

Review

Production Efficiency of Advanced Liquid Biofuels: Prospects and Challenges

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Abstract: Renewable sources are becoming more critical in light of global warming and the recent energy crisis. As a renewable energy source, biofuels may play an essential role in this process, especially in the transport sector. Advanced biofuels provide a great opportunity, as their potential feedstocks do not compete with food production. Based on a systematic literature review, this study aims to provide a comprehensive overview of the prospects and challenges of advanced liquid biofuels. Out of the identified 508 articles, 188 were abstract-screened, providing 67 articles for in-depth screening. Finally, 57 articles were reviewed. Although advanced biofuels are not yet economically viable, it is evident that every step of the production process can be optimized. Moreover, technological advancements, such as the use of novel catalysts and co-catalysts, nanotechnology, and genetic and metabolic engineering, offer great opportunities for enhanced production efficiency, which is key for their production to be profitable.

Keywords: feedstock; catalyst; genetic engineering; metabolic engineering; nanotechnology



Academic Editors: Wojciech Czekala and Jakub Frankowski

Received: 23 January 2025

Revised: 11 February 2025

Accepted: 18 February 2025

Published: 19 February 2025

Citation: Mizik, T.; Igbeghe, C.B.; Deák, Z. Production Efficiency of Advanced Liquid Biofuels: Prospects and Challenges. *Energies* **2025**, *18*, 1008. <https://doi.org/10.3390/en18041008>

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1. Introduction

Renewable sources are becoming more critical in light of global warming and the recent energy crisis. Fossil energy resources are scarce, and their use significantly contributes to global warming through the emission of different greenhouse gases, particularly CO₂. As a renewable energy source, biofuels may play an essential role in this process, especially in the transport sector. Road biofuel demand is expected to increase in the next couple of years, mainly due to higher blending rates and the slower uptake of electric vehicles (EV) in certain countries, especially in Brazil, India, and Indonesia [1]. Additionally, hard-to-abate (HtA) transport sectors that are heavily dependent on fossil fuels, such as aviation and maritime shipping, are lagging behind in decarbonization, and given current trends, emissions from these sectors could increase by more than 50% by mid-century [2]

First-generation or conventional biofuels are often criticized for the food vs. fuel contradiction [3–5], and they will not be analyzed in this study. Advanced biofuels provide a great opportunity, as their potential feedstocks do not compete with food production; therefore, they have a crucial role in decarbonizing the transport sector. It can be distinguished between three generations of advanced biofuels, namely lignocellulosic, algae-based, and genetically modified algae-based biofuels; however, genetically engineered algae-based production could be called the fifth generation [4]. Another promising, fourth-generation

feedstock is the syngas. The fundamental benefit of producing synthetic fuel out of it is the use of existing CO₂ in the natural environment [6].

There are two main types of liquid biofuels: ethanol (additionally biobutanol and biomethanol) and biodiesel. Both are renewable and provide better environmental performance than their fossil counterparts, as mainly the previously absorbed CO₂ is emitted during their burning. Their other common characteristic is that they are direct substitutes for fossil fuels; however, biodiesel blending rates can be higher without the need for significant modification of the vehicles. The share of biocomponent is indicated by a combination of a letter (“E” stands for ethanol and “B” stands for biodiesel) and a percentage number showing the maximum volume share of the biofuel.

Production efficiency is closely related to the economic performance of the biofuels. It seems that their large-scale production is still an ongoing issue; however, the immaturity of the advanced biofuel industry provides many opportunities for technological advancements that can improve economic performance [7]. This can be realized along the entire production chain starting from feedstock selection/production to every technical aspect of production. Challenges due to scalability, feedstock availability, and economic viability in biofuel production have fueled the creation of new generations of biofuels with enhanced technologies [8–10]. From this aspect, extensive research and development, as well as knowledge transfer/sharing are of utmost importance [11,12]. Progress is inevitable. For instance, lignin solubilization provided a greener and more efficient pretreatment method [13], while the use of biomass-based heterogeneous catalysts in microalgae-based biodiesel production resulted in significant energy savings and negative CO₂ emissions [14].

This study aims to provide a comprehensive and systematic overview of the current state of advanced liquid biofuels, their prospects, and challenges, with particular attention paid to production efficiency issues. As the focus of the article is on liquid biofuels, other alternative fuels will be excluded. The key novel elements of the review include:

- Analyzing the latest advancements across different generations of advanced biofuels, and
- Identifying and discussing various optimization techniques and technological innovations that can improve the production efficiency of advanced biofuels.

The structure of the article is as follows. The second chapter provides an overview of the advanced biofuel industry, including future projections. The third section presents the article selection method of the systematic literature review. The fourth section contains the results of the literature review based on the in-depth analyzed articles. The last section concludes and provides the limitations of the study and the potential future research paths.

