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Review

Emerging Technologies for Enhancing Microalgae Biofuel Production: Recent Progress, Barriers, and Limitations

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Abstract: The world has heavily relied on fossil fuels for decades to supply energy demands. However, the usage of fossil fuels has been strongly correlated with impactful problems, which lead to global warming. Moreover, the excessive use of fossil fuels has led to their rapid depletion. Hence, exploring other renewable and sustainable alternatives to fossil fuels is imperative. One of the most sustainable fossil fuel alternatives is biofuel. Microalgae-based biofuels are receiving the attention of researchers due to their numerous advantages compared with those obtained from other types of feedstocks. Hence, it is essential to explore the recent technologies for biofuel produced from microalgae species and define the possible challenges that might be faced during this process. Therefore, this work presents the recent advancements in biofuel production from microalgae, focusing on emerging technologies such as those using nanomaterials and genetic engineering. This review focuses on the impact of nanoparticles on the harvesting efficiency of various microalgae species and the influence of nanoparticles on biofuel production. The genetic screening performed by genome-scale mutant libraries and their high-throughput screening may assist in developing effective strategies for enhancing microalgal strains and oil production through the modification of enzymes. Furthermore, the barriers that limit the production of biofuels from microalgae are introduced. Even though microalgae-based biofuels are perceived to engage with low negative impacts on the environment, this review paper touches on several environmental issues associated with the cultivation and harvesting of microalgae species. Moreover, the economic and technical feasibility limits the production of microalgae-based biofuels.

Keywords: microalgae; biofuel; fossil fuels; nanotechnology; genetic engineering; barriers and challenges

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1. Introduction

The rapid growth of the global population has inflated energy demand. Thus, the rigorous usage of fossil fuels has led to their depletion and diminution [1]. The rapid depletion of fossil fuels can mainly be attributed to their non-sustainable and non-renewable nature. Furthermore, fossil fuels have a high environmental impact [2], contributing to the generation of around 2 billion tonnes of CO_2 in 2021 and about 300 million tonnes of CO_2 in 2022 [3]. As a result, researchers are intensively searching for novel and viable alternatives to fossil fuels. Biofuels are now considered an attractive alternative to fossil fuels [4–6].

Biofuels can be converted from various biomass sources, including fruits or crop wastes, plants, food crops, algae, and manure [7,8]. Biofuels are converted from biomass sources via various processes, including pyrolysis [6], anaerobic digestion [7], or dark fermentation [8]. Pyrolysis produces bio-oil from biomass and then utilises it to generate biofuels. However, this process presents several shortcomings such as increased costs. Hence, co-pyrolysis technology has been developed to diminish the need for bio-oil modification [6]. Anaerobic digestion significantly contributes to enabling a circular economy by using a digestate as a feedstock for nutrients, biofuels, biochar, algal cultivation, and polyhydroxyalkanoates (PHA) [7]. Dark fermentation produces biofuels without the presence of light by employing anaerobic bacteria extracted from substrates with high carbohydrate levels [9].

Biofuels are categorised into first-generation, second-generation, third-generation, and fourth-generation [10]. First-generation biofuel is generated from edible feedstocks, including sugar cane, vegetable oils, starch, soybean oil, palm oil, and corn oil. Nevertheless, using edible feedstocks to produce biofuels has inflated the prices of food items and increased deforestation [11,12]. To mitigate these issues, second-generation biofuels were produced from the fats of non-edible feedstocks, including animal fats, wastes, and cooking oil [13]. Even though second-generation feedstocks are cost-effective, they contain impurities that hinder the conversion processes required for biofuel production [14]. Moreover, second-generation lignocellulosic biomass can efficiently produce biofuels; however, the pretreatment techniques that are employed to convert lignocellulosic biomass into biofuels result in the production of various compounds that inhibit micro-organisms and enzymes from production [15]. Hence, microalgae were chosen as the feedstocks for third-generation biofuels [16]. In both the third- and fourth-generations, the lipids and biomass residuals found in microalgae and macroalgae species are converted into biofuels [10]. Biofuels are becoming more attractive energy sources than fossil fuels due to their lower emissions of pollutants, lower contributions to global warming, and renewability and sustainability [17,18]. Fossil fuels are accountable for the release of 29 gigatons per year of CO_2. Hence, the accumulated CO_2 emissions from fossil fuels are up to 35.3 billion tons [19]. On the other hand, algae (which are used for biofuel production) release high levels of oxygen (from 10 to 45%) with significantly low levels of sulphur. Moreover, algae-based biofuels are sustainable, reliable, non-polluting, and accessible [20]. Algae-based biofuels can generate up to 25% of the global energy supply [21]. Due to these advantages, there is a focus in research on producing biofuels from microalgae [22].

Microalgae are unicellular microscopic organisms found in terrestrial and aquatic ecosystems. Microalgae can flourish in various environments due to their high tolerance to pH, temperature, light intensities, and salinities. The main components for microalgae growth are sunlight, CO_2, nutrients, and water [23]. Microalgae capture CO_2 from the atmosphere and generate oxygen in the air [24]. It has been evident that about 1 kg of biomass produced by algae can account for 1.83 kg of CO_2 [20]. Moreover, the biological system of microalgae enables them to produce organic compounds from sunlight [25]. There are approximately 80,000 microalgae species, and around 40,000 of these species have been studied on a large scale for commercialization [26].

Numerous researchers have studied microalgae, in awe of its multifaceted applications [27]. Microalgae species can produce various essential biochemicals that can be utilised as feedstock for multiple products, such as proteins, carbohydrates, and lipids [28]. Nevertheless, innumerable biochemicals produced from microalgae species have still not been explored [27]. Microalgae applications can mainly be categorised into industrial, food, environmental, and pharmaceutical applications [29], as shown in Figure 1. Figure 1 displays the main categories of microalgae applications. Microalgae species produce various types of carotenoids, enzyme polymers, lipids, peptides, polyunsaturated fatty acids, antioxidants, and natural dyes, which can be utilised in multiple industrial items. Moreover, microalgae are commercially employed to produce cosmetics and pigments [30,31]. Microalgae have vast applications in the food industry: they produce animal and aquatic feed, nutritional supplements, and functional foods from biomass residuals. Biomass residual is high in proteins but low in lignin; thus, it can be utilised for the production of various biorefinery-based commercial products, including cosmetics, nutritional supplements, and pharmaceutical products [27] For environmental applications, micmroalgae play an essential role in wastewater treatment, where they purify the water as they take up the nutrients found in wastewater, such as phosphates, nitrates, and other organic constituents [32]. The potential of microalgae-mediated wastewater treatment systems to remove different micropollutants is attracting attention. Pharmaceuticals and antibiotics pollute aquatic systems and have dangerous consequences for flora, wildlife, and ecosystems. Microalgal systems are useful because they can break down pharmaceutical and antibiotic contaminants while recovering nutrients from wastewater. The primary processes for the degradation of organic pollutants by microalgae are adsorption, degradation, and accumulation. Research has concentrated on several methods, including improved reactor design, the utilization of algal–microbial consortia, and combining wastewater treatment systems with resource recovery and by-product accumulation technology to increase the effectiveness of treating wastewater [33]. Furthermore, microalgae are used for CO_2 sequestration and pollutant reduction [34]. Microalgae species generate bioactive compounds, such as acetylic acids, vitamin B, and lutein [35], which can be utilised in the pharmaceutical industry to develop antibiotics and antimicrobial, antiviral, and anti-inflammatory drugs [36].

