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Review Article

Synthetic Biology Guides Biofuel Production

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The advancement of microbial processes for the production of renewable liquid fuels has increased with concerns about the current fuel economy. The development of advanced biofuels in particular has risen to address some of the shortcomings of ethanol. These advanced fuels have chemical properties similar to petroleum-based liquid fuels, thus removing the need for engine modification or infrastructure redesign. While the productivity and titers of each of these processes remains to be improved, progress in synthetic biology has provided tools to guide the engineering of these processes through present and future challenges.

1. Introduction

The desire for the discovery of renewable liquid fuels, as well as commodity chemicals, has escalated due to the environmental impact, supply security, and decreasing total reserve of petroleum-based fuels and chemicals [1]. Petroleum consumption reached 37.1 quadrillion BTU in the United States in 2008, of which a large majority (71%) was used as a liquid fuel in the transportation sector [2]. This has lead to an increased focus to find sustainable replacements or supplements to petroleum derived diesel fuel, jet fuel, and motor gasoline [3–5]. The largest effort thus far has been the production of ethanol, which is often used as a supplement to gasoline but is also available in high percentage blends such as E85. Ethanol production via fermentation reached 9.2 billion gallons in the United States in 2008, an increase of over 40% from 2007 [2]. According to the newest Renewable Fuels Standard (RFS2) set aside in 2010, the mandate for renewable fuels production is 36 billion gallons by 2022. These renewable fuels are classified into 4 categories: cellulosic biofuels, which must be derived from renewable lignocellulosics and achieve a 60% lifecycle greenhouse gas (GHG) emission reduction over their petroleum-derived counterparts; biomass-based diesel (50% GHG emission reduction); advanced biofuels, which consist of any renewable fuel other than corn ethanol that reduces GHG emissions by 50%; total renewable fuel, of which any fuel that achieves a 20% reduction of GHG emissions is counted [6]. Of the biomass-derived diesel fuels, biodiesel has gained momentum as a supplement or replacement to traditional petrodiesel, with production reaching just under 700 million gallons in 2008 [2]. Biodiesel can be made by several methods, but is most commonly synthesized by the transesterification of oils and fat triglycerides with methanol to make fatty acid methyl esters (FAME).

Although each of these fuel alternatives provides initial platforms for biofuel development, their increased commercialization to replace petroleum is not without its limitations. Ethanol is incompatible with the current fuel infrastructure, and the supply of raw materials for biodiesel production from plant oils and waste animal fats may become a concern. These opportunities for refinement have lead researchers to look for alternative fuels and production processes to replace petroleum derived fuels, including fermentative alcohols [7–12], nonfermentative higher chain alcohols [13], isoprenoid [14] and lipid fuels [15–18], and fuels synthesized directly from CO_2 via photosynthesis [19, 20]. These microbial-based processes are critical first steps in designing processes to provide renewable drop-in liquid fuels.

Aiding in the design and continued development of these processes, among a host of others, has been synthetic biology. Synthetic biology aims to design, synthesize, and characterize new biological elements, or redesign natural systems, that can be lumped together in a "toolbox." These elements

can include promoters [21–23], regulatory proteins and RNAs [24–28], and scaffolds [29, 30]. With this "toolbox," synthetic biologists assemble these individually characterized parts into hierarchal structures to perform new, novel, or nonnative tasks [31], such as synthetic oscillators [32] and toggle switches [33]. This "toolbox" also allows for the investigation of several designs to achieve the same function, often with varying levels of success, as in the case of heterologous 1-butanol production [7, 9, 10]. This differs from traditional engineering approaches in that the design focal point is on the core components, which can be fine-tuned to meet strict guidelines for specific tasks [34]. Much of the work accomplished in biofuel research until now has relied on the identification of target pathways and the design of synthetic expression systems for enzymes responsible for fuel production. As these technologies progress and mature, the design, implementation, and optimization of new functions, as well as the upgrading and rewiring of existing components, will be essential for the successful discovery and production of new biofuels, as many challenges still limit their productivity. This review will investigate recent progress made in the microbial production of biofuels to supplant petrodiesel and motor gasoline and will discuss how existing and newly developed synthetic biology tools may aid in the advancement of these processes.