2. Current State and Prospects of Advanced Biofuels

The global biofuel market is dominated by conventional, first-generation production (Figure 1). In terms of volume, the USA, Brazil, the EU, Indonesia, and China are the top producers with an 85% share. The EU is the main producer of advanced biofuels, followed by the USA, China, Brazil, and Indonesia. The share of advanced biofuels is already 31% in the EU, supported by the renewable energy policy aimed at achieving a zero-emission transport sector [15]. It is expected to be further supported by the revision of the Energy Tax Directive that provides tax incentives for advanced biofuels [16]. China has the second-highest share of advanced biofuels (26%), mainly due to the continuous reduction in subsidies for conventional biofuel production [17]. The USA has the third highest value, as 11% of its total production qualifies as advanced biofuel, while Brazil has 7%. Their biofuel economy is driven by renewable energy policies (the USA and Brazil) and national institutions (Brazil), such as the Institute of Sugar and Alcohol [18]. The granted price premium of cellulosic ethanol also helped the commercialization of US production [19].

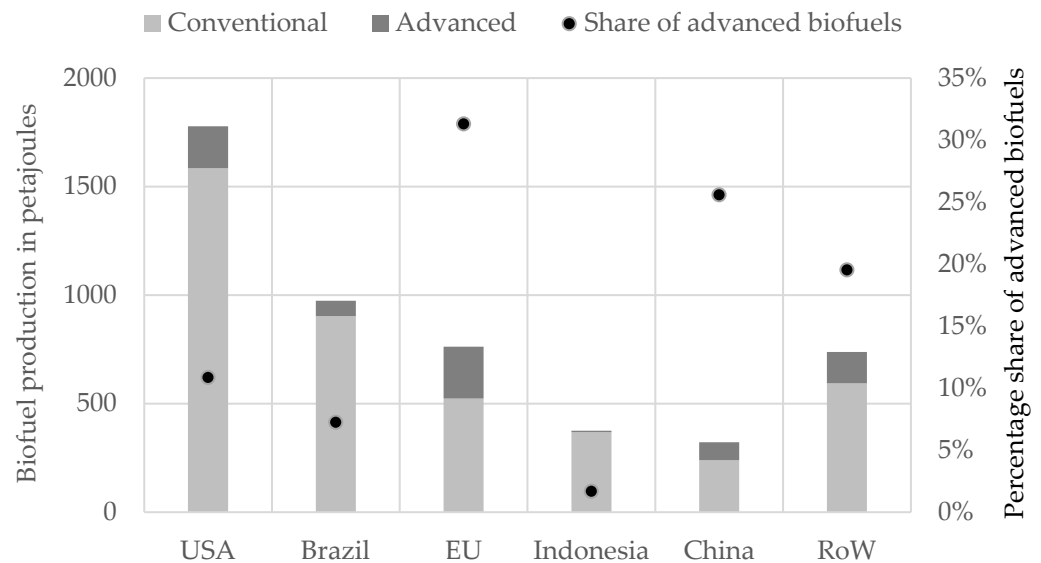


Figure 1. Composition of global biofuel production, 2021–2023. Source: Authors' composition based on [12].

The most significant barrier to advanced biofuel commercialization is the high production cost. Investment costs are high, especially when economy of scale matters, e.g., in the case of thermochemical conversion, while conversion efficiencies are generally low [20]. It makes the unit cost of production higher compared to fossil fuels. As a comparison, the production cost of fossil fuel is EUR 8–14 per gigajoule, biomass feedstock costs EUR 17–44 per gigajoule, and waste feedstock costs EUR 13–29 per gigajoule [21]. Biofuels are typically blended with fossil fuels; therefore, there is no price difference between biofuels and fossil fuels. However, even high-level blends can only be cheaper if they are supported by different policy measures; for example, when the biofuel price can be adjusted to account for its lower energy density. This is the reason why enhanced productivity and knowledge transfer are one of the most critical issues of advanced biofuel production. Commercialization itself may decrease production costs by 10–40%, even in the medium term [21]. Although comparable data for 2024 is not available, we calculated those values by using the OECD Data Explorer [22]. According to our corrected dataset, global biofuel production seems to be increased by 2.6% due to Indonesia (+6.8%), the rest of the world (5%), the USA (4.5%), and Brazil (1.7%), while the EU and China faced some decrease, 3.3% and 2.4%, respectively.

It should be highlighted that energy policies play a vital part in advanced biofuel production due to the price advantage of fossil fuels. Blending mandates encourage production and contribute to R&D in the sector, making production more efficient. A good example was the RED II, which incentivized advanced biofuels by double counting; however, it was terminated only in three years by RED III [15]. Due to the strong European climate-related commitments, the use of renewable energies is particularly important for the member states [23]. The Renewable Fuel Standard plays a similar role in the USA; however, its terminology for “advanced” is different, because it is linked to at least 50% lifecycle GHG reduction [24]. Advanced biofuels, especially third and fourth generations, have larger upfront investment costs compared to even first-generation refineries. Therefore, targeted R&D incentives in every part of the production process are essential for making them more efficient, as well as for positively forming public opinion toward the use of biofuels [25].

According to the OECD/FAO projection, conventional biofuel production is expected to increase by 23% in the next 10 years, while advanced biofuel production may

show an even larger, 40% increase (Figure 2). However, it should be highlighted that installed/nominal capacities are larger than actual production [12,16]. Moreover, there are many uncertainties besides excess capacities, such as policy uncertainties (blending mandate level, tax exemptions, or subsidies), technological advancements, oil prices, or the pace of EV uptake [26].

