Cyanobacteria as a Biocatalyst for Biofuel Production

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Photosynthetic microorganisms such as algae and cyanobacteria exhibit significant potential as third-generation biofuel catalysts, devoid of the limitations associated with contemporary biofuels. Cyanobacteria, a type of photosynthetic prokaryotes, exhibit significant potential for the direct conversion of carbon dioxide (CO_2) into biofuels, chemicals, and various other valuable compounds. There has been a growing interest in the concept of utilising biological processes to convert carbon dioxide into fuels and chemicals.

Keywords: cyanobacteria ; fossil fuels ; genetic manipulation ; synthetic biology approaches ; value-added products

1. Introduction

Cyanobacteria, the only prokaryote which possess the ability of oxygenic photosynthesis, play a critical role in various important biological processes, including nitrogen fixation, oxygen generation, and global carbon sequestration. These organisms have the potential to be employed in the development of a microbial manufacturing system that has the capability to produce valuable products by absorbing carbon dioxide from the environment and harnessing solar energy. According to Pierobon et al. [1], these photosynthetic microorganisms exhibit extensive ecological, metabolic, and chemical properties, which indicates that these organisms are highly promising in various biological applications. Cyanobacteria possess significant quantities of phycocyanin, lipids, and carbohydrates, rendering them promising candidates for the production of commercially valuable compounds. These chemicals are commonly produced via a specialized induction process, which may encompass physical stresses, such as variations in pH, light intensity, temperature variations, or chemicals (as stress parameters), such as nitrogen deprivation, carbon dioxide, and salt concentrations. Cyanobacteria serve as environmentally friendly and sustainable alternatives, offering promising prospects for the production of efficient biofuels, medicines, nutraceuticals, and potential therapeutic applications in the near future [2][3][4]. Earlier, the primary concern was the enhancement of integrative processes and cultural approaches, which included the development of optimised photo-bioreactor designs [5], strategies to minimise energy consumption during downstream processes for cyanobacteria harvesting [6], and approaches for extracting compounds of significant value [7]. Cyanobacteria are a perfect platform for complex metabolic engineering projects because they grow quickly and are relatively simple bacteria. Utilising a more comprehensive array of molecular tools has facilitated the exploration of cyanobacteria's potential in the domains of metabolic engineering and biotechnology [8][9][10]. The capacity to manipulate endogenous genetic material has expanded with scientific progress [10][11].

Selection and introduction of targeted genes and random mutagenesis are a few traditional techniques applied to increase the production of a desired compound [12]. A potential disadvantage associated with conventional methods is the substantial time commitment required for both the design and implementation phases. The resolution of these challenges can be accomplished through the application of metabolic engineering and systems biology techniques to boost microbial output. This strategy often involves the utilisation of mathematical models to simulate and forecast the behaviours that arise inside intricate systems [13]. The utilisation of systems biology methodologies, which rely on the implementation of standardised and thoroughly characterised modules or biological components like promoters, RBSs, riboswitches, and terminator libraries, presents a viable approach for the manipulation of organisms to enhance microbial production [6][14].

The CRISPR/Cas9 system, formally referred to as clustered regularly interspaced short palindromic repeats with associated protein 9, has rapidly evolved as a highly efficient and state-of-the-art technology within the past decade [15][16]. The utilization of CRISPR methodology has been extensively utilised to augment cell metabolism, control biosynthetic pathways, and enhance the rates and yields of metabolite production [17]. The application of CRISPR-Cas9 technology to genetically edit genes in the model microalga *Chlamydomonas reinhardtii* was first demonstrated by Jiang et al. [18]. In contrast, prokaryotic cyanobacteria possess genomes of relatively limited size and have undergone comprehensive sequencing. Several examples of cyanobacteria with desirable characteristics, including increased growth rates and

substantial lipid content, are *Nostoc* sp. PCC 7120, *Synechocystis* strains PCC 6803 and PCC 7002, as well as *Synechococcus* strains UTEX 2973 PCC 6301 and PCC 7942. In contrast to eukaryotic microalgae, the operating systems for genetic engineering in these particular organisms are significantly less complex. As a result, a multitude of efforts have been made to enhance the synthesis of fatty acids and other environmentally friendly substances, as demonstrated by the investigations carried out by Santos-Merino et al. [19] and Eungrasamee et al. [20]. Shabestary et al. [21] limited the optimal cell density of biofuel producing *Synechocystis* cultures utilising inducible CRISPR interference technology.

