological challenge. Government policies, such as subsidies, tax incentives, and blending mandates are important in encouraging biofuel production and adoption. Large-scale viability can often be determined by the cost of the feedstock, technologies employed in the processing of these feedstocks, and the demand in the market. Effective policy frameworks coupled with investment in innovation could assist in making biofuels more competitive as compared to conventional fossil fuels.

6.1 Market Study and World Production.

The production of bisodiesel has increased by 18.1 billion liters in 2010, compared to 3.9 billion liters in the same year and is projected to more than 41 billion liters in 2025. Nevertheless, it is also very sensitive to any changes in prices of crude oil that may also lower its economic competitiveness unless supported by policy (Rouhany and Montgomery, 2018). Moreover, the expensive prices of feedstock and production bottlenecks all remain factors in shaping economics of production and scalability. Government incentives and blending of mandates and carbon reduction policies often balance out the cost disadvantages. As such, a trade-off between technological development, cost cutting mechanisms and conducive regulatory frameworks will be necessary to sustain growth in the biodiesel industry.

6.2 Carbon Taxes and Blending Mandates

Biofuel consumption is mainly driven by government policies. Blending requirements (e.g., B20, E10) guarantee demand at all price levels (Rouhany and Montgomery, 2018). Carbon taxes also work well, by increasing the cost of fossil fuels and encouraging the transition to renewable biofuels (Zhang and Huang, 2024).

6.3 Social Benefits: Rural Growth and Employment

Biofuel cultivation and production provide substantial economic benefits to rural areas through jobs in farming, transport and processing (El-Araby, 2024; Dubey and Avinashe, 2024; Oloyede et al., 2024). This can rejuvenate the agricultural sector and minimise foreign energy imports (Maina et al., 2013; El-Araby, 2024; Huang et al., 2021).

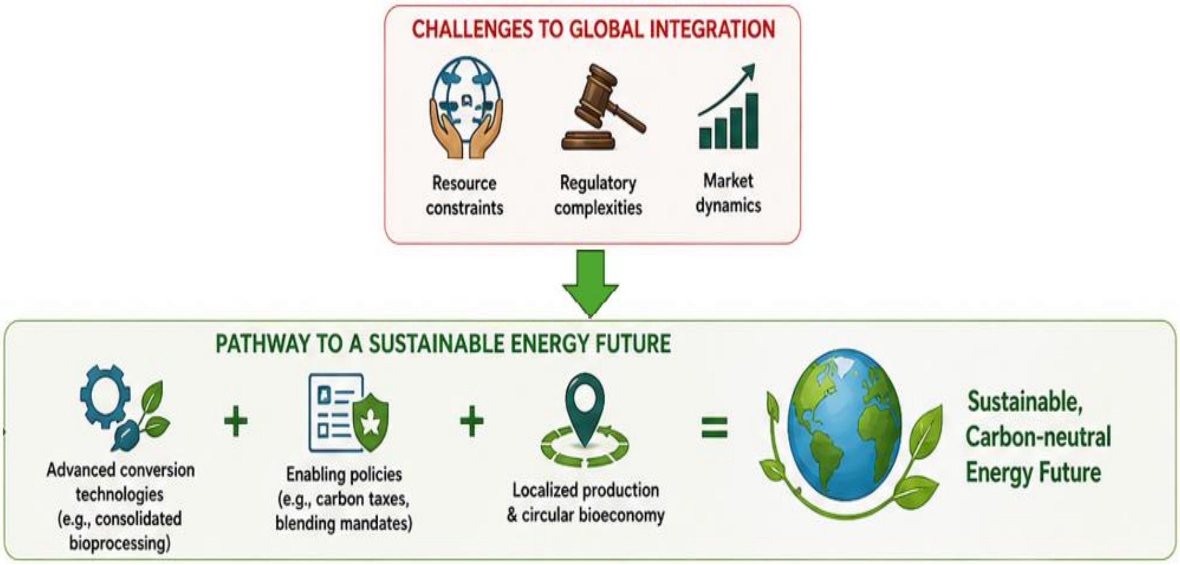

Figure 3: Challenges and the Pathways to a sustainable energy future

7. Applications and Performance

Biofuels are widely used in transportation, power generation, and industrial applications due to their renewable nature and lower environmental impact. Their performance depends on factors such as fuel composition, engine compatibility, and combustion efficiency. Continuous improvements in production technologies have enhanced fuel quality, making biofuels a more reliable and sustainable alternative to conventional fossil fuels. Biofuel are increasingly being targeted for specific applications.