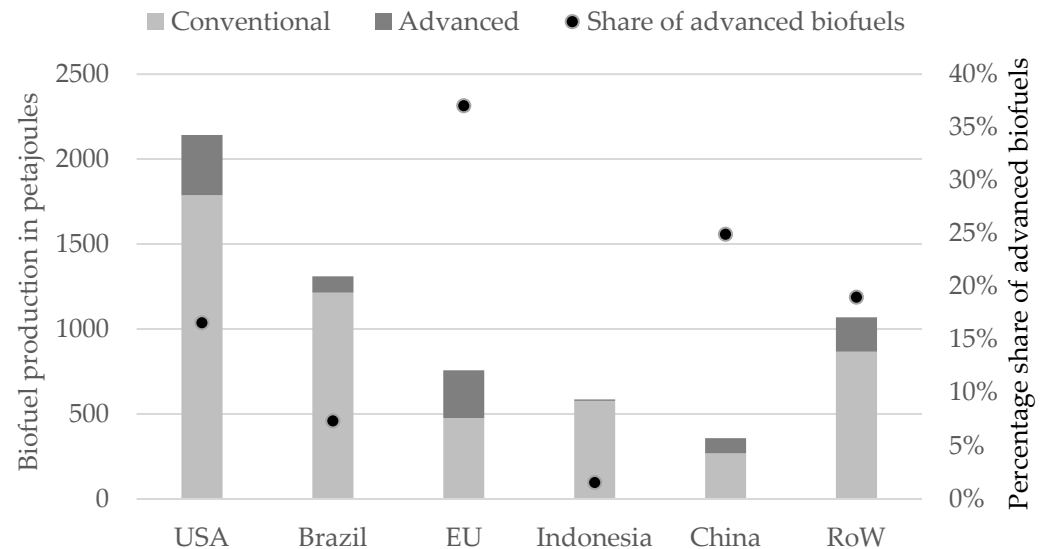


Figure 2. Projected composition of global biofuel production, 2033. Source: Authors' composition based on [12].

The order of the leading producers will likely remain the same; however, their aggregated share will decrease to 83%. The highest overall production growth of conventional biofuels is expected to occur in developing countries, such as Indonesia (42%) and Brazil (36%), while the EU will produce 9% less conventional biofuels. Although the share of advanced biofuel production remains the highest in the EU (37%), it is expected to enormously increase in the USA, by 84%. Based on the OECD/FAO projection, Brazil will be the third largest advanced biofuel producer relegating China to the fourth place.

Cane bagasse (ethanol) and used cooking oil (biodiesel) are expected to be the primary feedstocks in the next decade [27], while production will still be dominated by fatty-acids hydrotreatment (HVO-fuels) applied mainly to used cooking oil [28]. As a co-product of sugarcane-based ethanol production, cane bagasse is a relatively abundant feedstock being about 30% of the sugarcane quantity processed [29]. The use of cane bagasse requires pretreatment. At this moment, hydrothermal pretreatment is the most effective strategy [30]. Used cooking oil is also an abundant, as well as a cost-effective feedstock; however, the lack of a proper collection system and, therefore, an unstable supply can result in production problems [7]. In this case, physical treatment is followed by chemical treatment. At this moment, transesterification provides the best outcome in terms of physicochemical properties, most notably its kinematic viscosity [31].

It is estimated that the advanced biofuel market will grow at 13.9% CAGR from the USD 1.46 billion value in 2024 to 2034 [32]. Another vital expectation is the high relative growth of aviation and maritime fuels, as 75% of new biofuel production will be used in those sectors by 2030 [1]. There are various reasons for this, such as the continuously increasing share of EVs and the higher efficiency of internal combustion engines. It is forecast that EVs will help to displace 12 million barrels per day of oil demand growth for road transport between 2023 and 2035, especially in developing countries [33].

As energy-related policies consider production as a basis for blending mandates, international biofuel trade is expected to decrease in the next couple of years and will

be around 11% and 8% of production for biodiesel and ethanol, respectively [26]. Local production and use further increase the environmental advantages of biofuels.

3. Methodology

As one of the most widely used databases, the Scopus database was used for the article selection process [34]. The keywords of the research were production, efficiency, advanced, and biofuel with the Boolean operator “AND” between them. This process provided the opportunity to find articles that contain these four keywords. It also helped us exclude articles that did not analyze all of them at the screening stage. The article selection process followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) method [35]. The date of the query was 10 January 2025. It covered the title, abstract, and keywords and provided 508 documents for further analysis. The applied limitations were the English language, journal articles, and reviews, and being up to date to provide an overview of the latest results related to advanced biofuels. Therefore, only articles published since 2020 were included. These limitations resulted in 188 items for abstract screening. At this stage, 121 articles were non-relevant. The main reasons for exclusion were no production, such as biofuel use or only its technical details, biomass production in general, and other types of potential biofuels, such as hydrogen, and first-generation production. Out of the 67 in-depth screened items, 57 were included in the systematic literature review (Figure 3).