2. Engineering Cyanobacteria as a Source of Value-Added Chemicals

In the past few years, there has been a notable endeavour to employ molecular techniques from many species with the aim of enhancing the capabilities of cyanobacteria and facilitating cellular reprogramming to achieve greater yields of valuable compounds [22].The careful choice of chassis cells is of utmost importance when constructing efficient and dependable facilities for the cultivation of photosynthetic cells, given the intricate nature of metabolic conversions and the anticipated advancements in engineering applications [23]. The ongoing discourse over the manipulation of cyanobacteria is situated within the wider context of the progressively advanced methodologies that have emerged in the domains of metabolic engineering and synthetic biology. These developments hold the potential to pave the way for cyanobacteria to make substantial contributions as crop species in the realm of next-generation biofuels.

There exist multiple strains of cyanobacteria that exhibit a high degree of susceptibility to genetic modification. Modifications of this nature can be executed via cis (chromosome editing) or trans (plasmid addition) mechanisms, both of which have been employed in synthetic biology experiments. The aforementioned approach has been employed in cyanobacteria, along with other bacterial species, in order to broaden the range of substances they can synthesise and to enhance overall production efficacy [24][25]. Cyanobacteria-derived biofuels have been recognised as an alternative resource and have been classified as third-generation biofuels. Cyanobacteria possess the ability to convert carbon dioxide and water into lipids that are rich in carbon content. These lipids serve as a suitable substrate for the synthesis of biodiesel with higher efficiency in comparison to agricultural crops; thus, they are preferred over the traditional oil-seed agricultural crops [26]. In contrast to microalgae, cyanobacteria have a higher tolerance potential for foreign genes when the latter are introduced into them. They have also established a good genetic framework, and their genes are much more resilient when manipulated; moreover, they have a well-regulated photosynthetic mechanism [27]. The study of multiple species of cyanobacteria for the generation of ethanol and the development of photosynthetic innovative biofuels using metabolic processes is made possible through the regulation of the genetic transformation procedures. The marine cyanobacteria *Synechococcus* sp. PCC 7002 and the two freshwater species *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* sp. PCC 7942 have been extensively researched for their ability to synthesise various fuel molecules.

2.1. The Production of Alcohol and Aldehydes

The utilisation of photosynthetic organisms in the organic synthesis of alcohol is a promising solution to address the constraints associated with petrochemical manufacturing. In a recent study conducted by Deng and Coleman [28], it was observed that *Synechococcus* 7942, a genetically modified strain of cyanobacteria, exhibited the ability to convert pyruvate into ethanol. This conversion resulted in a concentration of 450 nmol/L of ethanol over a period of 7 days. The process involved the utilisation of heterologous enzymes, namely pyruvate decarboxylase and alcohol dehydrogenase. In a research investigation focused on *Synechococcus* 7002, it was observed that a decrease in the ratio of NADPH to ATP within the intracellular membrane led to an augmented synthesis of ethanol. This phenomenon was attributed to alterations in intracellular carbon allocation, subsequently resulting in the accumulation of glycogen and soluble sugars. The aforementioned process led to the synthesis of 1-butanol [29]. In the research-based study carried out by Mishra et al. [30], *Synechococcus* 7942 was utilised as a model organism to showcase the CoA-dependent photoautotrophic synthesis. To do this, the butanol pathway genes from *Clostridium acetobutylicum* were introduced into *Synechococcus* 7942. Specifically, the *EtfAB* and *tlh* genes were replaced with the *Treponema denticola* and autoB genes (Thiolase) from *E. coli*. In contrast, the introduction of the mature culture into typical photosynthetic conditions resulted in a limited production of butanol. Conversely, subjecting the culture to prolonged periods of darkness and oxygen deprivation led to a subsequent increase in butanol accumulation, reaching a level of 14.5 mg L^{-1} within a span of 7 days [31].

2.1.1. Ethanol Pathway

Alcohol dehydrogenase (*Adh*) and pyruvate decarboxylase (*Pdc*) are integral enzymes within the ethanol biosynthesis process. The initial enzyme uses a non-oxidative decarboxylation process to convert pyruvate into acetaldehyde and carbon dioxide, whereas the subsequent enzyme utilises acetaldehyde to generate ethanol. The original experiment for