2. Current Biofuels Research

2.1. Traditional Fermentative Processes. Ethanol, isopropanol, and 1-butanol are the only naturally produced alcohol biofuels. Isopropanol can be used directly as a fuel supplement to gasoline or as a feedstock for the transesterification of fats into biodiesel [35]. Both isopropanol and 1-butanol are produced in a mixed product fermentation in various strains of *Clostridium* [36], with maximum production levels reaching 2 g/L and 20 g/L, respectively [37, 38]. With a renewed interest in alternative fuels, the production of isopropanol and 1-butanol has been recently investigated in genetically tractable heterologous organisms. These organisms, such as *Escherichia coli* and *Saccharomyces cerevisiae*, facilitate the design and optimization of new biofuels processes by combining an increasing synthetic biology toolbox with a well-studied metabolism. Isopropanol production in *E. coli* has surpassed that of *Clostridium* by assembling the pathway for acetone production and a secondary alcohol dehydrogenase [8, 12]. The production of 1-butanol, however, has proven to be more difficult. Initial efforts were able to produce ~0.5 g/L using *E. coli* as a host [7]. Construction of a new strain harboring a single construct resulted in an increase in production to 1.2 g/L [9]. In addition to *E. coli*, 1-butanol production has been investigated in *Pseudomonas putida*, *Bacillus subtilis*, and *S. cerevisiae* [10, 11], although production in *E. coli* has thus far shown the most promise. Each of these processes, however, is far from industrial feasibility, as yields (~0.05 g/g) and productivities (~0.01 g/L/h) must increase significantly to match the same figures for corn ethanol (~0.5 g/g and 2 g/L/h). The advancement of these processes is thought to be limited by the low activity of pathway enzymes due to poor expression, solubility, or oxygen sensitivity, as well as the metabolic imbalance introduced by these heterologous pathways. While productivity in each of these platforms is low in comparison with Clostridial fermentation, the ability to engineer and manipulate these user-friendly hosts will facilitate the development of these processes.

2.2. Nonfermentative Higher Alcohols. The production of biofuels from native organisms can present unique challenges to synthetic biologists as oftentimes the availability of genetic tools and physiological knowledge of the hosts is limited. Additionally, the engineering of heterologous hosts for biofuel production may decrease the overall fitness of the cell and require delicate pathway balancing that is oftentimes difficult [7]. It is therefore advantageous to use native pathways to generate immediate precursors for biofuel production. This was accomplished in *E. coli* by using the hosts' amino acid biosynthesis pathways to generate 2-keto acid precursors, which can be converted to alcohols through a single heterologous reaction (Figure 1). These alcohols can serve as direct replacements to gasoline, or can be polymerized to form a variety of potential fuel molecules [39]. Expression of keto acid decarboxylase (KDC) from *Lactococcus lactis* enabled *E. coli* to convert these 2-keto acids to aldehydes, which can be reduced to alcohols using alcohol dehydrogenase (ADH) [13]. A total of 6 of these higher chain alcohols were detected after expression of KDC. The production of isobutanol, in particular, was able to surpass 20 g/L after host optimization and amplification of genes responsible for the synthesis of 2-ketoisovalerate, the precursor to both isobutanol and valine [13]. Subsequent efforts have also been made to engineer the production of 1-propanol and 1-butanol [40], 2-methyl-1-butanol [41], and 3-methyl-1-butanol [42].

An alternative and more direct route to 2-ketobutyrate, the citramalate pathway from *Methanococcus jannaschii*, was utilized to produce 1-butanol and 1-propanol independently of threonine. By leveraging a growth selection based on a requirement for 2-keto acids, the directed evolution of citramalate synthase was able to enhance the activity of this heterologous pathway and increase the production of 1-propanol and 1-butanol by 9-fold and 22-fold, respectively [43]. These pathways were also expanded to produce longer chain alcohols from nonstandard 2-keto acids. These longer chain alcohols can be used as commodity chemicals and also possess advantageous fuel properties similar to other high chain alcohols. The leucine biosynthesis pathway was engineered to catalyze the elongation of larger substrates by mutation of LeuA [44]. Similarly, KDC was also designed to fit larger substrates, after which *E. coli* was able to produce several longer chain alcohols from C5–C8 from these nonstandard 2-keto acids [44].

