

Review

# Role of Biofuels in Energy Transition, Green Economy and Carbon Neutrality

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**Abstract:** Modern civilization is heavily reliant on petroleum-based fuels to meet the energy demand of the transportation sector. However, burning fossil fuels in engines emits greenhouse gas emissions that harm the environment. Biofuels are commonly regarded as an alternative for sustainable transportation and economic development. Algal-based fuels, solar fuels, e-fuels, and CO<sub>2</sub>-to-fuels are marketed as next-generation sources that address the shortcomings of first-generation and second-generation biofuels. This article investigates the benefits, limitations, and trends in different generations of biofuels through a review of the literature. The study also addresses the newer generation of biofuels highlighting the social, economic, and environmental aspects, providing the reader with information on long-term sustainability. The use of nanoparticles in the commercialization of biofuel is also highlighted. Finally, the paper discusses the recent advancements that potentially enable a sustainable energy transition, green economy, and carbon neutrality in the biofuel sector.



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**Keywords:** biofuels; sustainability; bioeconomy; solar fuels

## 1. Introduction

Our planet is experiencing more natural calamities that are severe in terms of intensity and duration. The use of non-renewable fuels as primary energy sources for several years resulted in increasing the speed of global warming and the emission of various air pollutants that are detrimental to the environment and public health. According to a review of five leading international datasets by the World Meteorological Organization (WMO), 2020 was one of the three warmest years on earth, tied with 2016 for first place [1]: another stark reminder of the accelerated pace of climate change, which is devastating health and lives around our world. Based on current policies, global energy demand is expected to rise by 1.3% per year until 2040 without dramatic energy production and recycling [2]. Progress must be made even sooner to reduce greenhouse gas associated with industrial development and energy usage. By 2070, the International Energy Agency (IEA) anticipates global transportation (measured in passenger kilometers) to be increased fourfold and car ownership rates to rise by 60%. According to the Energy Technology Perspective study, the demand for passenger and freight aircraft will triple [3].

The idea of using biofuels appears to be feasible to bring our planet on the pathway to meet energy-related sustainable development. Henry Ford (1896) pioneered bioethanol, while Rudolf Diesel was an innovator in peanut oil. Biofuel is one of the sustainable energy sources obtained from processing various feedstocks such as plant, algae, or animal waste. Biodiesel (fatty acid methyl ester, or FAME, fuels derived from vegetable oils and fats, including wastes such as used cooking oil) and bioethanol (produced from corn, sugar cane, and other crops) are the two most popular biofuels. Since liquid fossil fuels dominate the transportation sector, replacing these fuels with renewable energy will significantly

contribute to the achievement of the comprehensive energy and sustainability goals. The most widely used biofuels are ethanol (from various sources), which is well suited to Otto cycle engines, and biodiesel (from multiple sources), which is better suited to diesel cycle engines. Biodiesel can be used as a fuel additive in compression ignition (diesel) engines, primarily in 20% blends (B20) with petroleum diesel. Biodiesel blend levels are often determined by the cost of the fuel and the projected advantages. Biomethane fuels in CNG buses demonstrate the sustainability concept while also improving overall environmental performance.

Several countries have now passed regulations approving biofuels to meet the potential transportation requirements [4]. The integration of biofuels will reduce a nation's reliance on conventional petroleum imports from other countries, which will help mitigate the impacts of the fluctuations in oil prices, boost the economy, and reduce carbon emissions. Moreover, biofuels encourage new entrepreneurs while simultaneously increasing economic activity globally. They also provide community-level growth alternatives for small and medium-size power grids [5].

Overall, global ethanol production decreased about 15% in 2020 and biodiesel production decreased by 5% in 2020. The International Energy Agency (IEA) estimates that worldwide transportation biofuel output will return to approximately 162 billion liters in 2021, similar to 2019 [6]. In 2025, biofuels are expected to provide roughly 5.4% of the energy requirement for road transport [7].

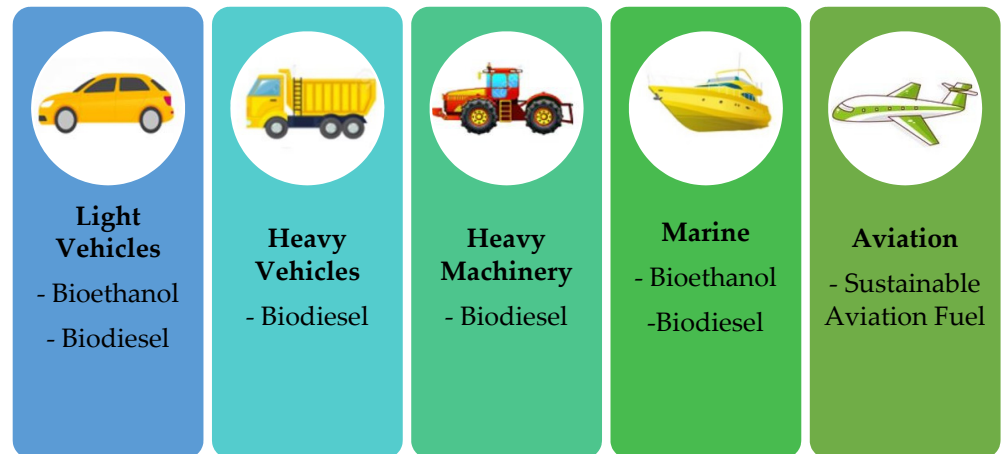
Dependent on feedstocks and technique, biofuels are grouped into multiple categories known as 1st, 2nd, 3rd, and 4th generation. Agricultural products or traditional biofuels are used to generate first-generation biofuels. Fermentation, transesterification, and anaerobic digestion are examples of comparatively well-established processes for producing these fuels [8]. The primary concern with first-generation biofuel is that it is primarily made from agricultural resources, which has a negative impact on financial, ecological, and political considerations because mass biofuel production necessitates more fertile land, resulting in far fewer lands available for human and animal food production [9]. Lignocellulosic feedstocks, agro-residues, and non-edible plant biomass constitute the second-generation feedstocks [10]. Biofuels of the second generation overcome the impact on the climate and social aspects. However, it has a negative energy yield, feedstock transportation issues, high downstream production costs, and modest greenhouse gas (GHG) reduction, limiting their use [11].

On the other hand, biofuels of the 3rd generation have gained broad interest as a substitute for biofuel production to address the problems associated with the first and second generations [8]. The most promising feedstock for renewable fuel production is macro and microalgae. Microalgae and macroalgae require sunlight [12], water, nutrients, and carbon dioxide to create energy biofuels. Algae biomass has the distinct benefits of not competing with soil, having low lignin content, requiring less energy, and competing less with food crops [13]. Genetically modified (GM) algae are used in fourth-generation biofuel (FGB), but there is still considerable concern about the negative environmental impacts.

### *Biofuels for Transportation*

Figure 1 depicts non-renewable biofuels used in the different transportation sectors. Biomethane is utilized to fuel CNG buses, demonstrating that the gas used to power CNG buses may be produced sustainably while also increasing overall environmental performance. The most promising bio-derived fuels for ship use are SVO and biodiesel. The importance of marine transportation in global freight distribution cannot be overstated. However, the maritime sector accounts for nearly 2.6% of worldwide GHG emissions. ExxonMobil has completed a successful sea trial of its first marine biofuel oil with Stena Bulk, which is a shipping firm bunkered in Rotterdam. The study showed that marine biofuel oil, which may reduce CO<sub>2</sub> emissions by up to 40% when compared to traditional marine fuel, can be utilized in a relevant maritime application without modification, allowing operators to make substantial progress toward their carbon reduction goals. The

aviation industry is responsible for 12% of all transportation-related GHG emissions and 2–3% of all anthropogenic GHG emissions [12]. Hundreds of demonstration flights have been flown by more than 20 airlines using a combination of regular jet fuel and aviation-grade biofuel generated from various feedstocks, including waste cooking oil and oil crops such as rapeseed, jatropha, camelina, and palm oil, to produce an alternate aviation biofuel.



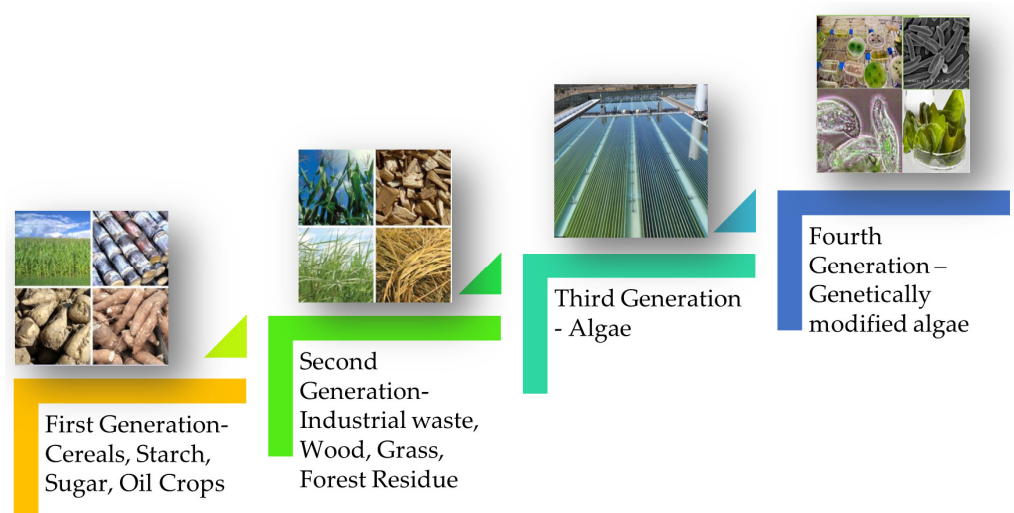
**Figure 1.** Biofuels as an alternative for non-renewable fuels in the different transportation sectors.

The purpose of this article is to shed light on the following aspects of biofuel:

- To investigate the benefits, limitations, and trends in different generations of biofuels.
- To assess the social, economic, and environmental effects for the long-term sustainability of biofuels.
- To highlight the recent advancements in the biofuel sector that potentially enable carbon neutrality, sustainable energy transition, and a greener economy.

## 2. Overview of 1G, 2G, 3G, and 4G Generations of Biofuels

Figure 2 depicts different generations of biofuels based on the feedstock and the development of the conversion process.