ethanol production was the utilisation of *Synechococcus* 7942 as the host organism, in which the *pdc* and *adhII* genes from *Zymomonas mobilis*, a known ethanol producer, were heterologously expressed. This expression was carried out under the control of the *rbcLS* promoter of *Synechococcus* 7942, despite its inhibitory effect. A quantity of ethanol corresponding to 5 mM (0.23 g L^{-1}) was generated following a cultivation period of 4 weeks, as reported by Deng and Coleman [28]. Additionally, endogenous alcohol dehydrogenase (*Adh*) and *Pdc* from *Z. mobilis* were also co-overexpressed in the *Synechocystis* 6803 strain. The expression of all four CBB cycle genes was regulated by the psbA2 promoter, while the expression of ethanol biosynthesis genes was controlled by the PnrsB promoter. Following a development period of 7 days at a light intensity of 65 µmol protons m^{-2} s^{-1} and a concentration of 2.5 µM Ni^{2+}, a notable rise in ethanol accumulation was detected in the strain that co-expressed *Fba* together with *Pdc* and *Adh*. The ethanol accumulation in this strain was around 750 mgL^{-1}, while the strain expressing only *Pdc* and *Adh* had an ethanol accumulation of approximately 400 mgL^{-1}. According to Liang et al. [32], strains that overexpress RuBisCO, FBP/SBPase, and TK enzymes produced ethanol within the range of 600 to 750 mg^{-1} L^{-1}. According to Roussou et al. [33], the strain that co-overexpressed FBA and TK exhibited a nine-fold increase in ethanol production compared to the strain that only overexpressed FBA, and a four-fold upregulation in comparison to the selective strain that only expressed TK.

2.1.2. CoA Pathway-Dependent Alcohols

The fermentation processes involved in the production of isopropanol and butanol (specifically 1-butanol) proceed via the acetone/butanol/ethanol (ABE) pathway. While isopropanol does possess a higher energy yield than ethanol, its applicability as a long-term substitute for petrol is limited. On the contrary, butanol possesses characteristics that closely resemble those of petrol and can be employed as a direct fuel source in petrol engines [34][35]. The genes associated with the butanol pathway derived from *C. acetobutylicum* were successfully expressed in *Synechococcus* 7942. This particular organism holds the distinction of being the first to demonstrate CoA-dependent photoautotrophic synthesis of 1-butanol. The *ter* gene from *T. denticola* and the *atoB* gene (Thiolase) from *E. coli* were utilised instead of *EtfAB* and *tlh*, respectively. Nevertheless, in typical photosynthetic conditions, the presence of butanol was observed in only a minimal amount. However, when the culture was subjected to both darkness and an anoxic environment, a further increase in butanol accumulation (approximately 15 mg L^{-1} in a week) was observed [31]. The initial reversible decarboxylation reaction involved in the synthesis of acetoacetyl coA potentially diminished the driving force necessary to channel the metabolic flow towards the desired product. Consequently, this may have led to a decrease in the size of the NADH pool, as NADPH is generated during photosynthesis. This reduction in NADH availability could have contributed to the observed decrease in the final concentration of the desired compound. The elimination of limitations arising from the reversible nature of the initial stage in the *Clostridium* butanol pathway and the presence of NADH in *Synechococcus* 7942 was achieved through the introduction of novel genes. The conversion of acetyl-CoA to acetoacetyl-CoA was achieved by considering the ATP-driven irreversible condensation of malonyl-CoA in the process of fatty acid biosynthesis. This was accomplished through the introduction of an acetoacetyl-CoA synthase (encoded by NphT7) derived from the *Streptomyces* sp. strain, as demonstrated by Lan and Liao [31]. The NADPH-dependent acetoacetyl-CoA reductase (*PhaB*) from *Ralstonia eutropha* and the (R)-specific enoyl-CoA hydratase (*PhaJ*) from *Aeromonas caviae* were substituted for *Hbd* and *Crt*, respectively. In order to circumvent the utilisation of *AdhE2* and compensate for its bifunctional nature, the substitution of *AdhE2* was carried out by expressing two additional enzymes, namely CoA-acylating butyraldehyde dehydrogenase (*Bldh*) and NADPH-dependent alcohol dehydrogenase (*YqhD*) from *E. coli. Bldh* is responsible for the conversion of butyryl-CoA to butyraldehyde, while *YqhD* serves as an alternative enzyme. In the study conducted by Kusakabe et al. [36], *Synechococcus* 7942 was selected as the initial subject to investigate the purpose of synthesising isopropanol. In this study, the IPTG-inducible promoter *PLlacO1* was employed to enhance the transcription of the genes thl and adc derived from *Clostridium acetobutylicum* ATCC 824, *ctfAB* (encoded by *atoAD*) from *E. coli* K-12 MG1655, and *adh* from *C. beijerinckii*. Propanol was not detected under the standard growth conditions. Nevertheless, the introduction of potassium acetate into the medium resulted in the detection of 21.7 mg L^{-1} isopropanol after a period of one week. This finding suggests that there were constraints on the carbon flow for acetyl-CoA. It is widely recognized that numerous species of cyanobacteria have the capability to synthesize acetate through the utilization of glycogen stores under conditions of inadequate oxygen and light availability. The strain responsible for isopropanol production was then cultivated in a controlled environment, lacking phosphorus, nitrogen, light, and oxygen. This experimental condition led to the production of 26.5 mg L^{-1} of isopropanol [36]. Hirokawa et al. [37] observed a significant enhancement in propanol yield (146 mg L^{-1}) by the manipulation of growth phase duration, production phase duration, and the transition from dark and oxygen deficient to light and oxygen efficient circumstances. This modification resulted in a six-fold increase in propanol production.