One distinct advantage in the production of these alcohols is the ability to apply existing amino acid production technology. Amino acids are produced microbially from several microorganisms, yet *Corynebacterium glutamicum* has been the most successful host for the production of many amino acids, including valine [45]. In addition to industrial success in amino acid production, *C. glutamicum*

shows an increased tolerance to isobutanol relative to *E. coli*, making the production of isobutanol from *C. glutamicum* promising. Optimization of the host by gene deletion and overexpression of isobutanol synthesis genes resulted in the production of 4.9 g/L of isobutanol from glucose [46]. Similarly, an amino acid strain development technique was adapted for the production of 3-methyl-1-butanol (3 MB), which shares a common precursor with leucine. Whole cell mutagenesis and selection with a leucine analogue resulted in a strain able to produce 2.8 g/L 3 MB, greater than a 5-fold improvement from wild-type (WT) [47]. Addition of an in situ extraction technique to remove 3 MB from the aqueous culture media resulted in the production of 9.5 g/L of 3 MB [47].

2.3. Isoprenoids. Isoprenoids represent a diverse group of hydrocarbons synthesized from the C5 isomers isopentyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These precursors can be produced from acetyl coenzyme A (CoA) via the mevalonate pathway or from glyceraldehyde-3-phosphate and pyruvate through the methylerythritol pathway (MEP) (Figure 1). Isoprenoids are found naturally as hormones, photosynthetic pigments, and a variety of other specialized secondary metabolites, and have been previously investigated as nutraceuticals [48] and pharmaceuticals [49]. Because isoprenoids possess vast structural diversity, including saturated, unsaturated, branched, or cyclic alkenes or alkanes, their potential as fuel candidates, such as isopentenol (C5) for motor gasoline, or farnesene (C15) for diesel fuel, is promising [3].

Recently, the production of isopentenol (3-methyl-3-buten-1-ol or 3-methyl-2-buten-1-ol), a C5 unsaturated alcohol, has been investigated in *E. coli*. Isopentenol can be produced by the dephosphorylation of the isoprenoid building blocks IPP and DMAPP (Figure 1). By screening a *Bacillus subtilis* genomic library for relief of prenyl diphosphate (IPP and DMAPP) accumulation, two isopentenol biosynthetic genes, *nudF* and *yhfR*, were identified [14]. By overexpression of *nudF* in a previously optimized strain [50], production of isopentenol reached 110 mg/L [14]. Although the production of isoprenoid fuels is still limited, synthetic biology can provide the framework for improving isopentenol production as well as the development of processes for other potential isoprenoid fuels.

2.4. Lipids. The fatty acid biosynthesis pathways are of great importance to the production of renewable fuels. Fatty acids are commonly used now in the synthesis of biodiesel, and the production of long chain alkanes, alkenes, aldehydes, and alcohols offer promise in the development of alternative diesel and jet fuels. The fatty acid elongation cycle begins with the carboxylation of acetyl-CoA to malonyl-CoA. After transacylation of acetyl-CoA and malonyl-CoA to acyl carrier protein (ACP), acetyl-ACP and malonyl-ACP are condensed into acetoacetyl-ACP. After a reduction, dehydration, and second reduction reaction, a saturated fatty acyl-ACP (butyryl-ACP) is formed. In each subsequent elongation cycle, malonyl-ACP is condensed with the saturated fatty acyl-ACP to add 2 carbons to the growing hydrocarbon. After elongation, these hydrocarbons can be released as free fatty acids by a thioesterase. Additionally, fatty acyl-CoA can be reduced to a fatty aldehyde, which itself can be reduced to a fatty alcohol, or decarbonylated to an alkane or alkene [51] (Figure 1).

The microbial production of biodiesel has been approached from two angles First, by producing short chain alcohols and performing the transesterification *in vivo* with exogenously added fatty acids, and second, by producing free fatty acids that can be harvested for transesterification *in vitro*. The *in vivo* production of biodiesel using endogeneously produced ethanol was recently demonstrated in *E. coli*. Expression of the ethanol production pathway from *Zymomonas mobilis*, along with a broad substrate range acyltransferase (AtfA) from *Acinetobacter baylyi*, lead to the production of 1.3 g/L of fatty acid ethyl esters (FAEE) after addition of exogenous oleic acid [18].