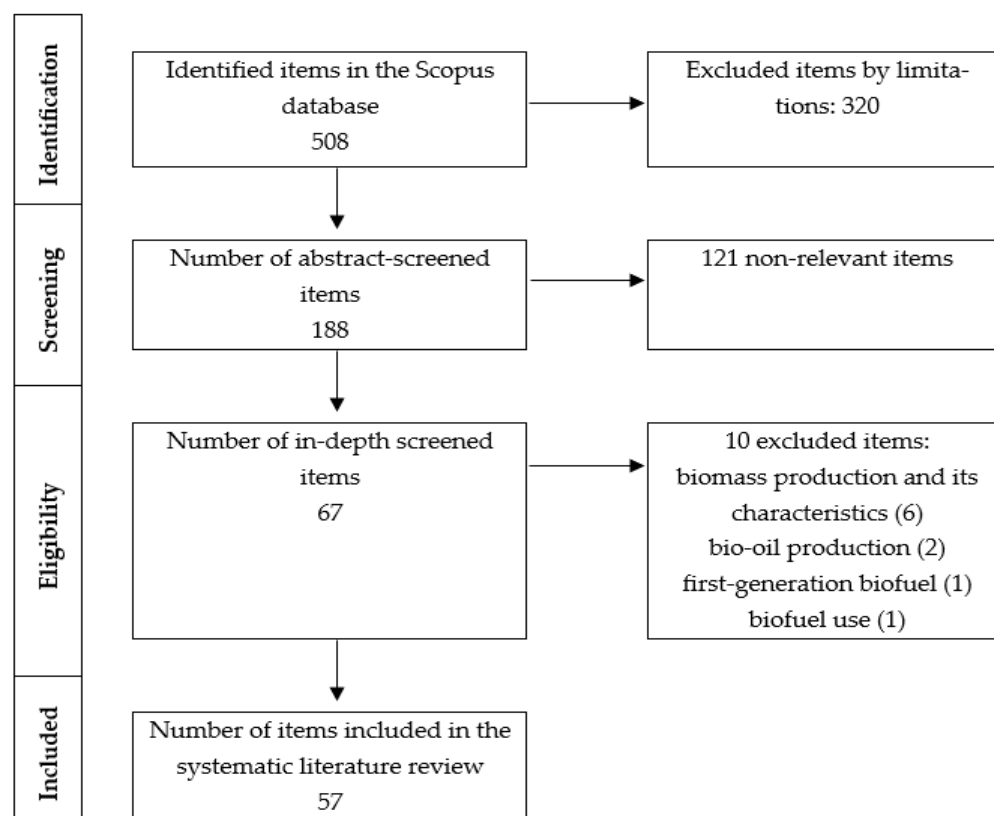


Figure 3. Stages of the literature selection process. Source: Authors’ composition based on the PRISMA method.

The systematically reviewed articles were published in 40 scientific journals. The most frequently used ones were the *Renewable and Sustainable Energy Reviews*, *Fuel*, *Bioresource Technology*, *Energies*, and *Energy Conversion and Management* (Figure 4).

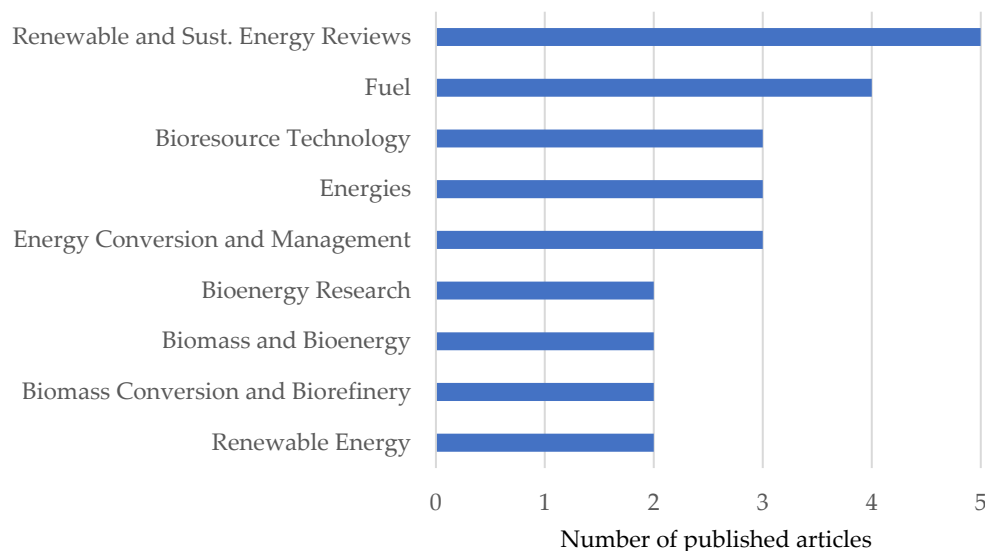


Figure 4. The most frequently used journals for advanced biofuel publications.

Regarding the year of publications, 2022 was the most active year with 18 publications, followed by 2024 (11) and 2023 (9), as shown in Figure 5. Despite the early date of query (10 January), 3 articles were already published in 2025.