2.2. Carotenoid Production

Carotenoids, tetraterpenoids, are found in all photosynthetic organisms, and they enhance light absorption and energy dissipation during photosynthesis. In addition to well-known compounds such as β-carotene, zeaxanthin, astaxanthin, and echinenone, cyanobacteria also synthesise specialised carotenoids, such as myxoxanthophyll and orange carotenoid proteins, which function as mechanisms for protecting against photodamage [38][39]. The commercial significance of astaxanthin and zeaxanthin in nutraceuticals stems from their antioxidant characteristics, while β-carotene is utilised as a pro-vitamin A supplement. The limited carotenoid content of cyanobacteria presents a challenge for their competitiveness in the market. However, the application of genetic engineering holds promises for enhancing carotenoid production in cyanobacteria [39]. The process of carotenogenesis is a multifaceted, yet extensively studied, metabolic pathway that enables cyanobacteria to produce a diverse array of terpenoids. The production of these chemicals is initiated with the precursor geranylgeranyl pyrophosphate. The genes responsible for encoding the synthases, desaturases, hydroxylases, and cyclases involved in the biosynthesis of carotenoids have been identified and documented in a study conducted by Sugiyama et al. [40].

The biosynthesis route initiates with the condensation of two molecules of geranylgeranyl pyrophosphate by phytoene synthase (*CrtB*), resulting in the formation of phytoene. Subsequently, phytoene desaturase converts phytoene into ζ-carotene (*CrtP*), which is further transformed into lycopene by carotene desaturase (*CrtQ*). In addition to the production of the typical phytoene desaturase (*CrtP*), certain genera, such as *Anabaena* and *Nostoc*, possess the capacity to directly convert phytoene into lycopene through the action of a specific phytoene desaturase (*CrtI*). The production of α- and β-carotene, which are the two principal carotenoids, is derived from lycopene. The conversion of α-carotene is facilitated by the enzyme lycopene cyclase (*CrtL* or *CruA*), while the production of β-carotene involves a two-step process by the same enzyme (*CrtL* or *CruA*), starting from γ-carotene [40][41]. According to Pagels et al. [39], a significant proportion of carotenoids consist of a hydrocarbon chain composed of 40 carbon atoms (C40), which encompasses eight isoprenoid units and several double bond conjugations.

Astaxanthin, a carotenoid known for its powerful antioxidant properties, has been recognised for its potential health advantages [42]. The strain of *Synechococcus* 7002 has been genetically modified to include the genes β-carotene hydroxylase *CrtZ* and *CrtW* from *Brevundimonas* sp. SD212. As a result, this strain exhibits a significant astaxanthin concentration of 3 mg/g dry cell weight. According to Hasunuma et al. [43], the stimulation of photosynthetic central metabolism in astaxanthin-producing cells seems to compensate for the limited availability of β-carotene as a light-harvesting pigment. The conversion of astaxanthin to β-carotene is the most probable mechanism for achieving this outcome. The study conducted by Hasunuma et al. [43] demonstrated that this method leads to a higher yield of astaxanthin compared to the natural synthesis process by *H. pluvialis*. Furthermore, cyanobacteria utilise the MEP pathway to synthesise carotenoids, which is critical for enhancing the production of desired chemical compounds. This enhancement is achieved through the process of carbon partitioning, facilitated by the incorporation of CO_2 [44]. The authors of this study modified the *Synechocystis* 6803 strain using genetic engineering techniques to enhance the biosynthesis pathway and reconfigure the intracellular metabolism. By these modifications, they successfully developed a far more efficient astaxanthin anabolic pathway, resulting in a remarkable 500-fold increase in its output. The study conducted by Diao et al. [45] involved the implementation of several key procedures to enhance the production of astaxanthin. These procedures encompassed the introduction of two vital enzymes, namely β-carotenoid ketolase and hydroxylase, along with the optimisation of screening techniques and carbon flux enhancements to augment the availability of precursors in the native MEP pathway. The outcomes of these interventions were observed to positively impact both photosynthesis and central metabolism, potentially attributable to the increased flux of astaxanthin, which reached a level of 29.6 mg g^{-1} cell dry weight. Furthermore, in their study, Gao et al. [46] demonstrated an augmentation in the synthesis of canthaxanthin (8.8 mg L) and echinenone (16%) through the overexpression of the *crtO* gene sourced from *Nostoc flagelliforme* in *Nostoc* sp. PCC 7120. The cyanobacterium *Synechocystis* was genetically modified to incorporate the MEP α-branch, resulting in the buildup of lutein. A *Synechocystis* strain deficient in the native cyanobacterial lycopene cyclase *cruA* was genetically modified by introducing a cassette containing four genes from *Arabidopsis thaliana*. These genes encode two lycopene cyclases (*AtLCYe* and *AtLCYb*) and two hydroxylases (*AtCYP97A* and *AtCYP97C*). The observed synlut strain exhibited normal growth characteristics similar to the wild-type strain, along with minor alterations in pigment production. Certain carotenoids are also effective in preventing several chronic diseases in humans. Changes in salinity and nutrients cause variations in carotenoid composition, which offers an effective way to boost specific targeted carotenoids [47]. These findings indicate that the absence of the *cruA* gene can be compensated for by the presence of *Arabidopsis* lycopene cyclases [48].