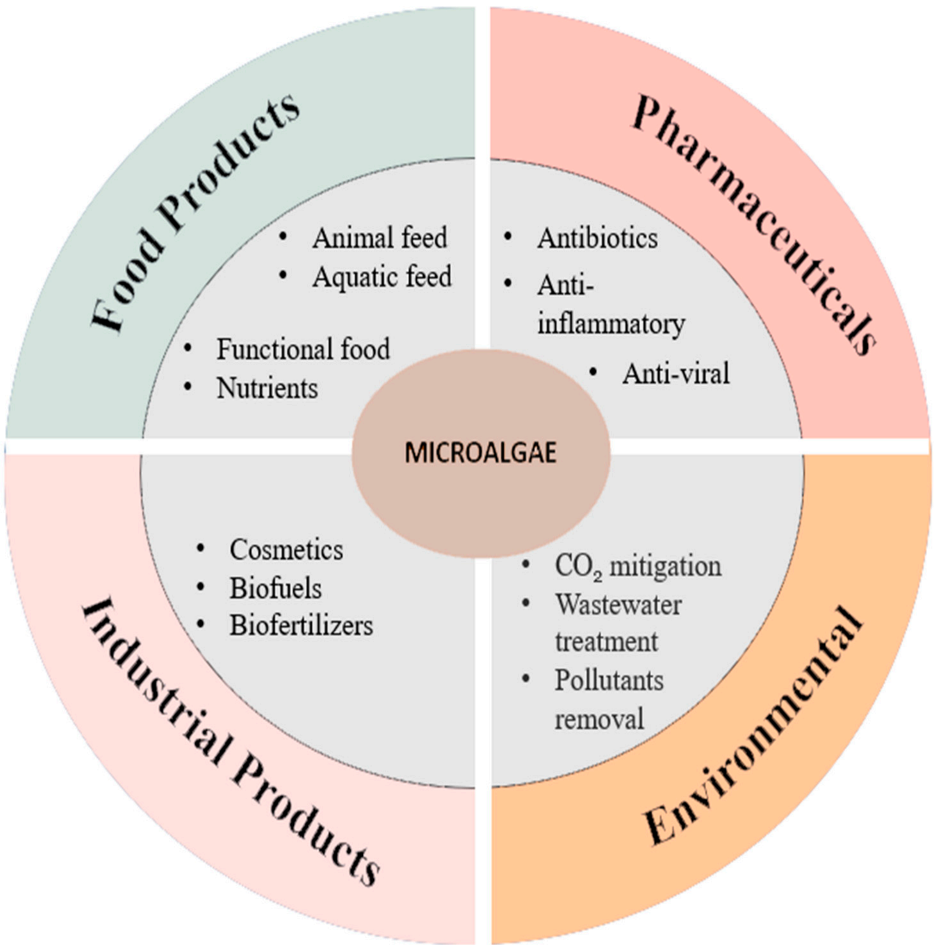

Figure 1. A display of the various applications of microalgae.

Biofuel is a type of fuel produced chemically from biomass as opposed to that obtained through a long geological process. Fuels in their liquid and gaseous forms are typically referred to as biofuels and are highly useful in the automotive industry. These biofuels are simple to mix with the currently available liquid fuels, such as gasoline and diesel [37]. Algae have been studied for their extraordinary capacity to produce a wide range of biofuels simultaneously with the production of value-added products and wastewater treatment. Lipids, which are mostly found in thylakoid membranes, can be present at significant levels. Their biofuels are both highly biodegradable and harmless. There are various forms of algal biofuels. Figure 2 shows a summary of the various processes for obtaining biofuels from microalgae. The carbohydrate portion of the biomass is utilised to make bioethanol through the fermentation process. At the same time, the oil content is used to make biodiesel through transesterification. The leftover biomass can be used to produce methane, fuel gas, fuel oil, or directly for electricity and heat [38], which can be accomplished via thermochemical conversion, biochemical conversion, and direct combustion. The thermochemical process includes gasification, pyrolysis, and hydrothermal liquefaction (HTL); each is performed at specified temperatures to produce the fuels. The biochemical process involves using microorganisms and/or enzymes to extract the organic part from the biomass. Direct combustion requires air for the direct conversion of algae into electricity/heat.

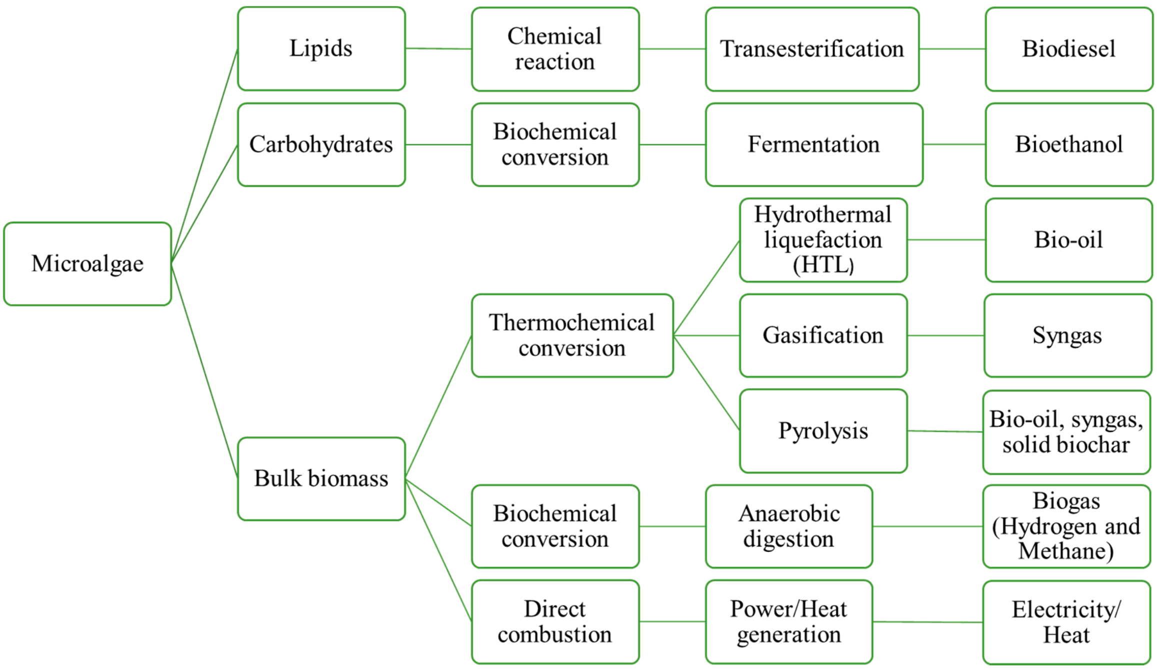

Figure 2. The conversion of microalgae into different biofuels.

Several researchers have summarised the progress achieved in the various aspects related to microalgae, including cultivation [39]; pretreatment methods [40–42]; the application of microalgae for wastewater treatments [41], CO_2 capture [41,42], such as fuel cells [43]; bio-photovoltaics [44]; and producing chemicals [17], and they have discussed the role of microalgae in achieving sustainable development goals [26]. Moreover, various researchers have summarised the progress achieved in the application of the different types of biofuels in hydrogen production [45] and biogas [46]. However, few works have summarised the progress achieved in using new technologies to improve biofuel production from microalgae, such as with nanoparticles and genetic engineering, which is the main target of this work. Furthermore, this work summarises the barriers and challenges facing biofuel production from microalgae.