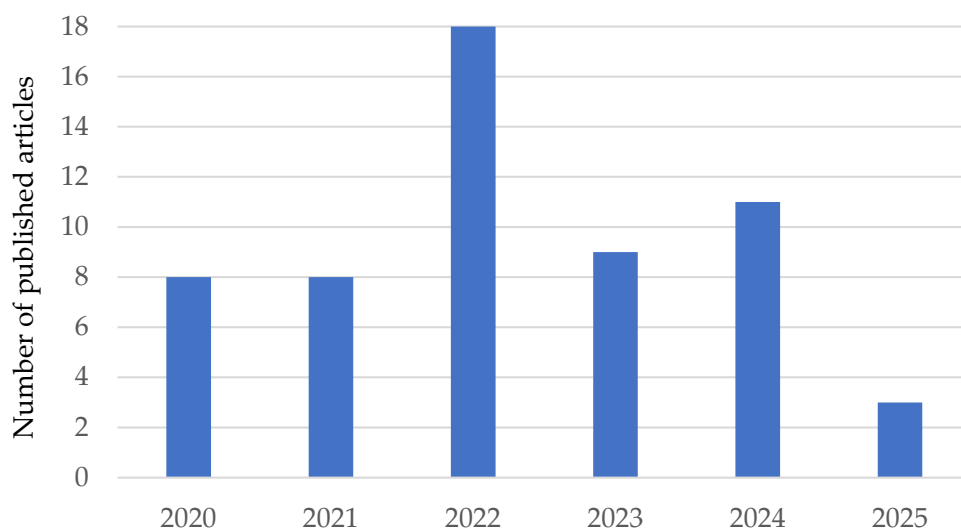


Figure 5. Yearly distribution of the systematically analyzed articles.

4. Results

Biofuel generations refer to the financial viability and scalability of the different fuels. While first-generation production is widespread and cellulosic biofuel production is also commercialized to a certain extent, algae-based biofuel production is still in the experimental phase. The systematically analyzed articles refer to this fact as most of the articles dealt with second-generation biofuels (Figure 6). It also means that 83% of the articles presented results related to second-generation biofuels.

The systematically reviewed articles were classified into three categories, corresponding to the different generations of advanced biofuels.

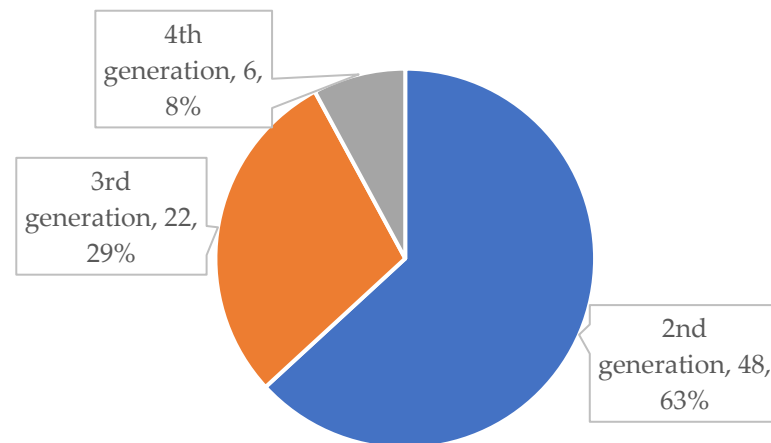


Figure 6. Share of different biofuel generations in the analyzed articles. Note: some of the articles analyzed more than one biofuel generation.

4.1. Second-Generation Biofuels

Diversifying feedstocks and using marginal or degraded lands are of utmost importance because raw materials can be the most significant cost element of biofuel production, especially in the case of biodiesel [36]. For example, it is between 57 and 86% in the case of Hydrotreated Vegetable Oils, while it is only 12–13% for cellulosic ethanol [20]. The average production cost of cellulosic ethanol is estimated to be approximately USD 4 per gallon gasoline equivalent [24], while gasoline prices are normally around USD 3 per gallon [37]. This cost issue is the reason why used/waste cooking oil is one of the best feedstock candidates; however, its collection can be a challenge. Waste-to-fuel technologies are of particular importance [38]. C4 crops (primarily grasses and sedges) perform particularly well with atmospheric CO₂ fixation and lignocellulosic biomass production [39]. The integration of biofuel production into existing industrial infrastructures provides many advantages, such as a wider variety of available feedstocks, the use of combined heat and power plants, leading to a reduction in the net demand for biomass feedstock utilization, and multiple outputs from the same biomass input [40,41]. Synergies with other processes in closed-loop approaches provide further opportunities for optimized technology deployment and enhanced resource recovery [41]. Biomass-to-liquids is a good example of how biochemical and thermochemical processes are integrated [42]. Co-processing, such as petroleum fractions with waste cooking oil, is also a promising option for more efficient production [8]. Integration of hydrothermal liquefaction with existing first-generation ethanol production improves the overall efficiency and economic viability of advanced biofuel production [43]. Combining the production of biofuels and other valuable compounds, such as isobutanol, isobutene, or hydrogen, can also increase the overall production efficiency due to their high metabolic fluxes [44].