2.3. Terpenes and Isoprenes (Isoprenoid Pathway)

Terpenoids are a class of organic chemicals that possess notable industrial utility, including several sectors, such as food production, fragrance manufacturing, cosmetic formulation, chemical synthesis, and the development of biofuels. According to Schempp et al. [49] and Mata-Gomez et al. [50], these substances possess diverse chemical characteristics and can serve as colourants, perfumes, feedstock, or precursors. According to Ko et al. [51], these compounds have the potential to serve as advanced fuels supplementing gasoline, jet fuel, and diesel. Isoprene acts as a viable precursor for the generation of second-order drop-in biofuel molecules via the process of oligomerization. Additionally, cyclic monoterpenes, such as limonene, exhibit greater energy density and possess physical features that closely resemble those of Jet A-1 aviation fuel [52]. DMAPP and IPP, consisting of five carbon atoms each, serve as the fundamental constituents for the synthesis of all naturally occurring terpenoids. According to Pattanaik and Lindberg [53], prenyl transferases are enzymes that facilitate the condensation of two C5 units, leading to the formation of hydrocarbons with varying carbon chain lengths and functional groups.

Cyanobacteria have the capability to synthesise terpenoids via the MEP route, which involves the utilisation of G3P and pyruvate derived from photosynthesis. Terpenoids fulfil diverse functions in cellular physiology, encompassing light absorption, modulation of membrane fluidity, and defence against elevated light intensity and oxidative stress [54][55]. Extensive research has been conducted on cyanobacteria as prospective solar-powered cellular systems aimed at the conversion of atmospheric carbon dioxide into terpene-based molecules. Multiple methodologies have yielded cyanobacterial platforms that exhibit the ability to synthesise diverse chemicals, such as isoprene [56], β-phellandrene [57], and limonene [58].

Tracy et al. [59] and Chuck and Donnelly [60], demonstrated that certain terpenoids, including farnesene, myrcene, and limonene, as well as their hydrogenated derivatives, exhibit compatibility with both diesel and aviation fuels. Isoprenoid compounds frequently exhibit a diverse array of structural characteristics and molecular masses, hence presenting the potential for using these terpenoids as viable substitutes for conventional fuels [61][62].

2.4. Limonene

Limonene is basically the oil extracted from the peels of citrus fruit. It is a cyclic monoterpene and has countless applications in corporate sectors. It has a potential of replacing fossil fuels, such as jet fuel blending, and thus has become an important source of next-generation biofuels derived from sustainable sources [60][61][63]. The nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120 produces limonene, making it an essential source for the generation of biofuel, as well as the production of high value chemicals such as limonene. The natural synthesis of limonene could be upregulated via genome modification to increase its yield.