2. Methodology

This paper focuses on reviewing the recent advances in the production of biofuels from microalgae and the challenges faced throughout this process. As a result, the collected papers were associated with the topic of biofuel production from microalgae species. The Scopus database was chosen as the main source for data collection. The following keywords were used to search for published papers related to this topic: “Biofuel” AND “Recent

Progress" AND "Microalgae" and "Biofuel" AND "Challenges" AND "Microalgae". After this search, the first keywords showed 38 papers related to the recent advances in algae-based biofuels. From these papers, it was evident that the main advancements in the production of biofuels from microalgae are related to genetic and metabolic engineering, nano-additives, and catalysts. Thus, a detailed search was initiated to thoroughly analyse these advancements, where the following keywords were utilised: "Biofuel" AND "Microalgae" AND ("Genetic" OR "Metabolic" OR "Nano*Additives" OR "Catalyst"). The search engine presented 1272 published papers related to these advancements, whereas the keywords associated with the challenges faced during the production of biofuels from microalgae presented around 829 published papers. After skimming through the papers, it was found that these challenges could be economical, technical, or environmental. Hence, another detailed search was initiated to explore all the possible barriers related to the production of biofuels from microalgae; therefore, the related keywords were: "Biofuel" AND "Challenge" AND ("Environment*" OR "Economic*" OR "Technic*"). For these keywords, the search engine showed around 3730 published papers.

Based on the obtained papers, an increasing trend in the number of published papers discussing the production of biofuels from microalgae was spotted. As shown in Figure 3, the number of published papers on this topic has been increasing since 2006. At first, the increase in the number of papers was small; however, since 2010, there has been a significant increase in the number of published papers. This increasing trend hints at the importance of the production of biofuels from various microalgae species. The importance of this topic is also reflected in Figure 4, which demonstrates that around 68.8% of the published papers are research papers that focus on studying the production of biofuels from microalgae.

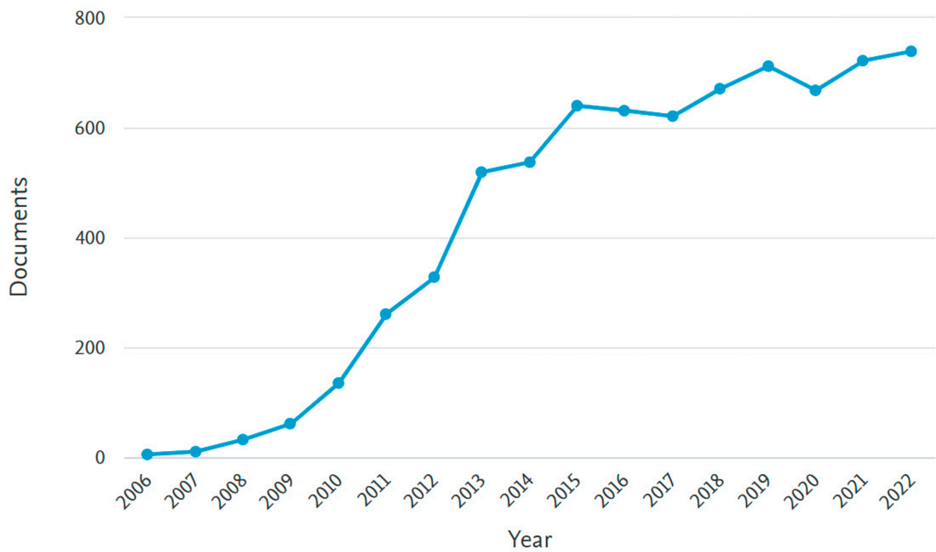

Figure 3. The number of published papers discussing the production of biofuels from microalgae from 2006 to 2022.

After determining the papers that are related to the production of biofuels from microalgae, the VOSviewer® software was employed to analyse these papers. Figure 5 was produced via the VOSviewer software to present a network visualization of the keyword co-occurrence in the selected papers. Each node presented in the network signifies a keyword. The node size designates the number of times the keyword was repeated. The connections between the nodes show the co-occurrence between the keywords, while the thickness of the connections between the nodes represents the number of times the keywords co-occurred together. The figure demonstrates an overall view of the co-occurrences between the keywords, where microalgae, genetic engineering, biomass, biosynthesis, and carbon dioxide seem to be the most dominant keywords that are most widely repeated by authors throughout the selected research papers.

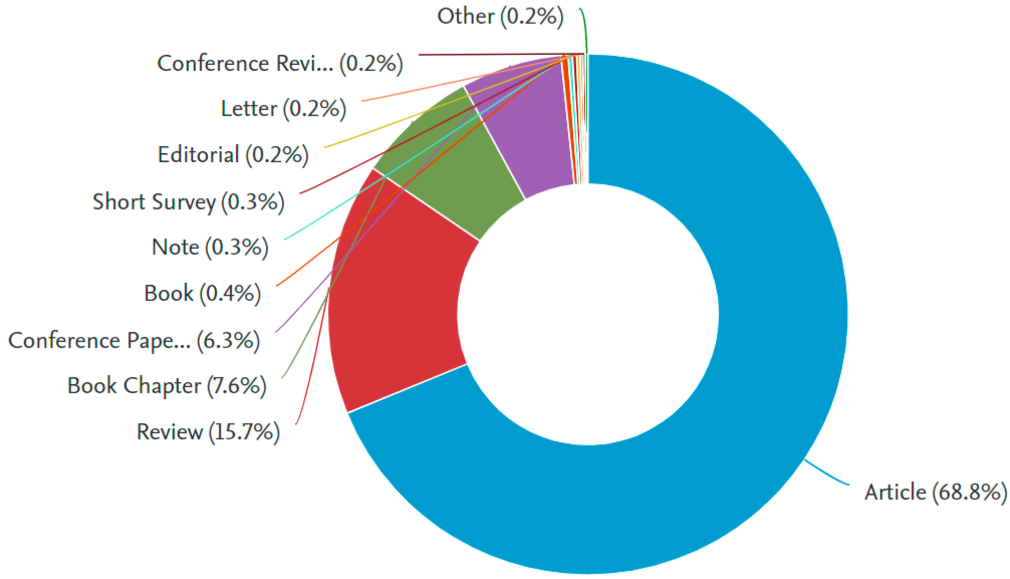

Figure 4. The type of published paper.

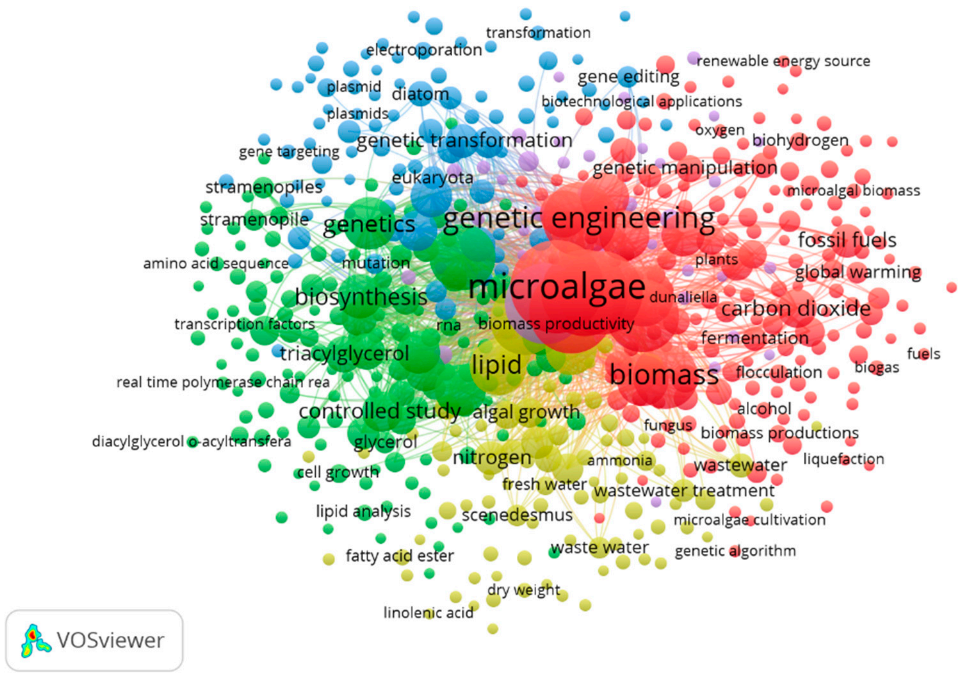

Figure 5. Visual demonstration of the network of keywords co-occurrences and connections obtained via the VOSviewer software.