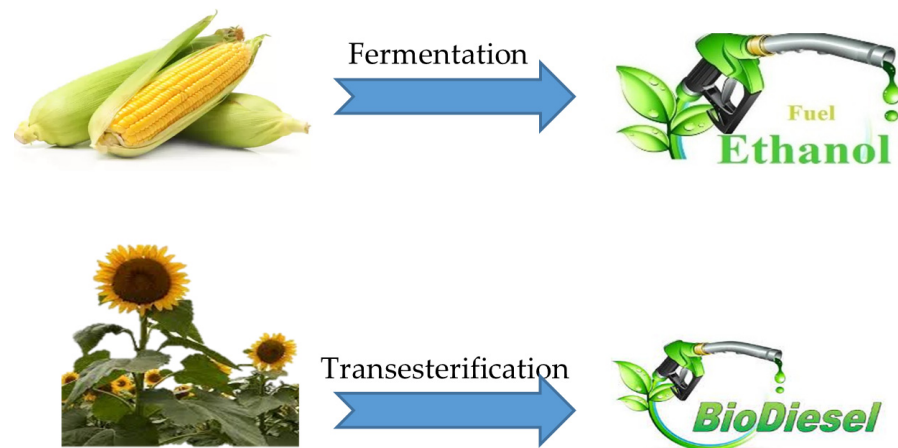


**Figure 2.** Biofuels generation.

### 2.1. First Generation (1G)

First-generation biofuels include biodiesel, bioethanol, and biogas, which are used commercially. Biodiesel is a diesel substitute produced by the oil transesterification of

natural sources as well as leftover fats and oils. At the same time, bioethanol is a gasoline substitute that is produced through the fermentation of sugar or starch as illustrated in Figure 3. First-generation biofuels are being evaluated based on two main claims: For instance, they explicitly attempt to compete with crops for feed. Second, their energy, economic, and environmental balance will not be as optimal as previously planned. According to many researchers, if food prices are influenced by biofuel production to the same extent, the number of food-insecure people in developed countries will increase to nearly 1.2 billion by 2025 [13].



**Figure 3.** First-generation biofuels.

Several studies have found that switching to first-generation biofuels may result in an increase in GHG emissions. Senauer [13] stated that agricultural use and fertilizer application will double emissions over the next 30 years rather than the anticipated 20% reduction in GHG emissions from biofuel. Furthermore, 1G biofuels such as ethanol require a large amount of maize, which requires a large amount of water ranging from 5 to 2138 liters (L) per 1 L of ethanol, depending on how and where ethanol maize is grown. [14]. This appears to have negative environmental consequences, as its intake from water sources can put those areas at risk of drought. Pursuing biofuel production in water-scarce locations would further strain an already constrained resource, mainly if a crop requires irrigation. Water resources and wetlands are expected to suffer as a result of increased water intake [15]. Chaudhary et al. [16] examined the ecological impacts of ethanol production in various parts of the world. It was demonstrated that the cultivation of sugar cane in Brazil suffers a greater loss of biodiversity than the production of sugar beet in France and maize (grain or stover) in the United States [16]. The expansion of 1G biofuels has been a source of social stress, particularly in developing countries where biofuel expansion has occurred in the absence of advanced facilities to control it. Biofuel-based community conflicts are typically related to land contract issues. Citizens in Tanzania, Mozambique, Ghana, Kenya, and Zambia have been reported to have lost access to their shared land due to extensive jatropha farming. Land leases are frequently at the core of biofuel-related community disputes [17]. The Indian government's and the biofuel industry's rapid adoption of jatropha threatens to drive millions of underprivileged rural farmers out of areas where they get their food, fuel, wood, fodder, and lumber [18]. Conventional agricultural production is already facing extreme water constraints; therefore, the regional and local water supply burden would be enormous with 1G biofuel. Policymakers would be hesitant to pursue biofuel alternatives based on conventional food and oil crops [19]. The pros and cons of the 1G biofuels are highlighted in Figure 4. The biofuel yield parameters from 1G feedstock is provided in Table 1.

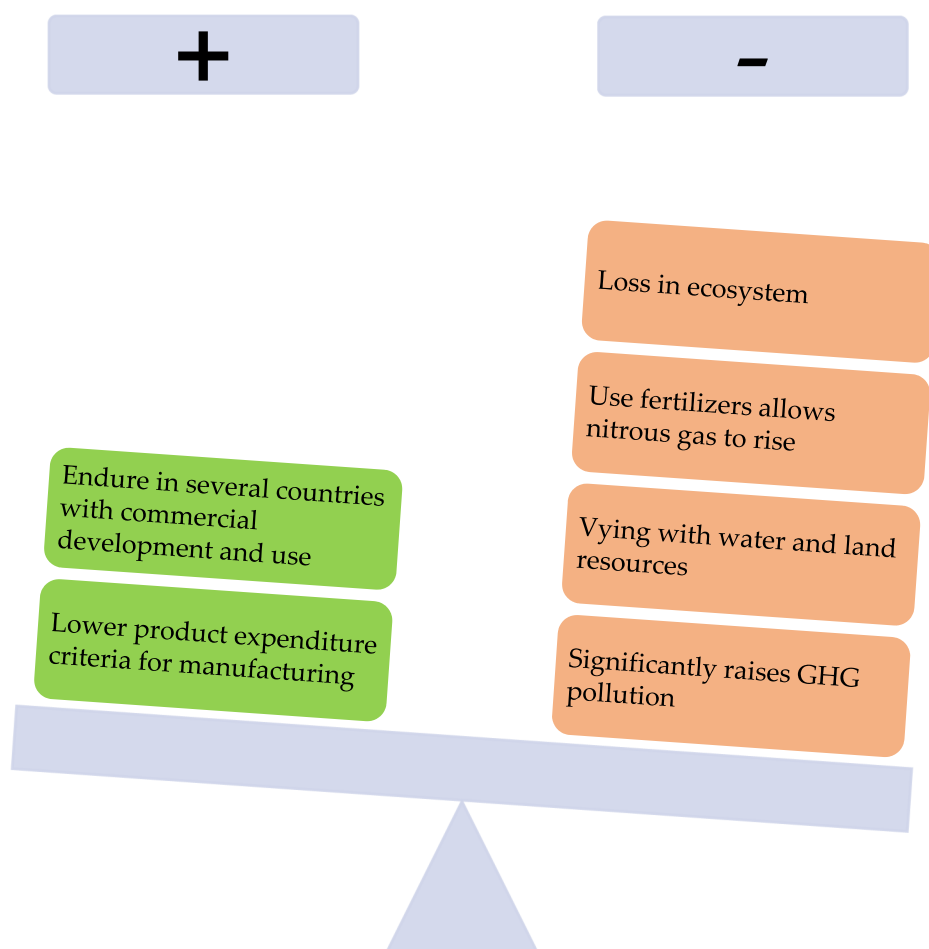


Figure 4. Pros and cons of first-generation biofuels.

Table 1. Parameters and yield of biodiesel, biomethane, bioethanol, and syngas for various feedstocks of first-generation biofuels.

Biofuels	Biodiesel	Bioethanol	Biomethane	Biobutanol	Syngas
Feedstock	Soybean	Corn	Corn	Corn	Rapeseed
Parameters	Transesterification, Hydrotalcite as basic catalyst, methanol/oil molar ratio of 20:1, reaction time of 10 h	Fermentation, Primary liquefaction, heat treatment (105–110 °C) for 5–7 min, α-amylase Secondary liquefaction: 95 °C, 1–2 h, enzymatic liquefaction, pH 4.5	Anaerobic digestion, pH-range—6.5–8.2. mesophilic (30–40 °C), or thermophilic (50–60 °C)	AB Fermentation, Strain: <i>C. acetobutylicum</i> , Temperature 34 to 39 °C for 40 to 60 h	Fast pyrolysis, Conversion to biochar Steam gasification Steam flow: 172 g min <sup>-1</sup> kg <sup>-1</sup> biochar Temperature = 750 °C
Yield	189.6 g/kg	449 g/kg	205–450 dm <sup>3</sup> kg <sup>-1</sup>	12–20 g/L	H <sub>2</sub> = 58.7% CO = 10.6%
References	[20]	[21]	[22]	[23]	[24]

### 2.2. Second Generation (2G)

Figure 5 provides the 2G biofuels feedstock. Second-generation biofuels address many of the issues related to first-generation biofuels. The prospects for fostering regional growth and improving the economic situation in developing regions is envisaged with 2G biofuels. Around the world, various strategies for the production of second-generation biofuels are being considered. Still, the focus is primarily on two distinct paths, either the thermo or bio route generated by biomass of cellulose and lignin, tree surplus, and



seasonal forage crop. Thermochemical manufacturing has the significant benefit of higher versatility of feedstock than biological production. The “thermo” route is focused on the heat processing of biomass under decreased oxidizing agent concentrations. Under the temperature range of 300 to 1000 °C, the bottom range will primarily produce solid biofuel called biochar. At elevated temperatures, pyrolytic oil and syngas are the most concentrated substances in the middle range. The “bio” approach includes the pretreatment of lignocellulosic material, enzymatic hydrolysis, and the fermentation of sugars by specific strains of microorganism. It is more challenging to convert lignocellulose to reducing sugars than it is to convert starch. Biological, physical (thermal), or chemical catalysts are used to pretreat biomass in the biochemical pathway as shown in Figure 6; hence, enhanced advances in the development of 2G biofuels are hampered due to the chemical and structural properties of the extracellular matrix. The biodiesel yield parameters from the 2G biofuel are highlighted in Table 2.

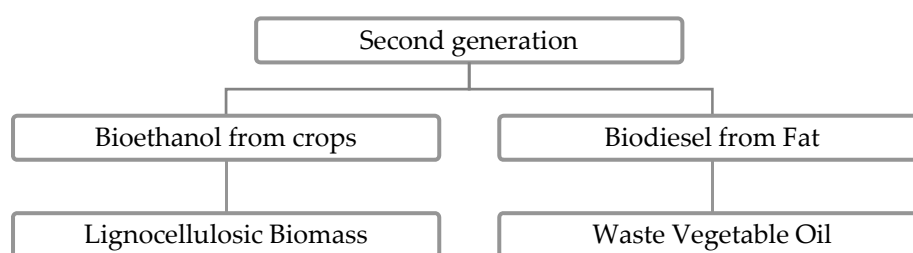


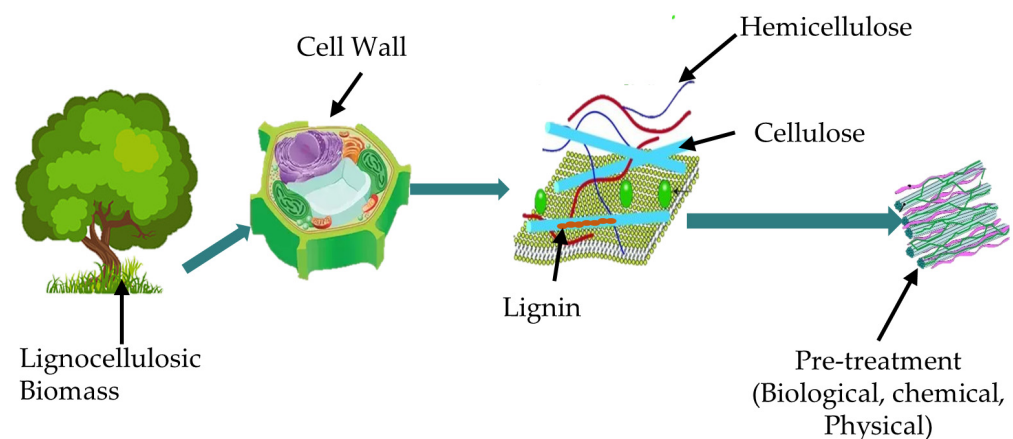
Figure 5. Feedstock of second-generation biofuels.

Table 2. Parameter and yield of biodiesel, bioethanol, biomethane, and syngas from various feedstocks of second-generation biofuels.