Research on the production of fatty acids have centered around the discovery of oligeanous algae, yeast, and even bacteria, in which the lipid content, mainly composed of triacylglycerols (TAG), can reach 60%–80% of the total biomass produced [51, 52]. The identification of the genetic elements involved in fatty acid synthesis and the implementation and development of synthetic biology tools to facilitate strain development will become critical for process refinement [51]. Recently, efforts have also been made to produce fatty acids from user-friendly organisms such as *E. coli*. The overexpression of an endogenous and exogenous thioesterase along with acetyl-CoA carboxylase in a Δ*fadD* strain resulted in the production of 2.5 g/L of fatty acids from glycerol [16]. By redesigning the expression of these genes the production of fatty acids was increased to 4.5 g/L at a 6% yield with a specific productivity of 0.04 g/h/g dry cell weight [17].

Although each of these approaches has been successful in producing precursors for biodiesel synthesis, the supply of raw materials (lipid) or downstream processing (transesterification) can be cost-intensive [18]. It would be advantageous, therefore, to create a consolidated process to reduce costs. Consolidated bioprocessing (CBP), which involves the simultaneous production of saccharolytic enzymes with the hydrolysis of pretreated biomass and the fermentation of hexose and pentose sugars [53], significantly reduces processing costs by converting abundant, inexpensive biomass into useful fuels or chemicals in a single step. Unfortunately, no organisms possess both the ability to digest lignocellulosic biomass and ferment sugars to fuels at high yields. The solution to this challenge is being approached in two directions: the introduction of high yield fuel production pathways into cellulolytic organisms, and the engineering of substrate (lignocellulosic) utilizing pathways into organisms with superior product formation. Much of the work until this point has focused around the production of ethanol [53, 54], until recently when this strategy was applied by engineering *E. coli* for the production of biodiesel (FAEE), fatty alcohols, and wax esters [15]. *E. coli* was chosen as a host due to its high fatty acid synthesis rate (0.2 g/L/h/g dry cell mass [51]) and straightforwardness in genetic manipulation. As in previous

studies, the ethanol production pathway from *Z. mobilis* (*pdc*, *adhB*) was overexpressed to produce ethanol for FAEE production. By combining this pathway with a cytosolic version of an endogenous thioesterase (‘*tesA*) and an ester synthase from *A. baylyi* (*atfA*), a fatty acid oxidation deficient strain of *E. coli* (Δ*fadE*) was able to produce 37 mg/L of FAEE directly from glucose [15]. To increase the production of FAEE, two CoA ligases, *fadD* from *E. coli* and *FAA2* from *S. cerevisiae*, were overexpressed along with another copy of *atfA* to bring production of FAEE up to 674 mg/L [15]. In order to mitigate the cost of processing of raw cellulosic biomass into refined sugars, this process was engineered to use xylan, a pentose polysaccharide component of hemicellulose. Expression of the endoxylanase *xyn10B* from *Clostridium stercorarium* and the xylanase *xsa* from *Bacteroides ovatus* as chimeras with OsmY allowed *E. coli* to grow on xylan as a sole carbon source [15]. Assembly of the xylan degradation pathway with the previously described FAEE production strain resulted in a strain able to produce 12 mg/L of FAEE from xylan [15]. Future work may focus on the development of secreted cellulases to increase the substrate utilization capacity of *E. coli*, in addition to optimizing the fatty acid pathway by prospecting for enzymes or expression systems with increased activity or stability. This work demonstrates the first consolidated process for the production of fatty acid based fuels and chemicals from complex polysaccharides, and while the process yields and productivity remained to be improved to merit commercialization, this work gives engineers and synthetic biologists the foundation to advance this process.

2.5. Direct Incorporation of CO_2. The role of photosynthesis in any biofuel production process is critical. Many current technologies, such as biomass derived biofuels and algal lipids, have received attention as viable fuel replacement technologies [55, 56], yet rely on intermediate stages to incorporate CO_2 or recover biomass to process precursors into useable fuels, which can increase costs. Photosynthetic organisms such as cyanobacteria, algae, and plants use light energy to generate reducing power to directly incorporate CO_2 into organic metabolites. The use of these organisms to directly produce fuels can limit production costs and CO_2 emissions during intermediate processing, and may also help reduce net CO_2 emissions by scrubbing CO_2 enriched flue gases from traditional power plants and producing useful fuels or chemicals, although their potential is not limited to this scenario. Initial efforts have focused on the production of ethanol in *Rhodobacter* [57] and *Synechococcus* [58].