3. Recent Progress in Biofuel Production from Microalgae

3.1. Genetic Engineering for Improved Biofuel Production from Microalgae

Technological advancements using genetic engineering and metabolic engineering can be performed to increase the yields from algal biomass. Oil production from microalgae can be improved by the modification of lipid synthesis enzymes, or other competing alternative routes, to direct the transport of carbon and reductive equivalents away from other pathways. The most popular method is the modification of certain genes that are responsible for different phases in a metabolic process. However, due to the complex control of lipid production in microalgae, this approach resulted in varying degrees of success [47,48]. *Chlamydomonas reinhardtii* is a green alga that has attracted attention for genetics research. Hence, the majority of the techniques for gene knockdown have been created for this species. However, diatoms and other algae that are more important and can be utilised for industrial purposes are now being increasingly developed as tools. Controlling the lipid synthesis for a specific strain can strongly affect the amount and quality of biofuel produced (biodiesel) [49]. The creation of effective tactics for improving microalgal strains may be aided by the genetic screening carried out by genome-scale mutant libraries and their high-throughput screening. As a result, such knowledge is absolutely necessary for microalgae strain bioengineering. Figure 6 shows the usual strategic flow from the combination of various datasets to the enhancement of the microalgal strain. The molecular technologies for stable transformation, selective screening, and accurate gene targeting are crucial for carrying out genetic manipulation in the process of strain enhancement with genetic engineering [50].

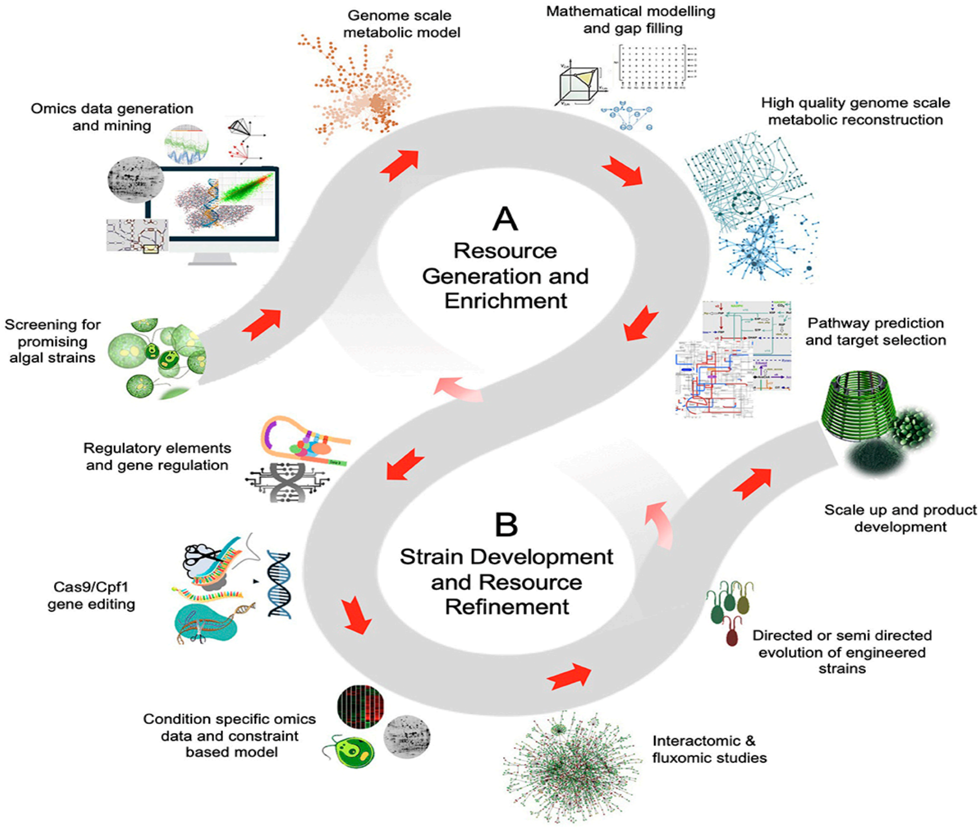

Figure 6. (**A**) Creation and enrichment of resources: High-quality curated data can be produced through high-throughput technologies, intensive bioinformatic analysis and computation, and substantial scientific interest in microalgae. (**B**) The development of microalgae strains can be aided by the application of cutting-edge technologies such as genome editing and high-throughput variant selection, as well as findings from metabolic models. The metabolic flux changes in mutants frequently imply that an organism evolved to optimise flux reconfiguration. The increased production of desired products may be the goal of the shift in the flux balance. Additionally, the knowledge gained through refined modelling and genomic-editing studies opens up new research opportunities [50], open access.

In microalgae, lipid production commences with acetyl-CoA carboxylase (ACC). According to Roessler's study, the enzyme activity increased by 2–3 times when the ACC gene from *Cyclotella cryptic* microalgae was overexpressed [51]. Sheehan et al. [52] came to the conclusion that increasing the expression of just the ACC gene might not be enough to improve the entire lipid production process. Enhancing lipid production might also result from blocking competing processes. An increase in lipid content was achieved by inactivating genes directly involved in fatty acid oxidation as well as those involved in triacylglyceride (TAG) and free fatty acid activation. Gene inactivation would have to be accomplished either through mutation or through the use of RNA silencing. In addition to possibly increasing fat storage, knocking down lipid catabolism genes may potentially negatively impact cell development and division [49].

Many microalgae commence TAG storage during the day and drain those stores at night to sustain cellular ATP requirements and/or cell division during diel light–dark cycles. Therefore, preventing oxidation would stop TAG from being lost throughout the night, but most likely at the expense of slower growth. Therefore, this approach might not be helpful for microalgae cultivated in outside open ponds, but it might be a good way to boost lipid production in microalgae grown in photobioreactors with continuous light and/or external carbon sources [49]. A study also reported the overexpression of malic enzyme (ME), which was identified previously for its role in metabolic pathways, such as lipogenesis, energy metabolism, and photosynthesis, resulting in enhanced lipid accumulation and a successful biodiesel provider [53]. Table 1 shows the improvement in lipids due to gene overexpression in several microalgal species.

Table 1. Lipid improvement due to gene overexpression in several microalgal species [54].