Biofuels	Biodiesel	Bioethanol	Biomethane	Biobutanol	Syngas
Feedstock	Palm oil	Sugarcane bagasse	Corn Stover	Rice straw	Corn Stover
Parameters	Transesterification, H <sub>2</sub> SO <sub>4</sub> —5% v/w, 95 °C/540 min	Fermentation, Acid (H <sub>2</sub> SO <sub>4</sub> ) hydrolysis, <i>Kluyveromyces</i> sp. IIPE453, Fermentation at 50 °C	Anaerobic digestion, Cellulase (Spezyme CP) T = 37 ± 1 °C, t = 30 days	Fermentation, <i>C.</i> <i>sporogenes</i> BE01, (37° C and 6.7 pH)	Gasification, Fluidized bed gasifier GA: steam; T: 600–710 °C; ER: N.A.
Yield	97 w/w	165 g/kg	135 dm <sup>3</sup> kg <sup>-1</sup> VS	5.52 g/L	H <sub>2</sub> : 26.9, CO: 24.7 CO <sub>2</sub> : 23.7 CH <sub>4</sub> : 15.3
References	[25]	[26]	[27]	[28]	[29]

New technologies have focused on genomes as well as structural and artificial genetics that would offer demanding opportunities for enhancing the digestibility of cell walls [30] and have the potential to raise PFCE from biomass radically. In species such as *Caldicellulosiruptor saccharolyticus* and *Acidothermus cellulolyticus* [31], enzymes have recently been used to degrade lignocellulose and speed up the process. The efficiency of cellulosic biofuels can also be significantly strengthened by supplying engineered microbes with the potential to digest lignocellulosic biomass even without the application of costly enzymes. Some see genetic modification (GM) techniques as key to achieving high yield, thus boosting the total energy stability of crop residues, for example, developing resistance to fertilizers and pesticides and flooding. However, GM discussions may result from socioeconomic and political decisions debates [32]. Second-generation biofuels are contemporary and innovative, but they do have a specific impact on sustainability. The balance of the life cycle of GHG emissions remains a problem depending on the location of the 2G biofuels produced, the conservation methods, the modes of transportation, and the methods of processing. The second-generation biofuels include waste from operations,

such as methane from garbage dumps or the conversion of waste from processes derived from fossil fuels [33]. A detailed study by Havlík et al. [34] (reported that 2G biofuel production powered by wood from clean sources would reduce overall emissions, considering the deforestation, agriculture water consumption, and increased crop prices especially with the rise of biofuel land area. Still, some other environmental requirements, such as ecosystem preservation, climate regulation, and fuelwood availability to the inhabitants, could be influenced by biomass feedstocks and land use [34].



**Figure 6.** Pretreatment of lignocellulosic material.

The bacteriological and physical properties of soils are also adversely affected by the removal of crop and forestry residues. For instance, according to Powelson et al. [35] the energy savings related to burying wheat bran in croplands are greater to those produced due to their removal for biodiesel production [35]. Various published data indicate that when forest deadwood is removed, the occurrence and variability of bird species decrease significantly. For cavity nesters (e.g., woodpeckers), the critical impact was recorded (e.g., woodpeckers). The eggs and offspring of insects are captured and expelled from the forest as waste is removed and shipped to energy plants, not only reducing insect proliferation but also removing an important source of bird food [36,37]. Evidently, over the past decade(s), the growth of cellulosic biofuel plants on an industrial level has become weaker than anticipated. The latest biofuel output cost statistics indicate that biofuels of the second generation on an energy-average basis are two to three times more expensive than fossil fuels [10].

Currently, producing 2G biofuel is cost-efficient, but there seem to be various technological challenges that need to be overcome before realizing their potential. During the pretreatment and extraction phase, the need for thermal energy and enzymes during cellulose-based hydrolysis increases the production value of bioethanol [38]. The treatment of co-products such as wheat bran raises significant sustainability concerns because pretreatment for enzymatic saccharification is required to overcome the issues associated with lignocellulosic biomass [39]. In addition to the production of sustainable, low pretreatment methods and highly productive fermentation processes, the incorporation of hemicellulose integration is a technical factor that should be taken into account when developing sustainable biorefineries [40]. Worldwide, several efforts are underway to commercialize the second-generation biofuels provided from both routes' extraction. The second-generation mainly operates on a pilot or prototype scale, as shown in Table 3.

**Table 3.** Commercialization status of advanced biofuel production route [41].

Conversion Process	Pilot/ Demonstration	Demonstration	Small Commercial	Commercial
HEFA				✓
Gasification—FT		✓		
Pyrolysis and upgrading	✓			
HTL and upgrading	✓			
Advanced sugar fermentation to hydrocarbons			✓	
Ethanol production from agricultural residues (pretreatment, enzymatic hydrolysis, and fermentation)				✓

In 2007 and 2012, the Canadian company Iogen corporation operated a demonstration plant and then planned to develop a production plant in Brazil that could manufacture ethanol in 40 million liters of production by sugar cane bagasse [42]. Even so, since their technology was not advanced enough or failed in their start-up process, several other firms had to close down [43,44]. When compared to the fossil energy, they could replace the basis cost of production. However, they are still just too expensive to manufacture. Changes in policy could expedite the transition from first-generation biofuels to the commercial deployment and adoption of second-generation biofuels. However, regulations must be designed to encourage the development of the most favorable biofuels while discouraging the production of “poor” biofuels [8]. Table 4 highlights the difference between first-generation and second-generation biofuels.

**Table 4.** First-generation vs. second-generation biofuels.

Conversion Process	First Generation	Second Generation
Possibilities for greenhouse gases mitigation	-	✓
Ability to reduced consumption of fossil fuels	-	✓
The viability of using marginal land to produce feedstock	-	✓
High manufacturing value	✓	-
Relatively simple conversion procedure	✓	-
High prospects for a net decrease in the use of non-renewable resources	✓	-
More output of land use	-	✓

### 2.3. Third-Generation Biofuel (3G)

First-generation and second-generation biofuels are not exclusively biological nor reliant on environmentally sustainable feedstocks. Furthermore, high-energy inputs are required in both feedstock processing and biofuel synthesis. Despite the popularity of 1G biofuels, they suffer from limitations due to disrupting the chain of food and feed, while second-generation feedstocks are losing reputation owing to the increased cost of the synthesis and chemical processing of biodiesel. The production of greener and more efficient biofuels is essential to meet the challenge of entirely replacing conventional fossil fuels with third-generation biofuels. The biofuel yield from 3G feedstock is highlighted in Table 5. Figure 7 provides the third generation feedstock.



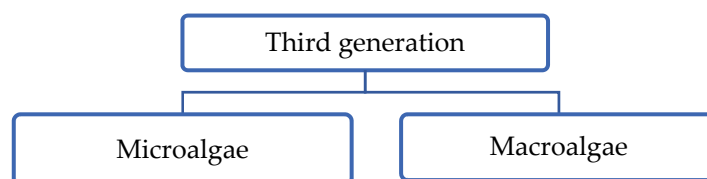


Figure 7. Third-generation biofuels.

Table 5. Parameters and yield of biodiesel, biomethane, bioethanol, and syngas synthesis from various feedstocks of third-generation biofuels.

Biofuels	Biodiesel	Bioethanol	Biomethane	Biobutanol	Syngas
Feedstock	<i>Spirulina platensis</i>	<i>Chlorococcum infusioenum</i>	<i>S. latissima</i> (macroalgae)	Macroalgae	<i>Spirulina</i>
Parameters	Transesterification, reaction temperature 55 °C, 60% catalyst concentration, 1:4 algae biomass to methanol ratio, 450 rpm stirring intensity	Fermentation, alkaline pretreatment, temp. 120 °C, <i>S. cerevisiae</i>	Anaerobic digestion, 53 °C for a period of 34 days, flushed with N <sub>2</sub> /CO <sub>2</sub> (80/20%), to obtain anaerobic conditions.	Fermentation, <i>C. beijerinckii</i> ATCC 35702, (37 °C and 6.0 pH).	Pyrolysis, Temp = 550 °C.
Yield	60 g/kg	260 g /kg	340 ± 48.0 mL g VS <sup>-1</sup>	4 g/L	H <sub>2</sub> = 29, CO = 24, CO <sub>2</sub> = 24, CH <sub>4</sub> = 18
References	[45]	[26]	[46]	[47]	[48]

Algae are at the forefront of the production of third-generation biofuel. When algae are used to produce biofuels, CO<sub>2</sub> emissions will be reduced in comparison to sources of fossil fuels, reducing rising temperatures. The production of 1 kg of microalgae, according to Chisti [49], involves the fixation of up to 1.8 kg CO<sub>2</sub>. This unique necessity for biomass production has evolved microalgae to focus on intensive bio-mitigation studies, which may vastly enhance life-cycle savings. The manufacture of biofuels through algae highly depends upon lipid value. Fast-growing algae are thought to have low oil content, while slow-growing algae have high lipid content [50]. Therefore, microalgal strains selection with greater efficiencies and a rapid growth rate of metabolites is essential [51]. Green microalgae (Chlorophyta) collect oil at a higher rate than other algal taxa such as cyanobacteria, brown algae, and red algae [52]. Owing to high lipids, approximately 60–70% [53] and high efficiency, 7.4 g/L/day for *Chlorella protothecoides* [54], species such as *Chlorella* are evidently targeted. Biofuel production through algal biomass could be commercially viable when algal products and waste are flexibly used. A variety of methods can be used to transform microalgae biomass into energy sources (Figure 8).

LCA analysis was conducted on the use of macroalgae for increased CO<sub>2</sub> fixation and biofuel generation [55]. It was showed that increased CO<sub>2</sub> fixation by macroalgae could provide an energy advantage linked with carbon recycling [55]. In the best-case scenario thus far studied, macroalgae can yield a net energy of 11,000 MJ/t dry algae compared to 9500 MJ/t for microalgae gasification. Lam and Lee [56] evaluated the energy-efficiency ratio (EER) of agricultural and microalgae-based biofuel manufacturing techniques. The EER is defined as the energy output divided by the energy intake. EERs for crop-based biofuel ranged from 1.44 to 5, whereas EERs for microalgae-based biofuel ranged from 0.35 to 434 [56]. Overall, the EER for microalgae-based biofuel generation was lower, but this ratio may rise if the process continues to develop. Microalgae-based biodiesel fuels have density, viscosity, flash point, heating value, cold filter clogging point, and solidifying point in common with petroleum-based biofuels. As a result, they meet both the American Society for Testing and Materials (ASTM) and the International Biodiesel Standard for Vehicles (IBSV) requirements [57]. Table 6 compares the properties of macro and micro-algae based biofuels.