The production of advanced biofuels such as isobutyraldehyde, isobutanol, and isoprene has recently been investigated in cyanobacteria. This was first demonstrated by transferring the 2-keto acid pathways to higher chain alcohols into the cyanobacterium *Synechococcus elongatus* (Figure 2). Isobutyraldehyde was chosen as an initial target since its boiling point (63°C) allows it to be easily stripped from the culture medium, thus avoiding any toxicity effects. Chromosomal integration of 2-ketoisovalerate biosynthesis genes (*alsS*, *ilvCD*) and 2-ketoisovalerate decarboxylase (*kivd*) resulted in the production of 723 mg/L of isobutyraldehyde from dissolved CO_2 ($NaHCO_3$) [19]. To improve the low activity of ribulose-1,5-bisphophate carboxylase/oxygenase (RubisCO), the *rbcLS* genes from a similar cyanobacterium were integrated downstream of the endogenous *rbcLS* genes. Production of isobutyraldehyde in this strain was elevated to 1.1 g/L, with a productivity of 6.2 mg/L/h [19], an encouraging figure considering microalgal biodiesel has been estimated to be near 4 mg/L/h [19]. The production of isobutanol was also investigated by expressing the $NADP^+$ dependent alcohol dehydrogenase YqhD from *E. coli*. The production of isobutanol reached 450 mg/L, and although encouraging, is currently thought to be limited by end product toxicity [19].

A similar study was also recently conducted in which the isoprenoid biosynthesis pathways were exploited for biofuel production in the cyanobacterium *Synechocystis* sp. PCC6803 (Figure 2). The volatile hydrocarbon isoprene, most notably produced in plants [59], is a potential feedstock for biofuel or chemical production. The *ispS* gene from kudzu vine (*Pueraria montana*), encoding for an isoprene synthase, which catalyzes the conversion of DMAPP to isoprene, was codon optimized and cloned into *Synechocystis* under the control of a light dependent promoter. Expression of *ispS* under high intensity light resulted in a small accumulation of isoprene (50 μg/day/g dry cell mass) [20] relative to healthy oak leaves (~1,650 μg/day/g dry cell mass) [60], demonstrating that while successful, this process remains to be improved.

A significant obstacle to biofuel production in photosynthetic organisms is the design of scale up processes. Photosynthetic organisms require more complex reactor designs and their growth and productivity in suboptimal conditions (temperature, salt concentration, etc.) is not well understood. However, two reactor designs in use today, the raceway pond and the photobioreactor, have shown promise in their ability to accumulate photosynthetic biomass using sunlight [61]. The raceway pond is inexpensive and simple to construct, but it is subject to contamination and is less photosynthetically efficient than a photobioreactor. Photobioreactors, having several designs [62], have increased capital costs compared to raceway ponds, but have superior productivities due to their increased biomass concentration, and therefore, greater photosynthetic efficiency. Hybrid systems comprised of both raceway ponds and photobioreactors have also been investigated to maximize the advantages of each design [62].

3. Synthetic Biology for Biofuels

Synthetic biology is an increasingly expanding discipline focusing on the design and construction of artificial systems to achieve a desired goal. These systems are derived from the assembly of standardized components in a hierarchal manner to create a population of programmed cells carrying out a desired function [31]. For biofuels, this is of particular interest as the production of these chemicals requires efficient integration of foreign genes and pathways into central metabolism. Delicate optimization and fine-tuning of these processes to maximize productivity and