Species	Genes Overexpression	Lipid Increase
Chlamydomonas reinhardtii	ACCase	TAGs increase by 2.4 times
Phaeodactylum tricornutum	G6PD	Lipids increase by 55.7%
Phaeodactylum tricornutum	GPAT1; LPAT1	TAGs increase by 2.3 times due to nitrogen depletion
Phaeodactylum tricornutum	G3PDH	Lipids increase by 1.9 times with a small decrease in growth
Phaeodactylum tricornutum	G6PD	Lipids increase by 2.7 times
Chlorella protothecoides	ME	Lipids increase by 2.8 times
Chlamydomonas reinhardtii	PSR1	Starch granules improvement, reduction in lipid amount
Nannochloropsis salina	bZIP	Enhancement in growth and lipid
Chlamydomonas reinhardtii	DGTA	Increased saturated fatty acids
Chlorella minutissima	GPAT; LPAAT; DGAT	A 2-time increase in lipid amount
Nannochloropsis oceanica	NoDGAT1A	A 2.4-time enhancement in TAGs
Chlorella pyrenoidosa	NAD(H) kinase	A 1.6-time enhancement in lipid amount
Chlamydomonas reinhardtii	LPAAT	A 20% enhancement in TAGs
Nannochloropsis oceanica	DGAT	A 69% enhancement in lipids

To better understand the genetic structure and metabolites of microalgae, researchers can capture and annotate any coding or non-coding RNA using transcriptome assessment with next-generation sequencing (NGS) [55]. The ability to uncover interesting metabolic pathways and possible targets for metabolic engineering in microalgae, as well as to facilitate functional genomic research for next-generation biofuel production, was proven by utilising transcriptome data from next-generation sequencing. The gene encoding for important enzymes responsible for the production and catabolism of fatty acids in *Dunaliella tertiolecta* (*D. tertiolecta*) were effectively identified based on the functional annotation of the transcriptome. The majority of the enzymes needed for the biosynthesis, elongation, and metabolism of fatty acids are present in the *D. tertiolecta* transcriptome. These results add to the biochemical and molecular knowledge required for the metabolic engineering of microalgal fatty acid production. Transcripts for enzymes involved in the degradation of TAG were also found. The TAG content can possibly increase because of the suppression of TAGL and other TAG-degrading enzymes. In addition, a large number of transcripts that encode enzymes involved in the biosynthesis and catabolism of starch were found in *D. tertiolecta*. Accumulated starch represents a desirable source for the ethanol, butanol, and hydrogen generation of a number of biofuels. The environmental and pretreatment process drawbacks associated with employing plant-based starch and lignocellulosic materials for ethanol production may be solved by producing biofuel from starch obtained from microalgae [56]. The sequencing and de novo transcriptome assembly for the microalga Eustigmatos cf. polyphem have also been also carried out. Metabolic pathways responsible for the biosynthesis and metabolism of carbohydrates, fatty acids, TAGs, and carotenoids have been rebuilt, and transcripts encoding important enzymes have been effectively identified. The significant number of transcripts identified offers a solid foundation for future genomic studies on oleaginous microalgae and supports comprehensive genome annotation. These discoveries make a significant contribution to the genetic engineering of this organism to increase the supply of feedstock for industrial microalgae biofuel production [57].

3.2. Catalysts

3.2.1. Catalysts for Oil Extraction

Recent studies have concentrated on catalytically improving the oil extraction process from algae. Ponnusamy et al. [58] investigated the photocatalysis process to extract bio-oil from *Nannochloropsis oculata* microalgal biomass, utilising solar radiation and nanoparticle catalysts to eliminate the dewatering and drying processes while obtaining the oil from algae, which saves money and energy. Furthermore, titanium dioxide photocatalysts are chemically and thermally stable, show attractive photoactivity characteristics in wet environments using solar energy, are affordable, and are less toxic [59]. In most cases, catalytic upgrading occurs between bio-oil and hydrogen gas reactions under conditions of high pressure, temperature, and reaction time. Distilled microalgae particles were catalytically upgraded using a Pd/C noble metal catalyst. The upgrading after the distillation process provided better quality for the produced biofuel [60].

Homogeneous Catalysts

Homogeneous catalysts are soluble in water at normal temperatures. By accelerating the water–gas shift reaction, the reaction prevents the production of char/tar while increasing the product yield. Na_2CO_3 is the most widely used catalyst, and it can increase BTEX (benzene, toluene, ethylbenzene, and xylene) production and change C5 into C18 aliphatic hydrocarbons; they are important components for gasoline and diesel fuels [61]. Generally, the working temperature mostly affects alkali catalyst performance regardless of the species assessed. Organic (HCOOH and CH_3COOH) and inorganic acid (H_2SO_4) catalysts have also been deployed [61]. Yang et al. [62] used H_2SO_4 and CH_3COOH in the catalytic process of HTL for *Enteromorpha prolifera* algae, which resulted in a maximum yield of 28% bio-oil. The possibility of using homogeneous catalysts on HTL in industrial settings is hampered by a few obstacles. Decarboxylation, isomeration, and fatty acid aromatization can be accomplished with relatively little efficiency using carbonate-based catalysts [61].

Heterogenous Catalysts

Heterogeneous catalysts are insoluble in water and can be recovered. They are preferred over homogeneous catalysts due to a smaller corrosion rate and better catalytic activity under harsh working circumstances, which frequently result in damage to the homogeneous catalysts. Despite their advantages, some circumstances can limit their effectiveness [61]. Xu et al. [63] revealed that the catalyst can be deactivated by the presence of impurities, including ash and excessive amounts of nutrients, after a given period of time during operation. Increased levels of S, N, and O derivatives result in deactivating the catalyst. A recent study investigated the use of waste-derived heterogeneous catalysts (which are calcined *Musa balbisiana* colla peel (CBPA)), calcined water hyacinth (CWH), calcined Carica papaya stem (CCPS), calcined Tectona grandis leaves (CTGL), and potassium-impregnated *Rhodotorula mucilaginosa* de-oiled biomass-activated carbon (K-RAC) for bio-ethanol production from algal biomass. The highest ethanol yield was 68.32%, which was successfully obtained using a CTGL catalyst as a base [64].

3.2.2. Catalysts for Biodiesel Production

For biodiesel production, acid–base catalysts are among the best candidates as a result of their capability to simultaneously catalyse the esterification of free fatty acids (FFAs) and the transesterification of triglycerides (TG) [65]. Catalysts, including basic and acidic homogeneous and heterogeneous catalysts, as well as biocatalysts, have been reported in the literature. Table 2 shows various basic and acidic catalysts used for biodiesel production from microalgae.

Table 2. Basic and acidic catalysts used for biodiesel production from microalgae.

Catalyst	Optimum Conditions	Microalgae Species	Conversion Efficiency (%)	Reference
Phosphotungstic acid HPW/ZIF-67	HPW/ZIF-67 weight ratio = 0.25. Oil: MeOH molar ratio of 1:20, 1 wt% catalyst concentration, 200 °C for 90 min.	*Chlorella vulgaris*	98.5	[66]
Co-based ZIF-67	MeOH:lipids ratio of 20:1, 3 wt% catalyst concentration, 550 °C for 30 min.	N/A	96	[67]
SiC/NaOH-GO	13:1 weight ratio of SiC/NaOH to GO, 5 wt% of catalyst loading, 65 °C for 6 min.	*Chlorella vulgaris*	96	[68]
BaO/CaO–ZnO	1:18 MeOH:oil molar ratio, with 2.5 wt % catalyst, 65 °C for 120 min.	*Spirulina platensis*	69.56% FAME	[69]
$Bi_2O_3/ZrO_{2(CTAB)}$	Lipid:MeOH ratio of 1:90 (g/mL), catalyst loading of 20 wt.%, 80 °C for 6 h.	*Nannochloropsis*	73.21% FAME	[70]
CaO	Oil:MeOH molar ratio of 1:150, catalyst loading of 9 wt.% for 1 h.	*Nannochloropsis oculata*	84.11% FAME	[71]