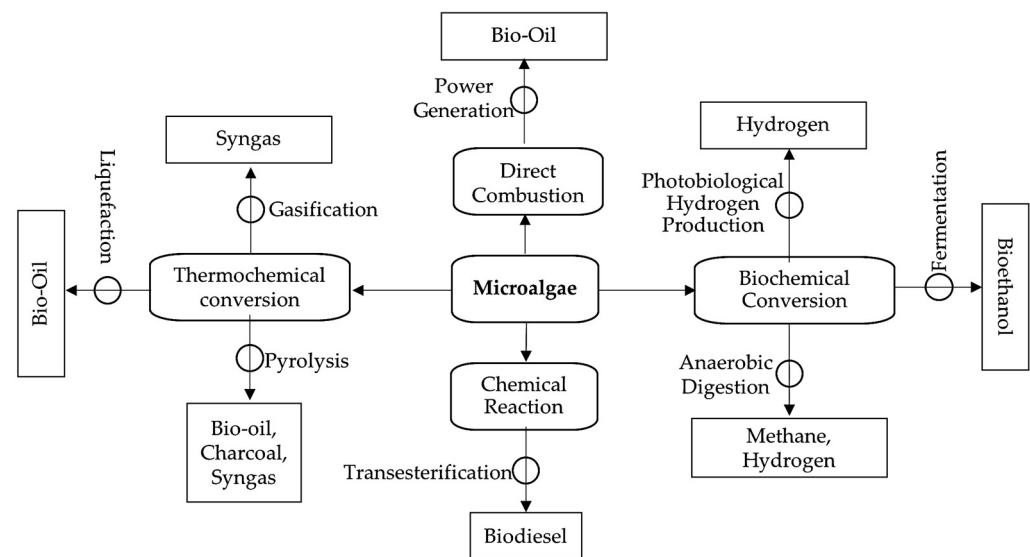


Figure 8. Biofuel generation from microalgae.

Table 6. Properties of micro and macro-algal biodiesel as compared to conventional diesel [58,59].

Fuel Property	Unit	Microalgae Biodiesel	Macroalgae Biodiesel	Biodiesel Standard EN 14214	Diesel
Cetane Number	-	46.5	58.23	51	53.3
Kinematic Viscosity @40 °C	mm <sup>2</sup> /s	5.06	4.3	3.5–5.0	2.64
Density @15 °C	kg/L	0.912	0.868	0.86–0.90	0.84
Acid Value	mg KOH/g	0.14	0.13	0.5 max	0
Flashpoint	°C	-	155	101 min	100 1-D 126 2-D
Cloud Point	°C	16.1	−4	-	4
Sulfur Content	mg/kg	7.5	8.9	10 max	5.9
Copper Strip Corrosion (3 h at 50 °C)	-	1	1	1	1

Approximately 30% is the algal biomass's oil portion, and the leftover 70% is the algae by-product. This by-product can be used for medical chemicals, cosmetics, toiletries, and fragrance products.

High energy and cost-intensive downstream processes, such as enzymatic hydrolysis and metabolic pathway extraction, remain primary techno-economic obstacles to the full commercialization of microalgal biodiesel production [60]. Efroymson et al. [61] suggested that by reducing the number of phases in the manufacturing and co-production of a more energetic fraction, the value of algal biofuels could be dramatically lowered. Many algae specimens are not appropriate for industrial cultures, as the structure of microalgal lipids in fatty acids may not be ideal when used as biofuel. In challenging situations, the accumulation of lipids leads to cell development and division being stopped, resulting in a clear limitation of the productivity of biomass [62]. Genetic manipulation engineering can deliver innovative routes to lipid and algal biomass production [63]. Through calculation, it was demonstrated that replacing 1% of US road fuel source with macroalgal biofuel only involves 0.09% area of the Exclusive Economic Zone (EEZ) [64]. Such a prospect is most likely to remain on a document before promoting strategies are carried out. Microalgae-derived jet fuels have also been extensively tested in commercial and military aircraft. Solazyme Inc. produced the world's first jet fuel made entirely of algae using the UOP HEFA process technology and fermentation. The US Navy has tested Solazyme's jet fuel [64]. To analyze the environmental effect of an algal-based BAF supply chain in the

United States, Agusdinata and Laurentis [65] combined LCA and multi-actors (stakeholder decisions) found that algal biofuels have the potential to reduce the country's aircraft industry's life-cycle CO<sub>2</sub> emissions by up to 85% by 2050 [65]. Massive algae processing also faces technical and logistical challenges, but respondents believe that algae-based biofuels can play an important role in the advancement.

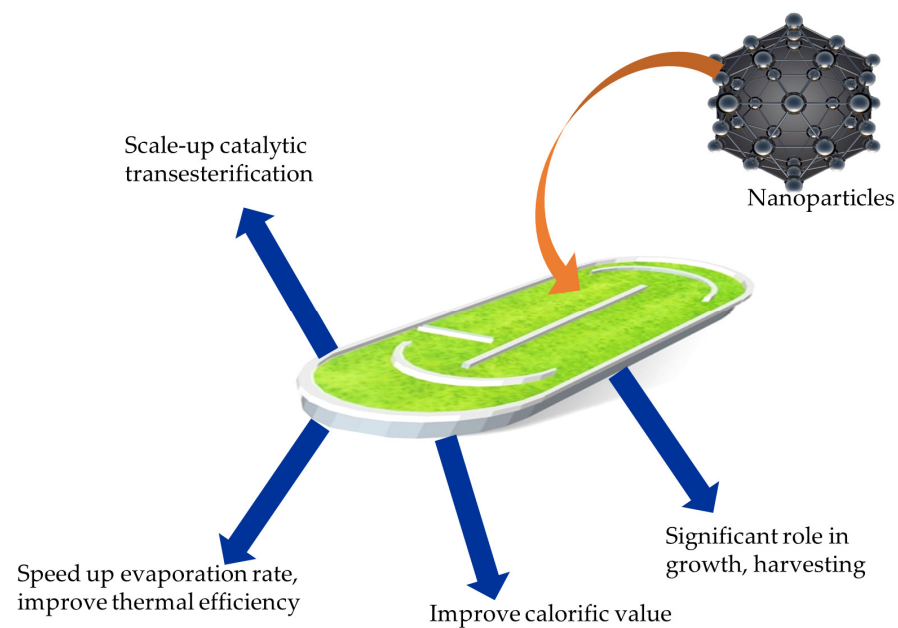
#### Emerging Trend of Nanotechnology

Nanotechnology, as a creative and ground-breaking technique, has a broad array of applications and prominent roles. NPs are reliable, cost-effective, and environmentally sustainable, with high stability, a faster synthesis rate, and a simple procedure. A nanoparticle is described as a structure with a diameter of 0.1 to 100 nm. As a result of their extraordinary physicochemical properties, nanoparticles are now being used strategically in biofuel development. Many nanomaterials with unique properties such as TiO<sub>2</sub>, Fe<sub>3</sub>O<sub>4</sub>, SnO<sub>2</sub>, ZnO, sulfur, graphene, and fullerene have been used in biofuel processing. Nanoparticle-aided microalgal harvesting has become the new trend to enhance energy usage, total microalgal concentration, quality, and process cost [66]. In a large-scale sample, the use of nanoparticles on microalgae harvesting claimed a 20–30% reduction in microalgae production cost [67]. The use of nanoparticles in bio-diesel blended fuels has improved efficiency and lowered emissions. More emphasis should be put on this in the future. Karthikeyan et al. have concentrated on preparing various biodiesel blends using CeO<sub>2</sub> additives in the hope of long-term applications in single-cylinder compression ignition engines that would benefit the whole population [68]. In the presence of ion–silica nanocomposites, algal oils have a high yield of production [69]. The Ames Laboratory has created a new technique dubbed “nano-farming” that extracts oil from algae using sponge-like mesoporous nanoparticles. The process does not damage the algae in the same way that other methods are being produced, lowering production costs and shortening the production cycle [70]. Table 7 highlights the use of nanoparticles in algal biofuels.

**Table 7.** Role of various nanoparticles in algal biofuels.

Nanoparticles	Functions	Ref.
Al <sub>2</sub> O <sub>3</sub>	Improved the <i>Chlorella</i> sp. microalgae growth by 18.9% after 4 days of exposure to a concentration of 1000 mg L <sup>-1</sup> Al <sub>2</sub> O <sub>3</sub>	[71]
TiO <sub>2</sub>	Improved total yield in microalgae processing (e.g., cell suspension, cell division, and cell harvesting)	[67]
Fe <sub>3</sub> O <sub>4</sub>	Increased harvesting productivity by 95% of <i>Scenedesmus ovalternus</i> and <i>Chlorella vulgaris</i> when grown with iron oxide NP	[72]
CaO	During scaled-up catalytic transesterification, the conversion efficiency of biodiesel was 91%	[73]
TiO <sub>2</sub> , CeO <sub>2</sub>	Enhance 10–11% of biogas yield from wastewater treatment	[74]

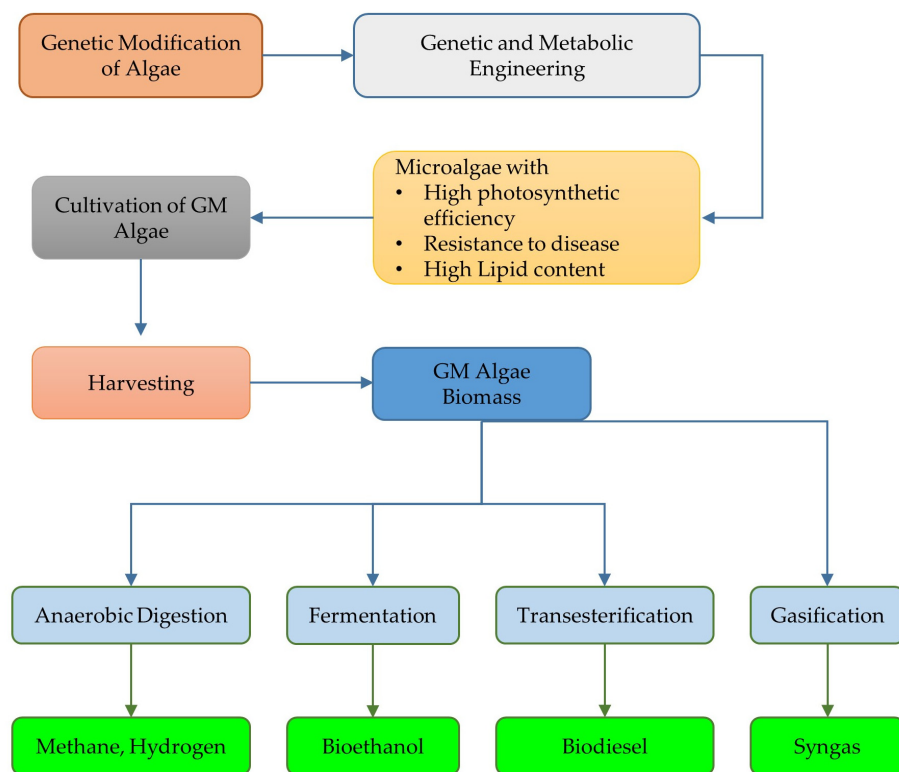
Although nano-additive applications were essential to microalgae growth, harvesting, biofuel conversion, and biofuel applications to enhance efficiency, there were still certain obstacles prior to the deployment of nano-additives for the commercialization scale. Nano-additives in micro-algal biofuels are limited to the laboratory and pilot size, which is a significant constraint. Despite its benefits, one of the primary difficulties with NPs is the high cost of manufacture, which has hampered the commercialization of nanofluids (Figure 9).