7.1 Transportation

Aviation is a prime candidate for "drop-in" biofuels (bio-jet fuel) such as HEFA-SPK and Farnesane that have demonstrated stable burning and reduced emissions in laboratory-scale gas turbine engines (Feser and Gupta, 2021). Likewise, bioethanol and biodiesel blends are under study for replacement of marine fuel oil to reduce emissions from the shipping sector (Kim and Lee, 2025).

7.2 Compression Ignition Engines and Performance

Detailed studies on compression ignition (CI) engines have demonstrated that biodiesel blends (e.g., B20) can have the same engine performance as petroleum diesel and significantly lower harmful emissions (Maina et al., 2013; Luján et al., 2009; Niculescu et al., 2019). The addition of alcohols (methanol, ethanol) to diesel-biodiesel can enhance combustion and emissions (Niculescu et al., 2019).

8. Obstacles, Limits and Research Needs

There are some major challenges such as the food vs. fuel debate continues to be a key policy issue for 1G biofuels (Latin, 2014; Solomon, 2010). The recalcitrance and production costs of 2G and 3G are currently high and prohibit commercialization (Moodley, 2021; Pompelli et al., 2025). Scaling up from lab/pilot to industrial scale is technically difficult (Sadatshojaei et al., 2020; Aitken and Antizar-Ladislao, 2013). The managing supply of a variety of biomass feedstocks is also a complex task (Blay-Roger et al., 2024). Ultimately more researchers are required to meet the need of the cost effective, environment friendly and sustainable biofuel for as a future energy.

9. Conclusion

The transition from a fossil fuel–dependent energy system to a sustainable and resilient bio-based economy is no longer optional but imperative. This review points out that although the first-generation biofuels have been very important in laying the foundation of the biofuel industry, its long-term viability is limited by food security issues and environmental areas of trade-offs. Second- and third-generation biofuels, on the contrary, because of the use of lignocellulosic biomass and microalgae, they provide better sustainability; nevertheless, economic and technological challenges still undermine their wide commercialization, in particular, in the factors of pretreatment, Conversion efficiency, and scalability.

The fourth-generation biofuels are considered a breakthrough technology, where genetic engineering, synthetic biology, and carbon capture strategies combine to generate a new form of fuel production that not only increases productivity, but also promises to result in carbon-negative fuel systems. Although those technologies promise a lot, they are still in their early stages of development and still need to be investigated to address such challenges as optimization of the yield, genetic stability, biosafety, regulatory approval.

The review highlights the need to shift towards a holistic approach that integrates several kinds of different feedstocks like agricultural residues and waste oils, into an advanced conversion technology including consolidated bioprocessing, and development of integrated biorefineries. Supportive policy frameworks to support market competitiveness and mass adoption such as carbon pricing, blending mandates, and incentives are also vital.

Finally, the future of sustainable energy is in the synergistic approach of utilizing technological innovation, resource efficiency and policy support. As it continues to evolve and become strategically implemented, biofuels have the capability to greatly cut down greenhouse gases, increase energy security, and socio-economically develop of the world, thus, becoming the first step to a future of a carbon-neutral and sustainable global energy supply.

Author Declaration Statements

Declaration: The authors hereby declare that the manuscript submitted for consideration is an

original work and has not been published or submitted elsewhere for publication. The authors

take full responsibility for the integrity, accuracy, and ethical compliance of the work presented

in the manuscript.

Conflict of Interest: All authors confirm that:

Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. - Not Applicable

All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged. – Not Applicable

Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable. – Not Applicable

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