Each proposed catalyst has pros and cons of its own. For example, basic and acidic homogeneous catalysts are cheaper and provide competent mass transfer; however, corrosion, nonrecovery, and the creation of soap are the main drawbacks affecting its use for mass production. While acidic and basic heterogeneous catalysts can be used to overcome these issues, they still have limited mass transfer, are expensive, and are weaker in FFA esterification and lipid transesterification. Bio-catalysis is an expensive process but can produce a pure product that eliminates side reactions [72]. Biochars have various benefits when employed as catalysts or catalyst supports, including low cost, a high surface area, and the ability to alter surface functional groups. Biochars can be utilised as effective heterogeneous acid or base catalysts to produce biodiesel after activation or modification. Sulphonated biochars are the most widely utilised heterogeneous catalysts for making biodiesel. It is simple to sulphonate biochar by impregnating it with concentrated H_2SO_4 under high temperatures or by subjecting it to gaseous SO_3, which results in the immobilization of -SO3H groups on the biochar's surface. Free fatty acid (FFA) esterification or the transesterification of triglycerides with alcohols to produce biodiesel can both be catalysed by these -SO_3H groups [73]. Recently, the catalytic activity of biochar produced from sugarcane bagasse, coconut shell, corncob, and peanut shell was assessed after surface functionalization. For effective catalysis, peanut shell that had been pyrolysed at 400 °C and had a sulphonic acid density of 0.837 mmol/g and 6.616 m^2/g of surface area was chosen. The effectiveness of transesterification was assessed using a catalyst loading of 1–7 wt% and a methanol–oil ratio of 6–30:1 at temperatures between 55 and 85 °C for 2–8 h. With a catalyst concentration of five weight percent and a MeOH:oil ratio of 20:1 at 65 °C after 4 h, a 94.91% biodiesel yield was produced [74].

Nano-catalysts are now being used in transesterification due to their advantages over currently employed conventional approaches. The CaO nanoparticle exhibited an improvement in biodiesel yield from 93 to 96%. Furthermore, a study reported the use of nano-catalysts for biodiesel production from algae, and with a 30:1 methanol to oil molar ratio and a reaction period of 3 h at 80 °C, the greatest possible fatty acid methyl ester (FAME) yield of 99.0% was obtained over a 3 wt% of Ca $(OCH_3)_2$ (nano-calcium methoxide) catalyst [75].

3.2.3. Nano-Additives

Nanotechnology is a notable research field with various applications in the energy sector, specifically in the bioenergy sector. Nanotechnology has induced revolutionary modifications to biofuel transformation and enhancement processes [10]. Nanotechnology is described as the creation and usage of devices and materials at the nanoscale, 10^{-9} m [76]. Nano-additives, including nanocrystals, nanomagnets, nanofibers, and nanodroplets, have been developed based on nanotechnology to enhance biofuel production and increase the efficiency of biofuel usage [77,78]. The traditional methods of generating biofuels via microalgae have posed several issues associated with the costs of cultivation and harvesting, land for large-scale microalgae cultivation, energy utilization for generating biofuels, and the environmental impacts of cultivating microalgae and producing biofuels. Consequently, nanotechnology has been integrated into the biofuel industry to remove some of the former limitations [79]. Nanotechnology can be applied to various stages of microalgae cultivation and produce biofuels due to its economic benefits, technical advantages, and positive environmental impacts. Based on the previous literature, nanotechnology has improved the cultivation process of microalgae and produced maximum biofuel yields. Different nanostructures and materials, including nanoparticles, nanosheets, nanotubes, and nanofibers, have been studied as efficient and productive nano-catalysts to directly and indirectly increase biofuel production [80,81]. Moreover, nanomagnets can be employed as an enzyme immobilization carrier, producing biodiesel, bioethanol, and biomethane due to their high resistance to magnetization and enhanced magnetic characteristics [82]. Moreover, immobilising cellulase on magnetic nanoparticles can be utilised for microalgae cell wall hydrolysis and lipid extraction. In a study carried out by Duraiarasan et al. [83], magnetic nanoparticles were employed to hydrolyse microalgae polysaccharide-walled cells for lipid extraction. The authors were able to hydrolyse the cell walls after exposing the immobilised cellulase to the added magnetic nanoparticles. The results of this study indicated that biodiesel production was optimised by 93.56% due to the added nanoparticles. Meanwhile, Zaidi et al. [84] studied the impact of microwaved magnetic nanoparticles on biohydrogen and biogas yields. The authors reported that the microwaved magnetic nanoparticles enhanced the production of biohydrogen and biogas, where biohydrogen production was 51.5%, and biogas yield was 328 mL. Apart from these significant advantages of magnetic nanoparticles in biofuel production, Khoo et al. [85] reported that preliminary studies are exploring the economic side and the applicability of integrating magnetic nanoparticles for large-scale biofuel production. Nanomagnetic powder can also be utilised to suspend microalgae cells in the photobioreactor cultivation process to group cells to uniformly distribute nutrients and lights in the reactor. Additionally, for easier accessibility to light, silver nanoparticles have been coated onto the surface of a photobioreactor [86]. On a similar note, nanospheres have been integrated by ultrasonication and irradiation techniques into the culture and harvesting processes, including lipid removal, hydrolysis, biofuel purification, and transesterification, to enhance biofuel production [87].

In general, nano-additive applications for enhancing the biofuel yield from microalgae species are classified into applications of nano-additives for cultivating microalgae, applications of nano-additives for converting biomass extracted from microalgae into biofuels, and applications of nano-additives for biofuel production from microalgae. The main goal of applying nano-additives for cultivating microalgae is to enhance biomass yield while substantially decreasing the required area for cultivation. Nanotechnology has also been pragmatic in immobilising enzymes to enhance the production yield. Additionally, the addition of nanoparticles to the harvesting process of microalgae has led to remarkable results, where all the harvesting efficiencies of the microalgae species surpassed 90%, and, in one case, modified Chu 13 nanoparticles were added to *botryococcus braunii* microalgae species, and the microalgae exhibited a harvesting efficiency of 100% [86]. Table 3 demonstrates the impact of various nanoparticles on the harvesting efficiency of different microalgae species. In addition to that, nanoparticles, such as optical fibres, can enhance the light conversion efficiencies in photobioreactors. Optical fibres lower the energy utilization for cultivating microalgae, decrease the costs associated with the need for additional lighting, and enhance the efficiency of microalgae [79]. Based on a study conducted by [88], the application of metal nanoparticles to localised surface plasmon resonance enhances the distribution of light at specific wavelengths. Torkamani et al. [89] validated this by suspending silver nanoparticles in plasmon photobioreactors and concluded that the metal nanoparticles had effectively scattered the blue light. In return, the light enhanced the photosynthetic processes of green and blue-green microalgae species. As a result, a noticeable increase of 30% in the biomass yield from the microalgae was reported. The benefits of nanoparticles are not limited to enhanced light distribution; they also increase the CO_2 intake yield and the CO_2 sequestration, which enhances biomass yields.

Biodiesel is one of the most widely utilised and known types of biofuels. Hence, various nanoparticles have been applied to the conversion processes of biodiesel for further enhancements in biodiesel yield and conversion efficiency [87]. Other nanoparticles, including nanofluids, solid nanoparticles, and nanodroplets, have displayed tremendous enhancements in the lubricity, catalytic performance, and heat transfer and mass transfer efficiencies of biofuels [90,91]. These enhancements boost the combustion of microalgae-based biofuel in the applications of various engines. As a result, it has been shown that nanotechnology has boosted the combustion efficiency of microalgae-based biofuel and lowered pollutants and greenhouse gas emissions by 72% [90,92].