**Figure 9.** Application of nanoparticles.

#### 2.4. Fourth-Generation Biofuel (4G)

The most promising advanced biofuels are those from the fourth generation of biofuels. The feedstocks of the fourth-generation biofuels are genetically engineered microalgae, microbes, yeast, and cyanobacteria; these microorganisms are genetically engineered. The best way to cut the price, nutrients consumption, and ecological footprint is to boost productivity and lipid accumulation. Ketzer et al. found that from a biological standpoint, a better energy return on investment (EROI) might be obtained by improving photo-conversion efficiency, which would result in higher biomass and energy yields. Increasing and altering the buildup or release of energy products (e.g., lipids, alcohol) is currently being researched [75]. Genome editing strategies are frequently used to improve the efficiency and lipid composition of algae. Currently, three types of genetic modification tools are commonly used for genomic editing of microalgae strains: zinc-finger nuclease (ZFN), transcription activator-like effector nucleases (TALEN), and clustered frequently interspaced palindromic sequences (CRISPR/Cas9) [76]. As a result of the complexity and difficulty of the experimental design of ZFN and TALEN, the CRISPR-Cas9 method is the most actively developed in microalgae [77,78]. Wang et al. performed precise CRISPR/Cas9-based genome editing of commercial algal strains such as *Nannochloropsis*, which accumulates oil as a source of plant-like fats for biofuel generation under nitrogen shortage [79]. Engineered ZFNs were utilized by Sizova et al. [80]) and Greiner et al. [81] to target the COP3 and COP4 genes in *C. reinhardtii*. The effectiveness of the ZFNs was only observed in the tailored model strain of *C. reinhardtii*. The most difficult challenge is to generate unique ZFNs with high specificity and affinity for the target sites [80,81]. Before executing the actual experiment, ZFNs must be validated using a gene-targeting selection method [80]. Using genetic and metabolic engineering, it is possible to connect the third and fourth generations compared to 3G biofuels where the main focus is on the production of biomass of algae to generate biodiesel. On the other side, the most attractive feature of fourth-generation biofuel is introducing the incorporation of modified photosynthetic microorganisms [82]. Figure 10 shows the process of biofuel production from genetically modified algae.



**Figure 10.** Fourth-generation biofuel production.

#### 2.4.1. Microorganisms Use in Fourth-Generation Biofuel

In biofuel processing, several microbes, yeast, cyanobacteria, and microalgae are used, with cyanobacteria and microalgae being the best choices for this reason. The selection of suitable strains, as all microbial species, cannot be genetically modified due to the complexity of structure, high nutrient demand, or environmental intolerance. Tables 8–10 provides the lipid yield, risk-mitigation and yield parameters of the GM algae

##### Green Algae

The *Chlamydomonas reinhardtii* has been genetically engineered to express many essential biofuel characteristics [83]. However, the production rate of biomass is low. Some examples of green algal such as *Chlorella*, *Parachlorella*, *Nannochloropsis*, *Scenedesmus*, *Botryococcus*, and *Neo-chloris* are rich in lipid content and hence mostly use for biofuel instead of having low biomass.

##### Blue-Green Algae

Cyanobacteria are among the first microorganisms to have lasted for a few billion years. They are a crucial source of atmospheric oxygen and play a vital role in the daily lives of ordinary people [84]. There are many possible uses for cyanobacteria, such as feed sources, agricultural biofertilizers, and wastewater treatment [85]. Compared to other photoautotrophs, biofuel production from cyanobacteria has a lot of potential as a biofuel platform, since they do not need fermentable sugar or arable land to grow. They will have far less competitiveness with farmland capacity to fix carbon dioxide gas. Genetic tractability, horizontal gene transfer, and competitiveness among genetically modified cyanobacteria and other microorganisms may impact natural ecosystems [61,86]. *Synechocystis* sp. PCC 6803, *Synechococcus elongatus* sp. PCC 7492, *Synechococcus* sp. PCC 7002, and *Anabaena* sp. PCC 7120 all have been used as model organisms for genetic engineering. The optimal production host, on the other hand, is challenging to forecast [87]. Fourth-generation processes include pyrolysis (at temperatures ranging from 400 to 600 °C [88]), gasification, and solar-to-fuel pathways in addition to genetic modification [89].

**Table 8.** Genetically engineered microalgae generate lipids for biofuel production.

Species	Attainments	Reference(s)
<i>Chlamydomonas reinhardtii</i>	Increased lipid content by 1.5 times 20% increase in triglyceride content	[83,90]
<i>Phaeodactylum tricornutum</i>	35% increase in lipid 1.1 times increase in triglyceride content	[91,92]

According to Snow and Smith [93], genetically engineered microalgae-induced complications include future environmental challenges such as ecological changes, toxicity, lateral transference of genes, and competition with native organisms [94]. The disposal of GMOs is one of the critical issues with their use. Since the intentional or unintentional release of chromosomal or plasmid DNA at specific concentrations might result in horizontal gene transfer through transformation, there are stringent rules for its disposal [95].

**Table 9.** Risk mitigation for GM algae.

Biological Method	Physical Method
Inactivation of a gene that regulates sexual behavior	To assess possible or unknown hazards, controlled field experiment should be conducted.
Upon escape, terminator gene expressed	UV, chemical, and heat deactivation during the harvesting of genetically modified algae.

To reduce the danger of large-scale GM algae being released into the environment, two main containment strategies are being considered: first, physically stopping the algae from escaping into the atmosphere, and second, genetically preventing the algae from replicating and competing in nature [96]. GE algae outperform native strains in the context of ecological compatibility and cost-effectiveness, making algal biofuels more viable. There is proof of their superiority and the absence of significant drawbacks on a lab and prototype size, but this must be demonstrated commercially, since it is necessary for the genetic stability of GE algae. Although CRISPR technology eliminates the fear of GMOs, it is not universally embraced. For example, gene-edited organisms must be subjected to the same onerous restrictions as traditional GMOs, according to a judgment by the European Union's Court of Justice ECJ [97].

**Table 10.** Parameters and yield of biodiesel, biomethane, bioethanol, and syngas synthesis from various feedstocks of fourth-generation biofuels.

Biofuels	Biodiesel	Bioethanol	Biomethane	Biobutanol	Syngas
Feedstock	<i>E. coli</i>	<i>Synechocystis</i> sp. PCC6803	-	<i>E. coli</i>	-
Parameters	Deletion of Aas gene in strain SS3B to produce strain SS34. Introduced DmJHAMT (Drosophila melanogaster Juvenile Hormone Acid O-Methyltransferase) inlet temperature 250 °C with split ratio 1:1; carrier gas: helium; flow: 5 mL/min; oven temperature: initial temperature of 160 °C, hold 3 min; gradient to 255 °C at 5 °C/min; hold 3 min; inlet temp: 270 °C, detector temp: 330 °C	Integration of pyruvate decarboxylase from <i>Z. mobilis</i> and endogenous alcohol dehydrogenase slr1192 under control of different promoters. Temperature: 37 °C, shaking at 80 rpm, induced by 0.4 mM isopropylthiogalactoside	-	Deletion of adh, ldh, frd, fnr and pta and insertion of bcd-etfAB from <i>C. acetobutylicum</i> . Cultures grown semi-aerobically in shake flasks at 37 °C for 24 h	-
Yield	0.56 g/L	5.50 g/L		0.37 g/L	
References	[98]	[99]		[100]	



### Industries Involved in Third and Fourth Generation Biofuels

The Scottish Association for Marine Science (SAMS) is working on MacroFuels, a Horizon 2020 project to develop improved biofuels from seaweed or macroalgae. The project's goal is to make a breakthrough in biofuel production. It also aims to develop technologies for fuels that can be utilized in heavy transportation and aircraft.

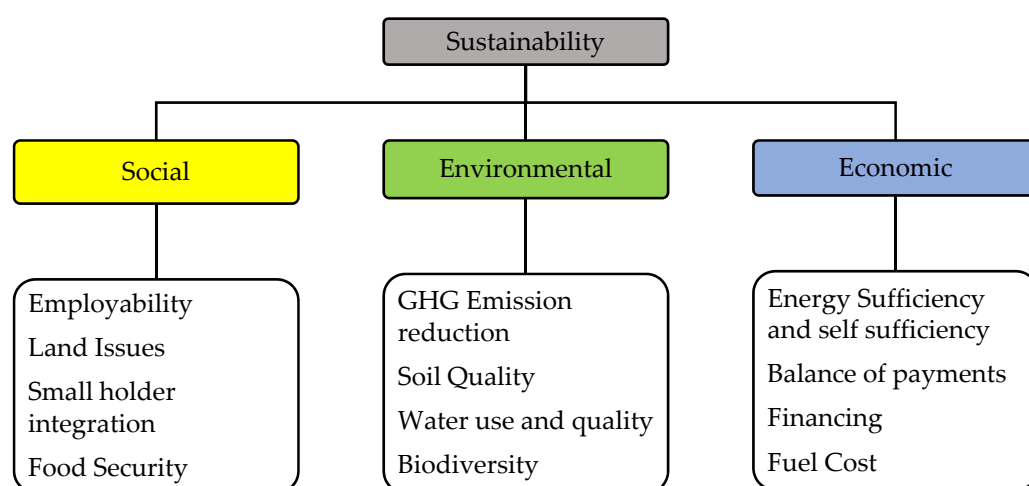
Craig Ventor's Synthetic Genomics is currently developing microbes capable of producing fuel directly from carbon dioxide (CO<sub>2</sub>). By 2050, it is predicted that the fourth generation will be wholly developed and play a significant role in the global power industry. A brief comparison of 3G and 4G biofuels are presented in Table 11.

**Table 11.** Comparisons between third and fourth-generation biofuels.

Biofuel Generation	Third Generation	Fourth Generation
Biomass used	Algae and microorganism	Engineered crops and solar fuels
Processing methodology	Biochemical conversion, Chemical reaction, Direct combustion, Thermochemical conversion	Genetically modified algae, Biochemical conversion, Thermochemical conversion
Generated fuel	Methane, Bioethanol, Biobutanol, Syngas, Biodiesel	Methane, Bioethanol, Biobutanol, Biodiesel, Syngas
Advantages	Easy to cultivate No competition for food crops	Biomass and production yield both are high. Increase CO <sub>2</sub> absorption capacity

### 3. Sustainable Assessment of Third-Generation Biofuels

The sustainability concept is multidimensional. It acknowledges that there are inherent relations between economic, social, and environmental well-being as shown in Figure 11. If any one of the dimensions changes, it will have an impact upon the other two dimensions. Sustainable biofuel production should include preserving biodiversity, sustainable water utilization, healthy air quality, soil conservation, social issues (such as storage, transportation, health effects, etc.), and most importantly, fair labor practices. On the one hand, biofuel contributes to the prospects of CO<sub>2</sub> reduction, improves air quality, and provides net energy gain.