After the feedstock selection, the next step of production is pretreatment, which can be physical, chemical, or biological. Each of them provides advantages and disadvantages. For example, mechanical pulverization and acid pretreatment are among the most commonly used physical pretreatment methods because, among others, they can dissolve some lignin; however, they also produce inhibitory products such as furfural [45]. In addition, mechanical crushing, ultrasonic treatment, and microwave treatment can be mentioned; however, they are energy-intensive methods [46]. Chemical pretreatment is highly efficient and easy to use; however, using high amounts of chemicals requires further treatments at a later stage of the process [47]. Biological pretreatment is energy extensive without producing inhibitors, but the process's stability or degradation efficiency can be improved [46]. Nevertheless, the positive impacts of different pretreatment methods, such as hot water and

steam explosion, have been intensively studied [48]. They can also enhance the accessibility of cellulose for enzymatic hydrolysis [49]. Effective pretreatment methods can enhance the hydrolysis rate and overall conversion efficiency of lignocellulosic biomass [50]. It should be emphasized that alkaline pretreatment is more effective than acidic pretreatment for bioethanol production from perennial grasses [51]. Recent innovations in pretreatment methods, such as ionic liquids and deep eutectic solvents, proved to enhance enzymatic efficiency [52]. The combination of different pretreatment methods also provides benefits, including significantly higher conversion efficiency and yield [53]. Pretreatment is followed by extraction with or without using a solvent. Carefully selected and optimally used solvents can increase the production yield of the feedstock.

Regarding biodiesel production, extraction is followed by transesterification for lipid conversion. Innovative methods, such as microwave-assisted and ultrasound irradiation transesterifications, result in faster conversion in comparison to conventional methods [9,54–56]. Combining microwave heating with other technologies, such as acoustic cavitation, significantly improves efficiency and product yield [57]. The use of alternative solvents, catalysts (such as biocatalysts), extraction techniques (such as enzymatic), and purification processes (such as acidified water washing) are the most significant options for increasing the efficiency of biodiesel production [36,39,58]. For example, syngas can be directly converted into jet fuel by the use of a unique catalyst, hierarchical zeolites [59]. The reusability of catalysts is also an important issue; for example, natural mineral rocks can be reused more with the ultrasonic method compared to the conventional method, and it provides faster biodiesel production [54]. Another option is using heterogeneous catalysts, which can simplify the separation process and make it more efficient [3,8]. The use of metal catalyst mixtures can enhance the conversion efficiency [60].

In the case of ethanol production, hydrolysis is the next step, followed by fermentation and purification. Moreover, the first two can be combined through simultaneous saccharification and fermentation [61], while consolidated bioprocessing combines enzyme production, hydrolysis, and fermentation in one single process [48,62]. Minicellulosome-based consolidated bioprocessing makes simultaneous hydrolysis of hemicellulose and fermentation of sugars possible [63]. Every step of this production process offers opportunities for further optimization by applying different temperatures, pH values, and catalyst concentrations [49,64]. Of these, the proper catalyst seems to be the key issue in ethanol production. Catalytic experiments suggested that anionic ruthenium complexes used with p-benzoquinone (BQ) as a co-catalyst can significantly enhance the overall reaction efficiency, as a 19% increase in alcohol yield was observed [65]. Using high solids loading in the enzymatic hydrolysis increases productivity and reduces the energy needed for the process [48]. The use of yeasts can also significantly improve production efficiency. Modified yeasts, such as *Saccharomyces cerevisiae*, can contribute to a higher free fatty acid yield and a more optimal production process [44,61]. It was also studied that if detoxifying cardoon hydrolysate was fermented with genetically modified *Escherichia coli* and membrane nanofiltration was used, the process was more effective than activated carbon adsorption [66]. Combining ethanol fermentation and anaerobic digestion increases energy conversion efficiency [67]. The other ethanol production route is dehydration, where the Pressure Swing Adsorption process with selective zeolites showed high anhydrous ethanol production efficiency [68].

Using exogenous redox mediators in butanol synthesis can improve electron transfer (bioelectrocatalysis) and redox balance in the bioconversion process, thus contributing to higher butanol yield [69]. Strain improvement through metabolic engineering and better fermentation (acetone–butanol–ethanol) conditions lead to increased butanol titer and yield [70].

Process optimization, including temperature, pressure, and the amount of hydrogen, results in significantly higher biokerosene yield [59,71]. Definitive screening design, a statistical method, provided promising results by process optimization [72]. Modern technologies, such as artificial intelligence and machine learning, can also be used to improve the parameters of production in a much shorter time [9,50,51]. Using nanocatalysts, such as magnesium oxide, can significantly enhance biodiesel yields [72,73]. They have better reusability and contribute to cost reduction [74].

Innovations are rising to improve the efficiency of second-generation biofuel production. They include marker-assisted breeding, nanotechnology, advanced multiomics, gene editing, and metabolic engineering for higher biomass production or synthetic biology for improved biomass conversion efficiency [4,39,41,75–77]. Nanotechnology enhances biofuel production efficiency and yield by using nanomaterials, such as catalysts, membranes, and transporters, in biomass conversion, fermentation, and purification [77]. Nanoparticles can facilitate the breakdown of complex cellulose into simpler fermentable sugars and increase the biofuel yield from lignocellulosic biomass [76,78], as well as improve reaction kinetics, selectivity, and stability in biofuel production [77]. Genetic engineering at gene or protein level can also help solve many problems, including high production costs, low yields, and inadequate quality [64]. Genetic modifications can also improve the ability of microorganisms to utilize a broader range of substrates and enhance transformation efficiency [10]. However, there is still a high need for robust microorganisms that can efficiently ferment both glucose and xylose sugars to improve overall conversion yields [48]. However, it should be noted that immature technologies, such as high-pressure gasification and low-temperature CO₂ removal, may provide the best efficiency [79].