Table 3. A summary of the impacts of various nanoparticles on the harvesting efficiency of different microalgae species.

Microalgae Species	Nanoparticles	Harvesting Efficiency	Findings	Reference
Chlorella ellipsoidea	Iron oxide, Fe_3O_4	97%	The added nanoparticles enhanced the efficiency of harvesting microalgae, enabled fast implementation, and decreased energy and water usage during microalgae harvesting. Hence, magnetic separation appears to be a prevailing technique for optimal microalgae harvesting.	[93]
Chlorella vulgaris	Iron oxide, Fe_3O_4	91%	The nanoparticles enhanced the microalgae harvesting process due to their prevailing efficiency and stability.	[94]
Chlorella sp.	Magnetic iron oxide	95%	A coating of dendrimer nanoparticles significantly enhanced the process of harvesting microalgae. Moreover, a positive correlation between microalgae harvesting and coating thickness was discovered.	[95]
Chlorella ellipsoidea	Iron oxide, Fe_3O_4	>95%	This study involved magnetic flocculant synthesis due to its ability to enhance harvesting efficiency without adversely affecting the environment.	[96]
Chlorella sp.	Chitosan/magnetic nanoparticles	99%	Not only did the nanoparticles attain a harvesting efficiency above 99% but they also did not result in any negative impacts on the growth rate of the microalgae.	[97]

Table 3. *Cont.*

Microalgae Species	Nanoparticles	Harvesting Efficiency	Findings	Reference
Chlorella vulgaris	Fe_3O_4	99%	Applying nanotechnology enabled the rapid harvesting of microalgae, where, in less than half a minute, about 99% of the microalgae cells were harvested. Additionally, the nanoparticles diminished the impacts of pH levels on the microalgae organic outputs.	[98]
Nannochloropsis sp.	Fe_3O_4	97.9%	The added iron oxide nanoparticles led to an enhanced microalgal cell magnetophoretic separation. Additionally, there was no need to modify the pH levels, as they did not impact the microalgal harvesting efficiency.	[99]
Nannochloropsis sp.	Nano-chitosan	97%	The addition of nanochitosan as the flocculant led to an enhancement of 9% in the biomass recovery and increased the microalgal growth level by 7%.	[100]

4. Barriers and Limitations

Despite the numerous advantages algae-based biofuels present, there are still several challenges faced for their broad commercialization. These challenges are categorised into techno-economic and environmental restrictions [101]. Environmental challenges can be further classified into land and water usage, greenhouse gas emissions, other pollutant emissions, and biodiversity loss.

4.1. Techno-Economic Barriers

Although biofuel production from microalgae technologies is promising, the associated high costs prevent the commercialisation of these technologies. Figure 7 depicts the economic differences between biofuel production from the first-, second-, and third-generations and fossil fuel production. The development of technological innovations that are economically viable, such as microalgal strain enhancement for enhanced oil output, is needed [48]. Moreover, research may not advance as fast it could due to methodological restrictions such as high equipment costs for scaling up [59].

Before biofuel production, the harvesting and dewatering of microalgal cells represent another barrier. Dewatering diluted cell suspensions is extremely difficult and time-consuming; some technologies are also costly [102]. According to studies, biomass harvesting costs between 20 and 30 percent of the cost of microalgal downstream operations. The technology gap is still one of the main challenges facing the commercialization of biofuel from microalgae. In addition, high water, nitrogen, and phosphorus consumption in large-scale biomass production are another challenge. When using wastewater as a source of nutrients, pollution from bacteria, pathogens, and chemical compounds present in wastewater is a rising concern [103]. Microalgal dewatering, drying, oil extraction, and free fatty acid and inorganic contaminant removal are energy-intensive and costly, and they provide the biggest technological obstacles in producing biodiesel from microalgae [14]. Additionally, comparing the energy consumption of the open pond, column photobioreactor (PBR), and tubular PBR throughout their respective life cycles, it was found that open ponds consume the least energy, while tubular PBRs consume the most during the culture phase. Although PBRs consume more electricity, which adds to the costs, they are still favourable and often achieve higher biomass concentrations [104]. One of the elements that should be optimised is the cost of utilising lamps to illuminate microalgae cells and deliver a sufficient quantity of light. Compound parabolic concentrators are one method of enhancing the illumination of microalgae. When *Chlorella vulgaris* was cultivated using a glass tube and a plain tube without a compound parabolic concentrator (CPC), the system with a concentrator was compared with the system with a plain tube. The system with a concentrator improved the irradiance level of the culture by 351% and the average light intensity of the cells by 462%. During the exponential phase, *C. vulgaris* grew more quickly in the tube system (1.14 div/day) than in the concentrator system (0.98 div/day); however, its cells were larger in the concentrator system (11.23 m) than in the tube system (6.28 m), and between days 2 to 4 of the cultivation, the CPC system produced more biomass than the tube system did in terms of organic dry weight and proteins, lipids, and carbs [105].

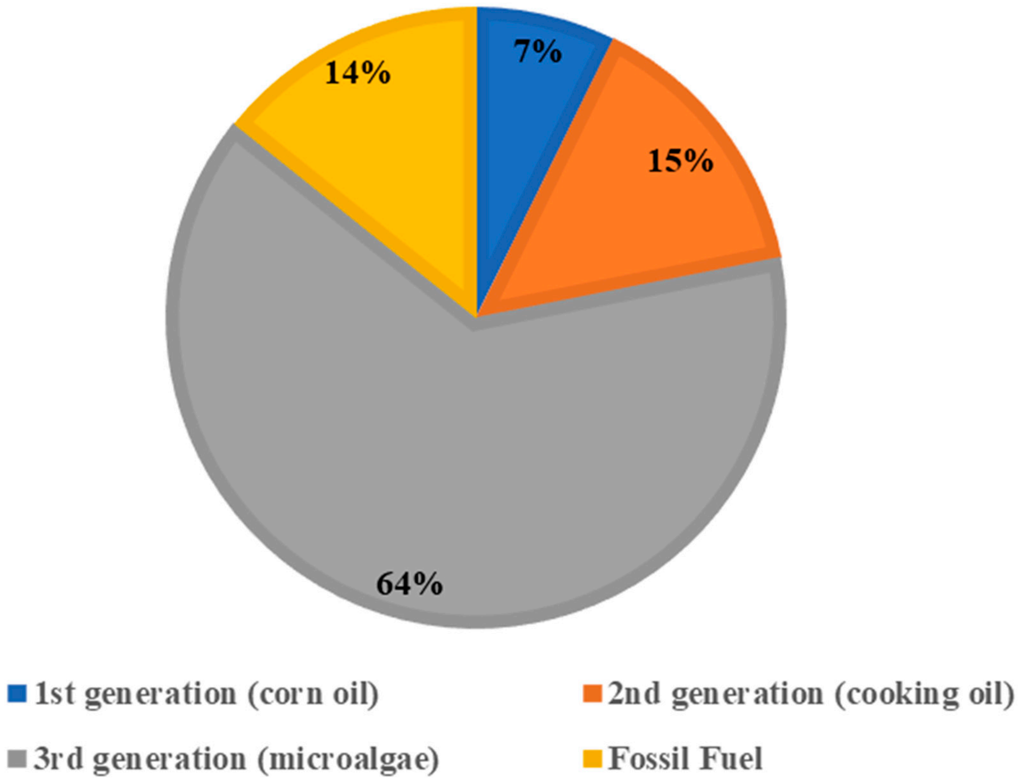

Figure 7. Economic comparison between first-, second-, and third-generations of biofuels and fossil fuels (data obtained from [106–108]).