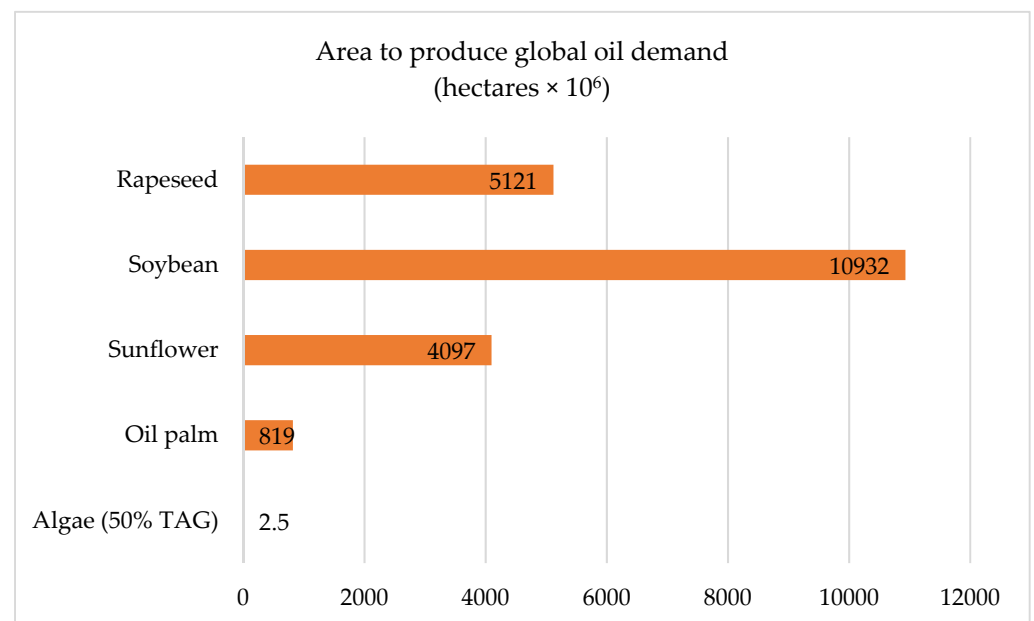
**Figure 11.** Sustainability aspects of biofuel production.

On the other hand, the continuous production of biofuels harms biodiversity, causes soil degradation, and affects food security. Since biodiesel production has risen steadily globally, food prices for vegetable oils have increased significantly [101]. Studies on marine algal biofuels have received interest in the last few decades. A potential solution to energy and environmental problems is a commercially feasible algal cultivation. It is cost-

effective, requires no additional land, uses less water, and reduces atmospheric CO<sub>2</sub>. The global efficiency, net productivity per hectare, avoided CO<sub>2</sub> emissions, net present value (NPV), and levelized cost of energy (LCOE) are the key metrics used for the sustainability evaluation of biofuels. Third-generation biofuels, with higher pollution reductions, aim to be more sustainable. These biofuels are focused on biomass sources that are not used for other primary purposes, such as food processing and cultivation. Algae demonstrate great promise as a possible future green energy source because of their environmental friendliness and high oil-yielding ability per given field.

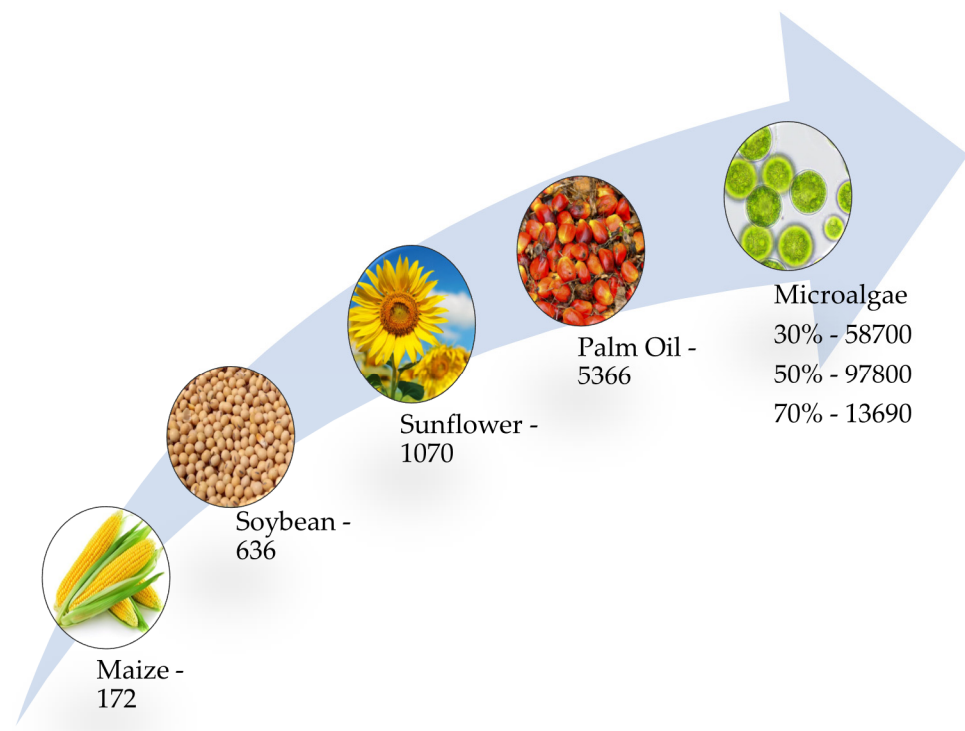
### 3.1. Land

The primary goal of biofuel conservation is to conserve land. Field use can be expanded from food to social growth and biofuels as the world population increases. As a resourceful evolution, third-generation (algal) biofuels may avoid food competition and land use. The fast growth rate of algae enables the massive cultivation in non-arable landmasses, thereby eliminating competition with land in use for crop production. Compared to conventional forests, agroecosystems, and other aquatic plants, microscopic algae have higher growth rates and efficiency. Unlike other agricultural biodiesel feedstocks, it requires much less surface area [49]. For example, brown seaweeds produce 13.1 kg dry weight m<sup>-2</sup> yr<sup>-1</sup> compared to 10 kg dry weight m<sup>-2</sup> yr<sup>-1</sup> from sugarcane [102,103]. Figure 12 highlights the land requirements of microalgae compared to other feedstock.



**Figure 12.** Land demand of microalgae oil compared to different biomass [104].

In addition, due to the limited dependency on farmland (compared to crop-based biofuels), algae lead to less habitat destruction. Consequently, by using microalgae as biodiesel feedstock, competition for agricultural land, particularly for human consumption, is significantly reduced [105]. Oil yields from microalgae can surpass those from oil plants such as rapeseed, palm, or sunflower per hectare as shown in Figure 13.



**Figure 13.** Comparison of microalgae oil yield (L oil/ha/yr) with other biodiesel feedstock [105].

In Malaysia, coastal areas and underutilized rice land are promising sites for massive microalgae cultivation [106]. Due to saltwater penetration, these lands are unproductive and therefore can be used to produce marine microalgae appropriate for saltwater [107,108]. The ‘Submariner’ research team has explored the possibilities of connecting both macro- and micro-algae development facilities to use an operational offshore wind farm in the Baltic Sea to reduce the burden on land availability [109]. The DOE estimates that if algae fuel replaced all the petroleum fuel in the United States, it would require only 15,000 square miles, which is a few thousand square miles larger than Maryland. This is less than one-seventh of the area devoted to corn production in the United States in 2000 [70]. Wigmosta et al. [110] examined the land, water, and resource availability in the United States and determined that about 43,107 hectares of land were suitable for algae culture in open ponds. This corresponds to a possible yearly output of 2.20 1011 L of algal oil, which is equivalent to 48% of the United States’ annual petroleum imports.

### 3.2. Water

Water use concern is the main drawback associated with first-generation and second-generation biofuels. Water is a limited resource, and a lack of it can severely affect well-being. In addition, current water problems are predicted to be intensified by climate change. As a result of the lack of freshwater sources worldwide and the inefficient usage of freshwater aquifers, only brackish water or seawater can be considered in broader application. The water footprint of a biofuel refers to the total volume of surface water needed for its production. Three types of green, blue, and gray algae are commonly considered for the water footprint of biofuel production [111]. Footprints in green and blue water refer to evaporation during the period of processing. The footprint of graywater applies to the water ultimately released as waste. The water footprint of microalgae and terrestrial plants was examined by Zhang et al. [112]. It was found that the green water footprint for microalgae processing was about one-quarter of the average green water footprint for three plant species. Microalgae biodiesel has a WF of about 3726 kg water/kg biodiesel. Still, it is possible to recycle about 84% of this water, taking the WF down to 591 kg water/kg biodiesel [113]. By using nutrients in wastewater and seawater, using algae reduces the

need for fresh water. Furthermore, by recycling and reusing the discharged water from the harvest process, up to 90.2% of the usage of topically discharged water can be restored to the manufacturing process [114]. Table 12 highlights the water footprint of biofuel feedstocks.

**Table 12.** A comparison of the blue–green water footprints of microalgae biofuel and other feedstocks.

Biofuel's Feedstock	Type of Water Footprint	Water Footprint	Biofuel's Feedstock
Sugar cane (Bioethanol)	Blue + Green	139	[115]
Rapeseed (Biodiesel)	Blue + Green	165	[116]
Microalgae (Biodiesel)	Blue + Green	14–87	[117]

### 3.3. Energy

The net energy ratio is the ratio of the energy of algal biofuel to the energy invested in algal production. Micro-algae have an energy content of 5–8 kWh/kg (18,000–28,800 kJ/kg) of dry weight [118]. The development of algal biodiesel could be feasible if the energy needed to generate the microscopic algae and the energy necessary to turn the microscopic algae into operational fuel is lower than that sum. As a result, the Net Energy Ratio can be written as

$$E_{NER} = \frac{E_{Out}}{E_{In}} = \frac{\text{Energy in Algal Biofuel}}{\text{Energy Invested}}.$$

Microalgae are solar-powered cell factories that turn carbon dioxide into potential bio-fuels [119]. Microalgae are a quickly evolving photosynthetic species capable of converting 9–10% of solar energy (average sunlight irradiance) into biomass, with a potential yield of around 77 g/biomass/m/day, which is about 280 tons per hectare per year [120,121]. In a highly efficient seaweed processing method, prices for energy return on investment from seaweed (0.44 to 1.37) for fermentation and ethanol distillation could be equivalent to corn (1.07) [122].

### 3.4. Socio-Economic Aspects

Jobs and profits, food security, economic progress, sustainable energy, economic viability, health and security, public acceptance, and equality of opportunity were defined as socio-economic measures. Both jobs and local revenue are vital factors of development in algal energy generation. To meet the challenge of long-term viability, the development of safer and more sustainable biofuels must be planned with third-generation biofuels. The growing of macroalgae is relatively easy; the crop can be harvested in around 6 weeks with modest initial capital expenditure. These features provide women with a significant opportunity to generate capital for themselves and their families [123]. About 116,000 households, representing over one million people, planted over 58,000 hectares of seaweed in the Philippines. It is worth noting that more than half of the seaweed-harvesting population is unskilled or semi-skilled [124,125]. Wild seaweed harvesting is a vital part of the history and practice of many nations. Women form a large proportion of the harvester workers in Brazil (assumed to be about 80%), and nori (*Pyropia* spp.) collection is typically performed by women in Japan.