4.2. Third-Generation Biofuels

In general, feedstock diversification provides many options [4]. Due to its fatty acid composition and other value-added products, microalgae is better for biodiesel production [36]. Besides removing heavy metals and sequesters, microalgal wastewater cultivation, especially in closed photobioreactors, provides 40–50% higher biomass productivity than traditional crop feedstocks. In these systems, a large variety of wastewater can be utilized such as municipal, industrial, domestic, agricultural, seafood, and even synthetic [80]. Several microalgae genera, *Chlorella*, *Scenedesmus*, *Synechocystis*, *Nannochloropsis*, *Dunaliella*, *Spirulina*, and *Acutodesmus*, are suitable for cultivation [80]. Advanced extraction techniques, such as ultrasound-assisted and enzymatic extraction, can also improve oil yield and quality [55].

There are various ways to improve algae-based biofuel production, such as optimized cultivation conditions, development, and use of innovative catalysts, as well as their right concentration, hydrolysis, extraction technologies, and purification processes [36,81–83]. Replacing the four-zone with the three-zone simulated moving bed process in red algae-based ethanol production resulted in a higher processing rate, better solvent usage, separation performance, and a greater level of purity [84]. Diluted acid treatment combined with other auxiliary methods, such as ultrasound or microwave-assisted pretreatment, increases the sugar yield of macroalgal biomass [83]. The marine yeast *wickerhamomyces anomalus* makes high-gravity fermentation possible, which results in higher ethanol yield [85]. Supercritical water gasification is a promising technology for the treatment of phycoremediation-derived algal biomass, especially if it is combined with other thermochemical conversion techniques, such as hydrothermal liquefaction or chemical looping gasification [86]. The optimal concentration of catalysts can increase biofuel production and positively impact the production of other components, such as different fatty acids [82].

Integrating hydrothermal liquefaction for biomass valorization and nutrient recycling enhances efficiency, as well as reduces the environmental impacts of production [87]. Although scaling up biofuel production is not always easy, for example, aqueous phase reforming with corn stover was able to produce a hydrogen surplus for biocrude upgrading [88]. Integrated production, such as industrial symbiosis, can enhance biofuel production with, for example, the simultaneous production of cellulosic and algae-based biofuels [41]. Combining different production methods and feedstocks can lead to further technological advancements. For example, two-stage culture strategies are used to maximize both biomass and biofuel production by addressing the balance between cell growth and biomass accumulation [89].

Nanoparticles can be efficiently applied at various stages of production, as they can increase cell growth (20–30%), harvesting efficiency (80–99%), and product extraction efficiency (85–99%) [81]; therefore, they enhance the efficiency of biofuel production [73,76]. Using metallic nanoparticles leads to a faster and more efficient carbohydrate release [74]. Nanoparticle-based flocculants can maximize recovery and significantly improve the efficiency of microalgae harvesting for biodiesel production [90].

The selection of suitable microbial strains, such as *Saccharomyces cerevisiae*, *Pichia stipitis*, or various *Clostridium* species, among others, and the optimization of fermentation conditions, especially temperature and pH, are critical for enhancing the overall conversion efficiency of sugars [62,83]. Strain improvement through genetic engineering can increase, among others, growth rate, yield, and stress tolerance [4,73,89], while salt-tolerant strains can improve seaweed hydrolysate [85]. Using genetically engineered cyanobacteria can improve biobutanol productivity compared to non-engineered strains [87]. Metabolic engineering strategies, such as improving carbon flux, promoter engineering, or directed enzyme evolution, proved to be positively impacting biofuel production [91].

4.3. Fourth-Generation Biofuels

There are similar efficiency paths to improve the efficiency of fourth-generation biofuel production as those presented in the previous subchapter. The most important ones are targeted genetic modifications for enhanced lipid productivity, more feedstock conversion process, development and use of innovative catalysts, extraction technologies, and purification processes [36].

Metabolic engineering strategies, especially for microbes, can be successfully applied to fourth-generation biofuel production. This includes approaches like DNA assembly, genome editing, and synthetic biology to enhance traits such as biofuel yield, tolerance, and productivity. Using omics technologies (genomics, transcriptomics, and proteomics) can lead to further optimization by gaining a better understanding of the modified host's complex metabolic networks and regulation [4,91,92]. Their aim can be to modify key metabolic pathways for optimized lipid production or to develop butanol-tolerant microalgae strains, which is critically important for scaling up biobutanol production [93]. Metabolic engineering also provides the opportunity for tailor-made biofuels with unique properties [92]. Genetic engineering offers the opportunity for increased sugar production making the biomass more suitable for biofuel conversion processes, and thus increasing the potential yield [62]. Fourth-generation biofuels are still in the research and development stage, and several issues must be resolved before they can be economically feasible [4]. Genetically engineered biofuels offer promising solutions but still face challenges related to low yields, high costs, and limited understanding of metabolic pathways that can be engineered [90].

5. Conclusions and Discussion

Advanced (lignocellulosic, algae-based, and genetically modified algae-based) biofuels provide a great opportunity for humanity to meet growing energy demand without compromising food production. Liquid biofuels (mainly ethanol and biodiesel) are direct substitutes for fossil fuels. The main barrier to their widespread production is their higher production cost, which can be addressed by increasing production efficiency. The main economic factors of advanced biofuel production are summarized in Figure 7. Due to the nature of the production process, raw material costs are the most significant factor, especially for biodiesel production.