4.2. Environmental Barriers

Globally, fossil fuels are intensively utilised as the prime source of energy. Hence, alternative energy sources must be adequate to meet global fuel demands. In 2008, in the United States, around 19 million barrels of oil were required daily. For biofuel feedstock such as microalgae to meet this demand, about 30 million acres of land must be available for microalgae cultivation [109]. Nevertheless, as third-generation biofuels, microalgae have fewer issues related to land use than first-generation and second-generation biofuels, as they can grow and reproduce on non-arable land, using wastewater and brackish water [110]. On the other hand, water is a significant limiting factor for utilising microalgae in biofuel production. For large-scale microalgae cultivation, it is essential to consider water utilization to prevent trade-offs between water and fuel [109]. The main factors impacting microalgae's water usage for biofuel production are the geographical location, conversion routes, and production systems. For instance, in the Netherlands, the water utilization of microalgae in a closed photo reactor was projected at 8 m^3 GJ^{-1}. In contrast, in Hawaii, the water utilization of microalgae cultivated in open pond systems was estimated at 193 m^3 GJ^{-1} [111]. Table 4 presents the average annual water footprint and cost of several fuel sources.

Table 4. Average annual water footprint and costs of several fuel sources.

Fuel Sources	Average Annual Water Footprint (m^3/GJ)	Cost ($/Gallon)	Reference
Microalgae-based biodiesel (open raceway)	From 14 to 87	3.50	[112–114]
Microalgae-based biodiesel (bioreactor)	From 1 to 2	3.50	[113,114]
Natural gas	0.11	0.6825	[109,115]
Soybean-based biodiesel	287	2.00	[113,116]
Petroleum-based diesel	From 0.04 to 0.08	5.34	[112,115]
Sugarcane ethanol	From 85 to 139	2.40	[113,117]

Microalgae-based biofuels are considered renewable and clean alternatives to fossil fuels. Nevertheless, multiple research studies have been conducted to assess the impact of microalgal biofuels (most specifically, biodiesel) on greenhouse gas emissions. Most studies have indicated that, currently, microalgae-based biodiesel results in higher greenhouse gas emissions than fossil diesel. This is due to the small microalgal harvest [118] and high energy utilization required for cultivating, harvesting, and drying microalgae [119,120]. Several studies have indicated that microalgae-based biofuels require abundant energy for several processes, including pumping, lipid extraction, dewatering, and thermal drying [119,121]. Nevertheless, the energy requirements for cultivating microalgae in raceway ponds are lower than 1 MJ MJ^{-1}, which is much lower than the energy requirements for photobioreactors [122]. In addition to greenhouse gas emissions, microalgae emit methane gas, nitrogen gas, biogenic halogenated, biogenic sulphur, isoprene, and volatile organic carbon [123]. Moreover, applying pesticides, such as herbicides and insecticides, leads to the release of organochlorine compounds, which result in ozone depletion [124]. In addition to pesticides, fertilisers used for optimum microalgal growth contribute to greenhouse gas emissions. Microalgae require nutrients, fertilisers, or supplements for optimal growth. Nevertheless, fertilisers are made of components extracted from fossil fuels and are non-renewable [125]. Other biofuel production processes, such as transesterification, harvesting, and drying, contribute to pollutant emissions [126]. The transesterification process is an energy-intensive process that requires chemicals for its operations. Mayol et al. [127] assessed the impact of several processes for cultivating microalgae by using artificial intelligence technologies. The authors reported that the transesterification process had the highest environmental impacts, with 19.40 million points. In comparison, the dewatering process showed the least environmental problems, with a score of 0.267 million points.

The impacts of microalgae are not limited to natural resources but also biodiversity. Microalgae cultivation poses a threat to biodiversity and the ecosystem. Deploying microalgae on a large scale requires the proper control and management of the cultivation process [128]. Hence, it may be a better choice to rely on native plant species for biofuel production rather than cultivating invasive species that might endanger biodiversity [129]. The mass cultivation of microalgae is also known as the controlled eutrophication process [128]. Nevertheless, the mass cultivation of microalgae can lead to the excessive richness of nutrients in the aquatic body, thus threatening biodiversity. As microalgal biomasses wither, their decomposed bodies take up oxygen from the aquatic body. This, in turn, leads to the asphyxiation of aquatic creatures and organisms that require oxygen for survival [130]. The mass cultivation of microalgae poses a significant risk to coastal biodiversity due to the over-exploitation of the microalgal species of coastal ecosystems [131]. Hence, eutrophication threatens biodiversity because of water toxicity, oxygen level reduction, and the opacity of water bodies [130]. As a result, microalgae-based biofuels lead to a loss of biodiversity because of the utilization and degradation of the habitats of many aquatic and non-aquatic creatures and the polluted land water from the extra nutrient load [132]. The cultivation of genetically modified microalgae has not been documented yet, most likely because of the numerous known and unknown dangers involved with cultivating it outdoors in open systems. There are a number of dangers associated with growing genetically modified microalgae, including the possibility of uncontrollable leaks. Upon proliferating, these algae may stop the growth of normal species and begin to compete with them. The dangers of toxic algal blooms, adverse effects on ecosystems, gene transfer, health and environmental effects, management control issues, and ethical issues are also the main issues with growing genetically modified microalgae [50].

5. Future Outlook

An ideal mix of technological advancements in systems, processes, and economic viability in practical implementation is required for commercial biofuel production. According to a previous study, algal biofuels are theoretically feasible, but more long-term (around 10 years) R&D is needed to obtain the desired high productivity. Additionally, it is thought that only open ponds can produce biofuels at the low cost required [20]. According to Belarbi et al. [125], biofuels from algae are promising, with the commercialization of algae fuels being attempted by a variety of new businesses. Transportation fuels made from algae could replace petroleum-based ones, significantly reducing carbon dioxide emissions. A few US businesses have recently been attempting to reduce the cost of photobioreactors (PBRs) to practically the same level as open ponds [20]. The most prevalent biofuel today is bioethanol, typically made from corn and sugarcane sugars. In just a few years, the amount of bioethanol produced worldwide has rapidly expanded from 1 billion to 39 billion litres, and it will soon surpass 100 billion litres. With the use of engineering techniques, efforts to create microalgae strains high in carbohydrates are growing. Numerous transformation techniques are now feasible because of genetics' quick advancement, and experiments support the use of genetic tools for various applications. Algal bioethanol technology has not advanced sufficiently as of now, but expectations for the near future are strong [22].

6. Conclusions

This review focused on recent advances in enhancing the biomass yield of biofuel extracted from microalgae as well as the barriers and limitations of applying these technologies. It is evident that biofuel productivity could be significantly increased with the incorporation of enabling technologies such as genetic engineering and the incorporation of nanomaterials. The application of the latter has a tangible effect on the yield of biofuel generation from microalgae. Various microalgal species were studied, and the addition of nanoparticles improved harvesting efficiency and biofuel production. In addition, transcriptome analyses were introduced for their role in the production of metabolites for microalgal species. The barriers concerning biofuel production are mainly techno-economic, as well as environmental. While the environmental challenges can be mitigated by growing algae in a protected environment, the energy and cost breakeven points of pretreatment procedures are far from ideal and need to be reduced. Overcoming these barriers is possible with more research and development, starting with microalgae cultivation and the biofuel production process, to reach a cost-effective solution to be adapted commercially.

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