Similarly, the bulk of seaweed harvesters in South Africa are women [126] whose annual average income was quoted as US \$5000; therefore, microalgae biofuel production, as a long-term sustainable sector, can also create opportunities for job development at all skill levels, close to traditional biofuels [127], and it also can be a safer choice when paired with existing complementary industries. The correlation regarding waste effluent bio-fixation and the production of usable co-products (e.g., feed, fertilizer) [128] may be economically advantageous to native communities in parallel, supplementing seasonal industry profits. Algae (seaweeds and microalgae) is recognized by the European Commission (2012, 2016) as such a good food safety choice that by 2054, the combined cultivation of algae could achieve 56 million metric tons of protein production, accounting for 18%

of the worldwide alternative protein industry [129,130]. Many algal biofuel companies have pilot plant job figures that can be registered. Wholesalers of algal biofuels products and technology, such as nutrients, CO<sub>2</sub>, polyethylene liners, PBRs, pumps, and workers from plants that have mutual storage services (e.g., CO<sub>2</sub>, nutrients) to biofuel facilities, are examples of indirect jobs [131]. Gallagher claims that [132] the economic viability of microalgae biofuel development seems reasonable and relies on government support and potential oil price. The list of sustainable indicators are presented in Table 13.

**Table 13.** List of sustainable development metrics for bioenergy with a focus on terrestrial feedstocks [133].

Category	Indicator	Sustainable System Design Goals
Social welfare	Occupation	High wages and more job opportunities
	Wealth of households	High-paying jobs and reducing fuel prices, raising household income
	Security of foods	Algal biofuel using non-arable land and opportunities for food co-products
Energy	Premium for energy security	Maximize the advantages of substituting algal biofuel for fossil fuel for oil security dollars.
	Volatility in the price of petrol	Reduce uncertainty of fuel prices.
External trade	Terms of commerce	Build situations such that fewer capital leaves a government agency to buy crude.
	Volume of commerce	Minimizing net fuel imports
Profitability	Investment return (ROI)	Build a constructive ROI
	Net present value (NPV)	Build a positive NPV
Resource conservation	Non-renewable energy resources depletion	Reduce the dependency on fossil fuels
	Fossil fuel energy return on investment (fossil EROI)	Raise fossil fuel EROI above 1 and finally above 3
Social acceptability	Public opinion	Show a promising estimation of a high percentage
	Transparency	Display a steadily rising or high value
	Efficient engagement from stakeholders	Maintain catastrophe level at current occurrence or based on comparable technologies
	Risk of catastrophe	

### 3.5. Environmental Aspects

The sustainable development focuses on eliminating GHG pollution from the atmosphere, reducing human health by using renewable energy sources such as biofuels and extracting toxic pollutants from the environment. Water contamination and freshwater retention shortages, combined with global warming, are now overwhelming global fears and threatening biodiversity. The third-generation biofuels offer valuable insight into the clean energy approach and long-term sustainability. Algae is one of the most efficient biological mechanisms for transforming sunlight into energy to absorb and convert carbon dioxide to biomass. Around half of the global carbon fixation is carried out by algae [134]. Ecological science has shown that the combination of the production of macroalgae with e.g., shrimp [103] and salmon [104], will remedy coastal eutrophication. Algal-based wastewater treatment offers an efficient and cost-effective tool to eliminate organic and inorganic contaminant wastes from wastewater. The wastewater from these sources can be divided into organic and inorganic compounds. The critical portion of organic waste is carbon-containing biodegradable substances. In contrast, inorganic waste includes nitrates, phosphates, and heavy metals [135]; hence, it provides a reliable and cost-effective tool to extract wastewater from organic and inorganic toxins. The author of [122] has recently shown how the rare metal indium could be extracted from e-waste (old electronic materials) that is frequently transported to developing nations, where workers (many of whom are minors) burn circuit boards to remove valuable and rare metals. This has grave health and pollution consequences. The brown seaweed *Ascophyllum nodosum* biomass was discovered to aid in the 'extraction' of the metal indium, which is essential as an input to modern electronics, particularly screens. Biofuels derived from microscopic algae are one of the most promising green energy options not just because of their reduced greenhouse gas emissions but also because of their CO<sub>2</sub> sequestration [136]. The CO<sub>2</sub> sequestration in microalgae is 10–50 times more than that of many terrestrial plants, while a higher concentration of CO<sub>2</sub> results in a higher yield of lipids. In contrast, fourth-generation biofuel is pushed to

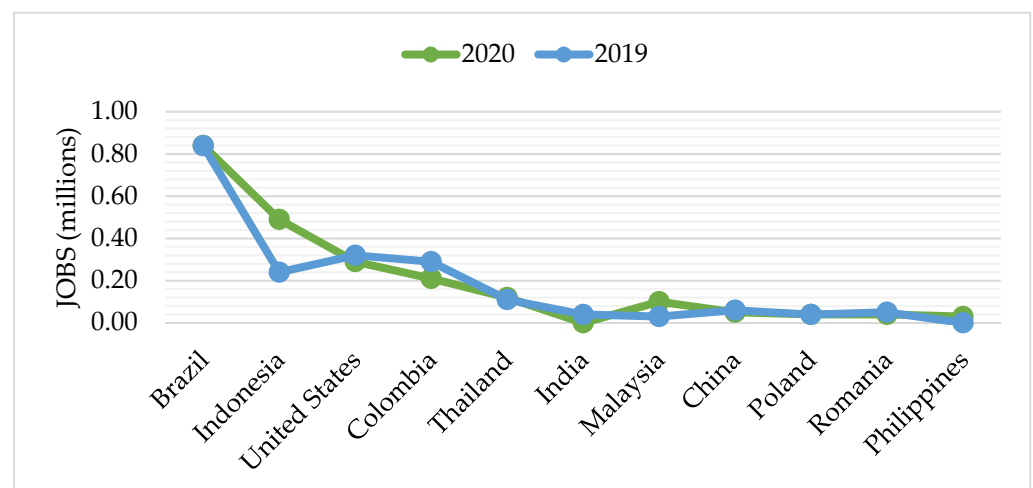
be carbon-negative with significant environmental benefits. Table 14 highlights the GHG reduction of genetically engineered microalgae.

**Table 14.** Fixation of greenhouse gases by genetically engineered microalgae.

Species	Method	Attainment	References
<i>Chlamydomonas reinhardtii</i>	Engineered small PSII antenna size <i>Chlamydomonas reinhardtii</i> by transforming a permanently active variant NAB1* (mutagenized NAB1) of the LHC translation repressor NAB1 to decrease antenna size by translation repression.	Photosynthesis efficiency has increased by 50%.	[137]
<i>Synechococcus elongatus</i> (Strain of freshwater cyanobacteria).	Genetically modified cyanobacteria generate and secrete carbonic anhydrase (Cas) in the medium. The secreted CAs converted dissolved CO <sub>2</sub> to HCO <sub>3</sub> <sup>-</sup> . The cyanobacteria absorbed HCO <sub>3</sub> <sup>-</sup> and converted it into biomass through photosynthesis.	Carbon intake has increased by 41%.	[138]

### 3.6. Current and Future Prospects of Biofuels

In 2019, global production of biofuels increased 5%, which was led mainly by a 13% biodiesel expansion (with Indonesia overtaking the US and Brazil to develop into the significant national producer (Figure 14). Meanwhile, bioethanol production increased by 2%. In 2019, global biofuel jobs were projected at 2.5 million [139].



**Figure 14.** Liquid biofuel employment in top 10 countries [139,140].

According to IEA, 3% annual production growth is projected for the next five years, but the decline in oil prices in 2020 (USD 30 per barrel) due to lower global demand stemming primarily from the COVID-19 pandemic decreased demand for biofuel crops [141]. Liquid biofuels are believed to be one of the most cost-competitive suppliers of high efficiency and a potential substitute for marine and aircraft fuels. The biofuel market was heavily affected by the COVID-19 pandemic in 2020. Global biofuel transport production is expected to be 144 billion liters in 2020, which is equivalent to 2,480,000 barrels per day (kb/d): an 11.6% decrease from peak production in 2019 and the first decline in annual production in several decades [139]. Figure 15 depicts the increase in yearly biofuel demand in various countries to meet the 2030 sustainable development scenario. Biofuel production in the United States and EU member states will fall short of SDS demand in 2030. While biofuel production in Brazil and India is estimated to rise, the SDS volume for 2030 must involve even faster growth. China and ASEAN countries are also experiencing production growth, which, if maintained, would meet the SDS's 2030 biofuel volume requirements [142].



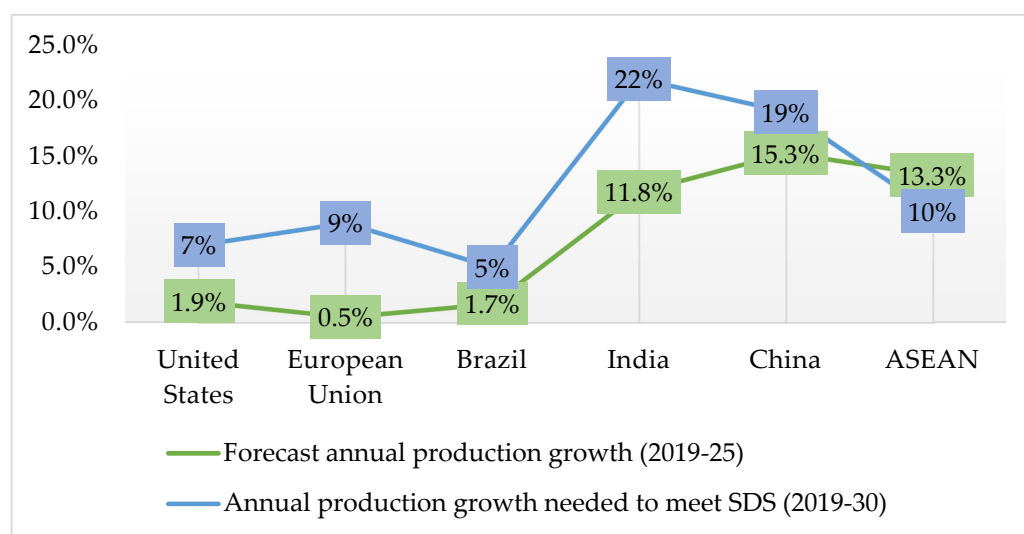


Figure 15. Biofuels annual production growth to meet sustainable development scenario, 2030 [142].

#### 4. Transition to a Circular Economy, Green Economy, and Bioeconomy

##### 4.1. Circular Economy

The fundamental principle that connects the ideas of circular economy, green economy, and bioeconomy is balancing economic, environmental, and social objectives. The circular economy is a possible solution to the optimal utilization of investments and ensures their long-term use. The processing units must demonstrate economic feasibility while minimizing waste and environmental effects to achieve a fully integrated circular bioeconomy. The new green deal from the European Commission focuses on priority areas where algae production may make a significant contribution: for example, the goals of the EU becoming climate neutral by 2050, the protection of biodiversity [143], and the development of a circular economy [144]. The circular economy is based on three basic principles:

- No waste, since products are renewable and biodegradable.
- Consumed resources are recovered without posing any security threats to the ecosystem.
- Energy for all processes is provided from renewable and sustainable sources.