The heterologous expression of limonene synthase (Lms) from *Schizonepeta tenuifolia* allowed the synthesis of volatile monoterpene limonene in *Synechocystis* 6803. Three native MEP pathway genes (*dxs*, *crtE*, and *ipi*) were co-expressed with a codon-optimized *lms* under the control of the P_{trc} promoter. Limonene was produced at a rate of 41 g L^{-1} d^{-1} by strains that just expressed *lms*, while 56 g L^{-1} d^{-1} was produced by strains that also expressed three additional MEP pathway genes [64]. A group of researchers has successfully engineered a filamentous cyanobacterium, namely *Anabaena* sp. PCC 7120, to produce and release limonene. This was achieved by expressing the limonene synthase gene from Sitka spruce plant into the cyanobacterium. Limonene is actively transported across the cellular membrane and then released into the surrounding headspace, facilitating its efficient separation from the biomass present in the culture. The coexpression of a synthetic DXP operon (dxs-ipphp-gpps), which includes three rate-limiting enzymes of the MEP pathway, was employed to redirect carbon flux from the Calvin cycle towards the production of limonene, in the context of limonene synthesis. When exposed to increasing levels of light, the yield of limonene increased by a factor of 6.8, while the maximum rate of synthesis increased by a factor of 8.8. The methodology described in this study has the potential to be utilised in the synthesis of a wide range of commodity chemicals and drop-in-fuels [65].

By modifying the ribosome binding sites (RBS) in *Synechococcus* 7942, Wang et al. [66] altered the trc promoter's initial RBS to boost limonene production. Consequently, strain L1113 demonstrated limonene production of 32.8 g/L/OD/d. Similar to this, a synthetic RBS inserted into the psbA promoter enhanced the production of limonene to 885.1 g/L/OD/d. However, before a process can become economically viable, production needs to be significantly boosted [63][65].

Acoording to Lin et al., [67] the genetic modification in *Synechococcus elongatus* UTEX 2973 enabled the production of limonene, a terpenoid compound widely utilised in commercial applications. A mutation was discovered in the gene responsible for producing geranylgeranyl pyrophosphate synthase *crtE*, which led to a significant 2.5-fold augmentation in the production of limonene. The strain exhibited a limonene production of 16.4 mg L^{-1} at a rate of 8.2 mg L^{-1} day^{-1}, which

is an eight-fold improvement compared to the prior limonene productivity observed in other cyanobacterial species. The investigation employed a combinatorial metabolic engineering strategy to optimise the genes implicated in the biosynthesis of limonene. The findings of the study indicate that augmenting the expression levels of enzymes in the MEP pathway plays a pivotal role in facilitating the production of limonene.

2.5. Fatty Metabolites (Fatty Alkanes, Fatty Alcohols, Fatty Acids)

Due to increasing apprehensions regarding global warming, biofuels have garnered recognition as potentially viable substitutes for fossil fuels due to their renewable nature and generally reduced carbon emissions [68]. Oleochemicals encompass a diverse range of fatty acid derivatives, including fatty acids, fatty alcohols, fatty alkanes, fatty acid methyl/ethyl esters, and waxes [69]. According to Marella et al. [70], these substances have many applications, such as biodiesels, lubricants, surfactants, and other purposes. Lipid-derived biodiesels have gained recognition as superior biofuel molecules compared to ethanol, primarily due to their high energy density and compatibility with the pre-existing liquid fuel infrastructure [71].

Alkanes are the major constituents of conventional petroleum; thus, alkane could be produced by several strains of cyanobacteria, and biosynthesis could be enhanced through genome modifications to create less environmental pollution, which is essential for the sustainable production of biofuels [72][73]. Firstly, the FAR enzyme converts fatty acyl-ACP to fatty aldehyde. Secondly, the polyketide synthase enzyme synthesises alkenes [73]. The optimization of fatty alkanes production was carried out in *Synechococcus sp*. NKBG15041c, which produces α-olefin through the expression of the *aar* and *ado* genes. Thus, alkane was synthesized from fatty acids and fatty aldehyde substrates through the action of ADO and AAR [74][75]. Overexpressing both aldehyde-deformylating oxygenase and acyl-acyl carrier protein reductase, Wang and colleagues created a number of mutant strains of *Synechocystis* 6803, with a maximum yield of 1.3% of DCW [76]. Some cyanobacterial strains under salt stress can also synthesize alkanes. Subsequently, alkane production was reported to be 1200 μg^{-1} DCW when *Anabaena* sp. PCC 7120 was cultivated under salt stress (nitrogen deficiency) conditions [77].

It is possible to create energy-rich fatty alcohol molecules from FAA and its active forms, such as fatty acyl-ACPs and fatty acyl-CoAs, which have the potential to be used directly as biofuel. The enzymes known as FAR were first discovered in the jojoba plant before being found in bacteria, plants, insects, and mammals. Different pathways are used in eukaryotic and prokaryotic systems to produce fatty alcohol, with fatty acyl-CoA reductase (*Acr*) converting fatty acyl-CoA to fatty aldehyde, which is then converted to alcohol by fatty aldehyde reductase (*Ahr*) [61][78].