Figure 7. Economic factors of advanced biofuel production.

The advanced biofuel literature is still dominated by cellulosic production as that is the closest to commercialization among the advanced biofuels. Technology development, using more cost-efficient feedstocks, technology learning, and a supportive policy environment are important to narrow the cost gap between biofuels, especially advanced ones, and fossil fuels [21]. Only this can speed up research and innovation in this field to switch from fossil fuel to renewable fuel shortly. Table 1 highlights some key factors constituting the strengths and weaknesses of the various biofuel generation categories.

Table 1. Summary of the Strengths and Weaknesses of the Various Generations.

Category	Strengths	Weaknesses
Second generation	Low cost Minimal land	Cultivation issues Alcohol consuming
Third generation	High yield Waste minimization	High cost Complex processing
Fourth generation	High efficiency Advanced extraction	Environmental concerns High cost

Table 2 schematically presents the production processes of advanced liquid biofuels and emphasizes the critical points of their production.

Table 2. Comparison of Advanced Liquid Biofuel Production and Critical Points of Production.

Second Generation				
Feedstock	Non-food biomass such as lignocellulosic biomass, agricultural residues, forestry waste, and non-edible oils			
Production Process	Ethanol	Pretreatment Enzymatic Hydrolysis Fermentation Distillation	Biodiesel	Pretreatment Transesterification Purification
Critical Points		Pretreatment and enzymatic hydrolysis		Feedstock variability (collection and transport), feedstock pretreatment, and conversion
Third Generation				
Feedstock	Algae and other fast-growing microorganisms			
Production Process	Ethanol	Cultivation Harvesting Pretreatment Hydrolysis Fermentation Distillation	Biodiesel	Cultivation Harvesting Oil Extraction Transesterification Purification
Critical Points		Pretreatment and hydrolysis stages		Cultivation and harvesting
Fourth Generation				
Feedstock	Genetically modified organisms			
Production Process	Ethanol	Genetic Engineering of Microorganisms Advanced Cultivation Ethanol Collection Carbon Sequestration	Biodiesel	Genetic Engineering of Microorganisms Advanced Cultivation Carbon Capture Lipid Extraction Transesterification Purification
Critical Points		Genetic engineering and large-scale cultivation (e.g., custom bioreactors)		

Additionally, extant studies have shown the high potential of subsequent generations in terms of commercial viability, as demonstrated by the average energy return on investment (EROI). Fourth-generation biofuels hold the most promise for the future; however, their commercial viability depends on breakthroughs in genetic engineering, bioprocessing, and energy efficiency. Table 3 presents a summary of the EROI range of advanced biofuels based on the analyzed studies.

Table 3. Comparison of Various Generations Based on Efficiency.

Biofuel Type	EROI Range	Supporting Literature
Second generation	2:1–15:1	[9,65]
Third generation	1.9:1–10:1	[43,54]
Fourth generation	5:1–30:1 (theoretical)	[9,36,84]

Based on the systematically reviewed literature, it is evident that literally every step of the production process can be optimized. Integration of different steps can make the whole process faster and more reliable. The use of advanced technologies, such as different and combined processing methods, the use of novel catalysts and co-catalysts, nanotechnology, genetic and metabolic engineering, the use of suitable microbial strains, as well as their genetically modified versions, are the most promising options for enhanced production

yields. They can even provide the opportunity for tailor-made biofuels with desired properties. Table 4 provides an overview of the key strategies related to the different generations of advanced biofuels.

Table 4. Key strategies discussed for improving the efficiency of advanced biofuel production.

Second Generation Biofuels	Third Generation Biofuels	Fourth Generation Biofuels
Diversifying feedstocks	High lipid content	Feedstock optimization
Using marginal or degraded land	Value-added co-products	Advanced processing
Integrating production into existing industrial infrastructures	Advanced extraction, hydrolysis, and catalytic techniques	Genetic/metabolic engineering of microbes
Innovative pretreatment methods, catalysts, and extraction/purification techniques	Genetic and metabolic engineering of microalgal strains	
Genetic and metabolic engineering of microbes		

There are limitations to the results. Although the Scopus database is a widely used database, other databases may provide other articles for the review. The aim of the article was to provide an overview of the most recent achievements of advanced biofuels and only articles published since 2020 were included.

There are various potential future research paths. First, every generation of advanced biofuels can be analyzed more deeply. Second, non-liquid advanced biofuels can be analyzed, such as gaseous fuels, most notably (green) hydrogen, and especially electricity. Comparing their economic and environmental performance could be of interest as well. Other aspects of advanced liquid biofuels, such as technical feasibility or economic viability, can be of interest, too. A detailed analysis of the regulatory framework of advanced biofuels is also an essential issue, as it is one of the most important initiators of advanced biofuel production.

Author Contributions: Conceptualization, T.M.; Methodology, T.M.; Validation, T.M., Z.D. and C.B.I.; Formal Analysis, T.M., Z.D. and C.B.I.; Investigation, T.M.; Resources, T.M.; Data Curation, T.M., Z.D. and C.B.I.; Writing—Original Draft Preparation, T.M., Z.D. and C.B.I.; Writing—Review and Editing, T.M., Z.D. and C.B.I.; Supervision, T.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

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