Vitamins, proteins, amino acids, polysaccharides, fatty acids, sterols, pigments, fibers, and enzymes with unique properties can be synthesized from microalgae. In a microalgae-based circular bioeconomy, production wastes are recycled and reintroduced as secondary raw materials, i.e., to convert waste materials into new products in microalgae-based production systems (microalgae biorefineries). Microalgae are helpful in a circular economy as they can be used for the bio-remediation of nutrient waste and provide biomass for various commercial uses. Microalgal farming on nonarable land or coastal ecosystems reduces water demands, recycles nutrients, and converts atmospheric CO<sub>2</sub> into nutrient-rich sustainable feedstocks. This lays the groundwork for a circular aquaculture-based industry as part of a larger circular bioeconomy [145] contributing to several UN Sustainable Development Goals. The circular bioeconomy principle is currently gaining attention as a critical component of green technology. Recently, combined activated sludge (AS) microalgae wastewater treatment systems have been suggested as a more energy and commercially efficient alternative to traditional solutions for removing carbon and nutrients from liquid streams.

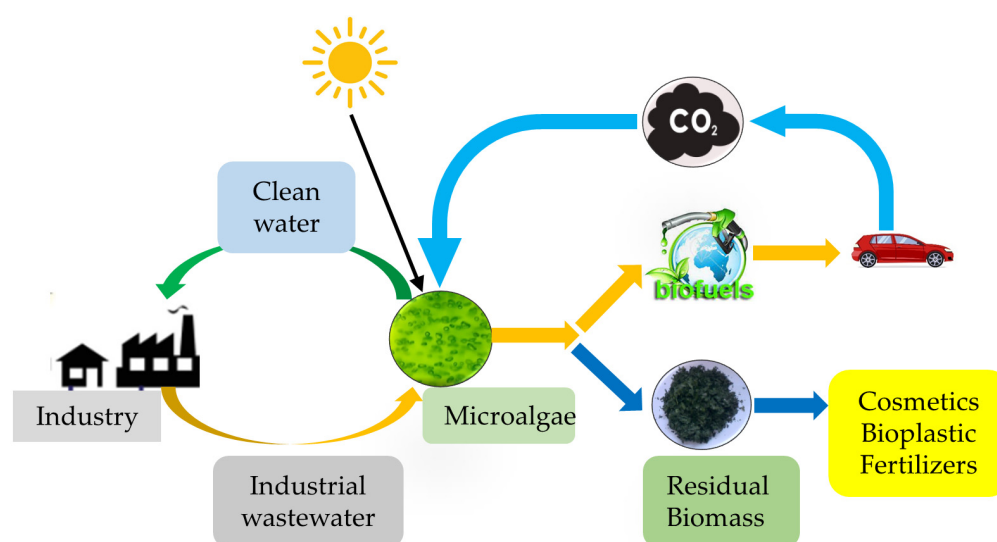
Furthermore, microalgae cultivation in wastewater leads to faster nitrogen and phosphorus removal, with up to 1 kg of dry biomass generated per m<sup>3</sup> of wastewater [146]. The EU goals for creating circular economy from waste sources [147] align with current urban water management paradigms [148]. Bioplastics are critical in transitioning the plastics sector from a wasteful linear economy to a circular economy. Algae-based bioplastic is considered to be a long-term solution for ensuring the circular economy practice. Bioplastic could produce natural materials via composting as the end of life cycle management [149].

Microalgae are a viable alternative source for making bioplastics. Several recent studies have looked at the production of bioplastics from microalgae biomass. According to Karan et al. (2019), the average requirement for microalgae cultivation to meet global plastic manufacturing is about 145 000 km<sup>2</sup>, which is only 0.028% of the Earth's surface area of 510,000,000 km<sup>2</sup> [150]. Polysaccharides agar, carrageenan, and alginate are used to make bioplastics from seaweeds, and seaweed waste from agar extraction has been suggested as a material filler [151]. The methods of producing PHA from genetically engineering algae is presented in Table 15.

**Table 15.** Methods of producing PHAs from genetically engineered algae.

Algae	Type of Product	Culture Mode	Polymer (Percentage of Dry Cell Weight)	Reference
<i>Spirulina plantesis</i>	PHB	Production of P(3HB) using CO <sub>2</sub> /acetate as a carbon source.	10	[152]
<i>Nostoc muscorum</i>	PHB	P(3HB) production under phosphate-starved medium + 1% (w/w) glucose + 1% (w/w) acetate with aeration and CO <sub>2</sub> addition.	21.5	[153]

A circular economy-based business model for obtaining several products from microalgae biomass for agricultural, nutrition, cosmetics, and aquaculture use is proposed in a study [154]. AlgaePro is developing technologies for growing microalgae in a circular economy approach, using biodegradables from urban waste, CO<sub>2</sub>, and waste heat from industrial sites [155]. Researchers in Italy and Slovenia are cultivating microalgae that absorb nutrients from agricultural wastewater as part of a European initiative called Saltgae. Once the water has been cleaned, the algae are dried and sold in cosmetics, animal feed, and fertilizers. Aquaculture of algae on industrial sites would enable a circular economy, turning wastewater into a viable resource [156]. A circular based economy using Micro/Macroalgae is presented in Figure 16.

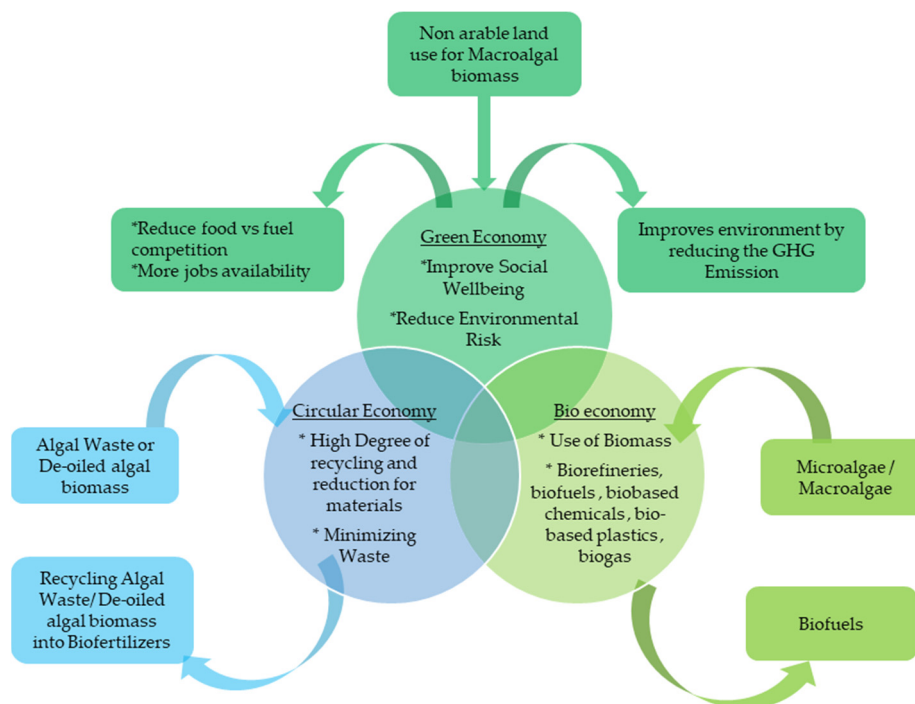


**Figure 16.** Systematic diagram of a circular economy.

#### 4.2. Green Economy

The green economy is a viable alternative to today's economic framework, which aggravates inequality, stimulates pollution, induces resource scarcity, and poses numerous environmental health risks. According to the UNEP [157] (a green economy is "low-carbon, resource-effective and socially equitable", with the ultimate goal of reducing environmental

impact and biodiversity loss as well as improving human well-being and social justice. The numerous benefits associated with algal energy, such as eco-friendliness and high productivity, lead to a green economy and sustainable growth by improving human health and quality of life [157]. The benefits of green economy using algae is presented in Figure 17.



**Figure 17.** Algal-based circular economy, green economy, and bioeconomy.

#### 4.3. Bioeconomy

Rapid urbanization, improved quality of life, and longer lifespans place demands on all manufacturing sectors producing food, chemicals, and fuels. As a result of the increased strain, land usage, drinkable water, fossil fuels, and other natural resources are anticipated to increase, resulting in unexpected climate change, biodiversity loss, and a decline in the capacity to manage ecosystems sustainably. The bioeconomy may offer a potential solution to this rising demand by substituting biomass-based commodities for depletable resources, reducing environmental impact. A bioeconomy is defined as “the development of long-term biological resources and the conversion of waste biological resources into value-added products such as food, feed, bio-based products, and bioenergy” [158]. The European Union introduced a plan for improving the bioeconomy in 2014, which was based on microalgae. Microalgae can significantly contribute to the economy, providing required biomass for human applications such as new drugs, cosmetics, food, and feed. The plan also included options for wastewater treatment and atmospheric CO<sub>2</sub> mitigation. Increasing the market development for microalgae-based products as long-term substitutes for currently available options will be critical to the success of a microalgae-based bioeconomy. The industrial units of the bioeconomy are biorefineries. The enormous potential of tiny microalgae favors a microalgae-based biorefinery and bioeconomy, generating huge opportunities in the global algae industry. Seaweeds can also be used as feedstock in biorefineries to produce fuels, pesticides, food additives, medicines, and other products, making them an essential part of the future bioeconomy [159].

## 5. Conclusions

Many developed and developing nations are steadily supporting biofuel production due to its potential benefits. This study examined the prospects of third-generation and

fourth-generation biofuels in the context of long-term sustainability. The following are some of the key conclusions from the study.

- Greenhouse gas emissions, environmental impact, loss of habitat, community conflicts, and substantial production costs are all associated with first-generation and second-generation biofuel. The use of edible biomass in first-generation biofuels has been of significant concern. It competes with the world's food requirements that limit its production to a few countries. The other limitation includes the high investment costs and poor efficiencies of feedstock conversion to biofuel. Second-generation biofuel has production limitations. Both the first and second generations have their strengths and weaknesses in terms of environmental and social impact. Hence, both generations will shortly be unable to meet the growing biofuel demand and energy transition targets.
- Developing third-generation and fourth-generation biofuels has broad implications on global socio-economic growth and sustainable development goals. It contributes to carbon balance, biodiversity conservation, sustainable water utilization, healthy air quality, soil conservation, and sustainable social enterprise.
- A large number of companies are investing heavily in biofuels to accelerate the global energy transition. Creating and applying sustainable biofuels standards will be more critical, with more entrepreneurs or companies committed to thinking that benefits will ultimately outweigh the risks.
- Nanotechnology has the potential to make next-generation biofuels feasible. The efficacy of biofuel can be significantly enhanced by incorporating nanomaterials into the process development. Magnetic nanoparticles, carbon nanotubes, metal oxide nanoparticles, and other Nano catalysts have the potential to become an essential part of long-term bioenergy production. However, most of the performance data are based on small-scale biofuel generation. Further research is needed to study the efficacy of nanotechnology in pilot-scale biofuel production.
- Biofuels from macroalgae and microalgae contribute to a circular economy by generating natural bio-products, such as proteins, pigments, fatty acids, and bioplastics. Therefore, algae-based green and bioeconomy opportunities include a new supply chain in manufacturing, cleaner fuel, food security, and GHG mitigation benefits.
- With the advancement of technology, extensive research, and development, it is reasonable to assume that third-generation and fourth-generation biofuel will become more appealing for commercial usage globally.
- If specific considerations related to sustainability requirements are met, third-generation and fourth-generation biofuels can be used realistically as a transitional approach. In the future, nature-inspired solutions hold more excellent prospects as sustainable energy sources for the planet.

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