Fatty alcohols can be readily used as an alternative to fuels since they are molecules that possesses high energy. The pathway for making fatty alcohols in cyanobacteria is similar to that for making fatty alkanes, in which the intermediate aldehyde serves as the substrate for the corresponding alcohol. In cyanobacteria, biosynthesis of fatty alcohols can occur when acyl-ACP or acyl-CoA is reduced. The production of fatty alcohols was enhanced in *Synechocystis* 6803 when a fatty acyl-CoA reductase was expressed, obtained from *Simmondsia chinensis.* Moreover, when the poly-3-hydroxybutyrate (poly-3HB) and glycogen were prevented from carbon partitioning, an improved production of fatty alcohol was observed [79][80]. When comparing cells treated with the chemical molecule butylated hydroxyanisole (C_{BHA}) to the control (Co; 163.62 ± 1.57 $mgL^{-1}d^{-1}$), the biomass and lipid productivities increased by 11% (181.60 ± 1.94 $mgL^{-1}d^{-1}$) and 48% (18.71 ± 0.20 $mgL^{-1}d^{-1}$), respectively. The biomass yields in CBHA-treated cells could reach up to 2 g L^{-1} [81]. Additionally, enhancement of fatty alcohol production could also be achieved in *Synechocystis 6803*, when the fatty aldehyde reductase obtained from *Marinobacter aquaolei* VT8 was expressed, followed by the elimination of reductase enzymes from alkane synthesis pathways [82].

Fatty acids have gained immense recognition for the biological synthesis of biofuels owing to their ability to produce compounds similar to diesel. They are crucial for hydrocarbon-based biofuel production, and converting cyanobacteria into cell factories through metabolic engineering can be beneficial for the next generation. Cell-free fatty acids (FFAs) are a beneficial method, as they eliminate the time and cost of cell recovery or biofuel extraction, leading to an increased FFA production and secretion in cyanobacteria [83]. Although there are limited instances of genetic transformation for microalgae, successful examples of genetic manipulation that improve plant oil production (and/or change the fatty acid composition) are reported in organisms such as *Brassica napus* and *Arabidopsis thaliana* [84]. FFAs are used as starting materials for biodiesel and substrates for fatty alcohol, alkene, and alkane synthesis. Fatty acids are a promising biosynthesis target due to their easy conversion into diesel-like compounds. The biological production of FFAs through metabolic engineering could meet heavy transportation fuel needs [85].

A thorough understanding of lipid metabolism and the molecular mechanisms underlying the increased triacylglycerol accumulation is needed for the efficient production of triacylglycerols. Genetic modifications of lipid pathways are possible

for cyanobacteria by upregulating fatty acid biosynthesis or by downregulating β-oxidation. To increase the production of mono-unsaturated lipids, knocking out or modifying the enzymes responsible for the synthesis of polyunsaturated lipids in the cell can be performed [86]. The enzyme ACC, encoded by *accDACB*, catalyses the carboxylation of acetyl-CoA to malonyl-CoA. This process produces fatty acyl-ACP through the action of the enzyme fatty acid synthesis II (FAS II). An intermediate of fatty acid synthesis, acyl-ACP, or fatty acyl-ACP, is a key precursor for phospholipid production [87]. In *Synechocystis*, the biochemical balance of fatty acyl-ACP is gained through the recycling of free fatty acids into fatty acyl-ACP. This reaction involves the use of an enzyme, AAS, encoded by *aas.* Similarly, this balance is reduced by the production of hydrocarbons such as alkane and alkene. This conversion is catalysed by acyl-ACP reductase and aldehyde dehydrogenase encoded by the *aar* and *ado* genes, respectively [75][88]. A set of genes, *PlsX*, *PlsY*, and *PlsC* is responsible for the direct conversion of fatty acyl-ACP to phospholipids, whereas *lipA* converts the phospholipids into free fatty acids [89]. Changing the expression pattern of these genes affects the production of lipids in *Synechocystis.* For example, three *Synechocystis* 6803 strains having *aas*-overexpression, *aas*-overexpression with *aar* gene interruption, and *accDACB*-overexpression with the *lipA* gene showed a significant increase in lipid production. Whereas overexpression of *aar/ado* results in increased production of alkanes, especially heptadecane.

AAS is one of the primary targets to be eliminated for FAA synthesis in cyanobacteria because it may recycle the FFA into ACP. Kaczmarzyk et al. [88] inactivated AAS, thereby increasing FFA production in *Synechocystis* 6803 and *Synechococcus* 7942. They catalysed the esterification of FFA to ACP and used the released fatty acids as raw materials to create complex lipids by recycling them.

Kato et al. [90] found that the balance between FFA production and exportation is crucial for cyanobacteria FFA productivity. They manipulated the *Synechococcus elongatus* mutant (dAS1T) by inactivating the *wzt* gene, which effectively exports O-antigen in the outer membrane of cyanobacteria. This inactivation increased FFA secretion by removing the O-antigen layer from the dAS1T cells. In another experiment, the expression of GPD1 from *Saccharomyces cerevisiae* and DGAT from *Rhodococcus opacus* increased the lipid production of *Synechocystis* 6803. The genes encode glycerol-3-phosphate dehydrogenase and diacylglycerol acyltransferase essential for cyanobacteria's lipid metabolism [83]. Therefore, several species of cyanobacteria, including *Synechococcus* 7942, *Synechocystis* 6803, and *Synechococcus* 7002, have all been genetically modified to produce fatty acids. Biosynthesis of lipid occurs when the hydrocarbon chains are elongated, which results from the condensation of the acyl-acyl carrier protein. The thioesterase enzyme causes the cleavage of acyl-ACP bond, producing free fatty acids FFA. Thus, acyl-ACP synthetase elimination promotes FFA buildup in the said microbial species by preventing FFA recycling [91][92]. Several genetically modified cyanobacteria along with their byproducts are listed in **Table 1**.

Table 1. Genetically modified cyanobacteria and their byproducts.

S. No	Cyanobacteria Species/Strain	Product(s)	Promoter(s) Used	Expressed Gene(s)	Titre	Vectors Used	Reference
1.	*Synechococcus* 7942	Ethanol	rbcLS	*pdc, adhII*	0.23 g L^{-1}	pCB4	[28]
2.	*Synechococcus* 7942	1-Butanol	Ptrc/PLlacO1	*atoB, hbd, ter, crt, adhE2*	14.5 mg L^{-1}	pEL5, pEL14, pEL17, pEL19, pEL30, pEL31, pEL32, pEL37	[31]
3.	*Synechococcus* 7942	Isopropanol	PLlacO1	*thl, atoAD, adc, adh*	21.7 mg L^{-1}	pTA216, pTA418, pTA372, pTA634	[36]
4.	*Synechococcus* 7942	Fatty acid	Ptrc/Prbc/Pcpc	*teA, acc, rucLS, rbcSS*	50 mg L^{-1}	pSE15, pSE17, pSE18	[93]
5.	*Synechococcus* 7942	Fatty acid	Ptrc	*tesA*	35 mg L^{-1}	pSE15, pSE16	[91]
6.	*Synechococcus* sp. NKBG15041c	Fatty alka(e)ne	Pcpc/Ptrc	*aar, ado*	4.2 µg g^{-1}	pKT230	[75]
7.	*Synechocystis* 6803	Ethanol	psbA2 /PnrsB	*Rubisco, fba, tk, pdc, adh*	750 mg L^{-1}	PEtOH	[32]
8.	*Synechocystis* 6803	Carotenoid	psbAII	*CrtP, crtB, crtR, ipi*	-	pPSBA2	[94]
9.	*Synechocystis* 6803	Isoprene	PsbA2	*IspS*	120 µg g^{-1}	pBA2A2	[95]

S. No	Cyanobacteria Species/Strain	Product(s)	Promoter(s) Used	Expressed Gene(s)	Titre	Vectors Used	Reference
10.	*Synechocystis* 6803	Limonene	Ptrc	*dxs, crtE, ipi, lms*	1 mg L^{-1}	pT31CTH	[64]
11.	*Synechocystis* 6803	Isobutanol	Ptrc	*kivd, adhA*	298 mg L^{-1}	pTAC-KA, pSL2035	[96]
12.	*Anabaena* 7120	Farnesene	Pnir/PpsbA1	*faS*	305.4 µg L^{-1}	pZR1188	[97]
13.	*Nostoc* PCC 7120	Hydrogen	patB	*Flv3B*	-	pRL25T	[98]
14.	*Nostoc punctiforme*	Fatty alka(e)ne	-	*npun_F1710, npun_F1711, npun_F5141*	12.9% of DW	pSCR119	[99]
15.	*Synechococcus* 7942	Farnesene	*Ptrc*	*faS, dxs, idi, ispA*	4.6 mg L^{-1}	pHCMC05	[100]

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