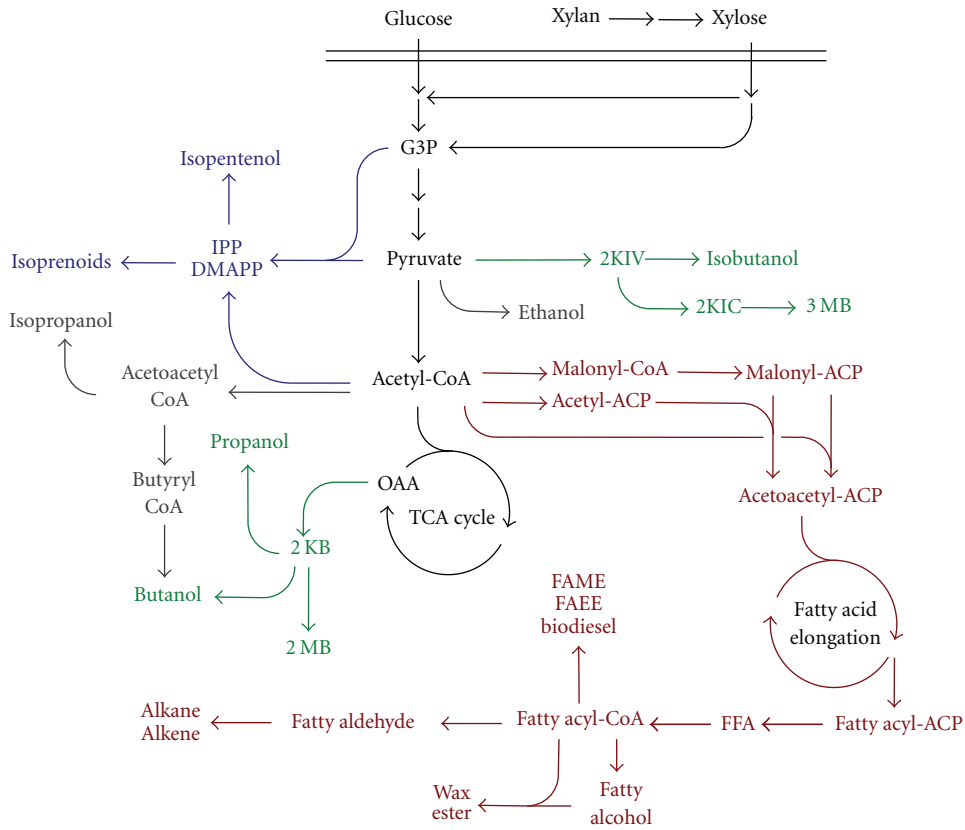

Figure 1: Metabolic schematic for biofuel production. Biofuel production pathways for traditional fermentative processes (grey), nonfermentative higher chain alcohols (green), isoprenoid fuels (blue), and fatty acid fuels (red) from central metabolism. Abbreviations: 2 KB (2-ketobutyrate), 2 KIV (2-ketoisovalerate), 2KIC (2-ketoisocaproate), 2 MB (2-methyl-1-butanol), 3 MB (3-methyl-1-butanol), ACP (acyl carrier protein), CoA (coenzyme A), DMAPP (dimethylallyl diphosphate), FAEE (fatty acid ethyl ester), FAME (fatty acid methyl ester), FFA (free fatty acid), G3P (glyceraldehyde-3-phosphate), IPP (isopentyl diphosphate), and OAA (oxaloacetate).

yield is of equal concern as the viability of any biofuel processes is extremely sensitive to production costs [3], such as raw material supply, total production, and downstream processing. Synthetic biology can provide tools and design principles to guide the development of such processes (Figure 3).

A goal of synthetic biology is to create a library of biological parts that can be used independently or as part of a larger assembly for a higher function. These biological parts can have simple or complex behaviors relayed through a variety of outputs such as gene expression. A simple but powerful example is the design of synthetically regulated promoters [21–23] to accurately control gene expression. More complex examples include the modulation of the expression of multiple genes through tunable intergenic regions (TIGR) [27], the activation and silencing of gene expression by riboregulators and ribozymes [24, 26, 28], or successive increases in gene expression through chromosomal amplification [63], which will become particularly important in the design of stable strains for industrial biofuel production. Additionally, thermodynamic models have been developed to rationally design ribosome binding sites to achieve robust expression levels varying by as much as 100,000 fold [64]. Preliminary designs for these biofuel gene expression systems will need to evolve to regulate and fine-tune the gene expression of these pathways, as was previously discovered for isoprenoids, a pathway discussed earlier for biofuel production [65]. Synthetic biology has also achieved the adaptation of posttranslation systems to regulate enzyme activity such as allosteric protein gates [25] and synthetic scaffolds [30].

The balance between the metabolic capacity of biofuel production pathways and host fitness will also play a key role in the productivity and yield of the process. This balancing of biofuel pathways, which aims to maximize the flow of carbon toward product formation without drastically altering the metabolic load or intracellular cofactors (NAD(P)H, ATP, ACP, etc.) can be achieved through the use of these synthetic biology tools. This was again demonstrated in the mevalonate pathway to isoprenoids by employing a synthetic scaffold to increase the metabolic capacity while limiting protein expression [29]. In addition to the balancing of biofuel production pathways, the expression of multienzyme complexes, which often require delicate balancing of catalytic subunits, is of great importance. The most direct example is the production of complexed cellulases, which will be crucial to increase the substrate utilization capacity of biomass-based biofuel processes.

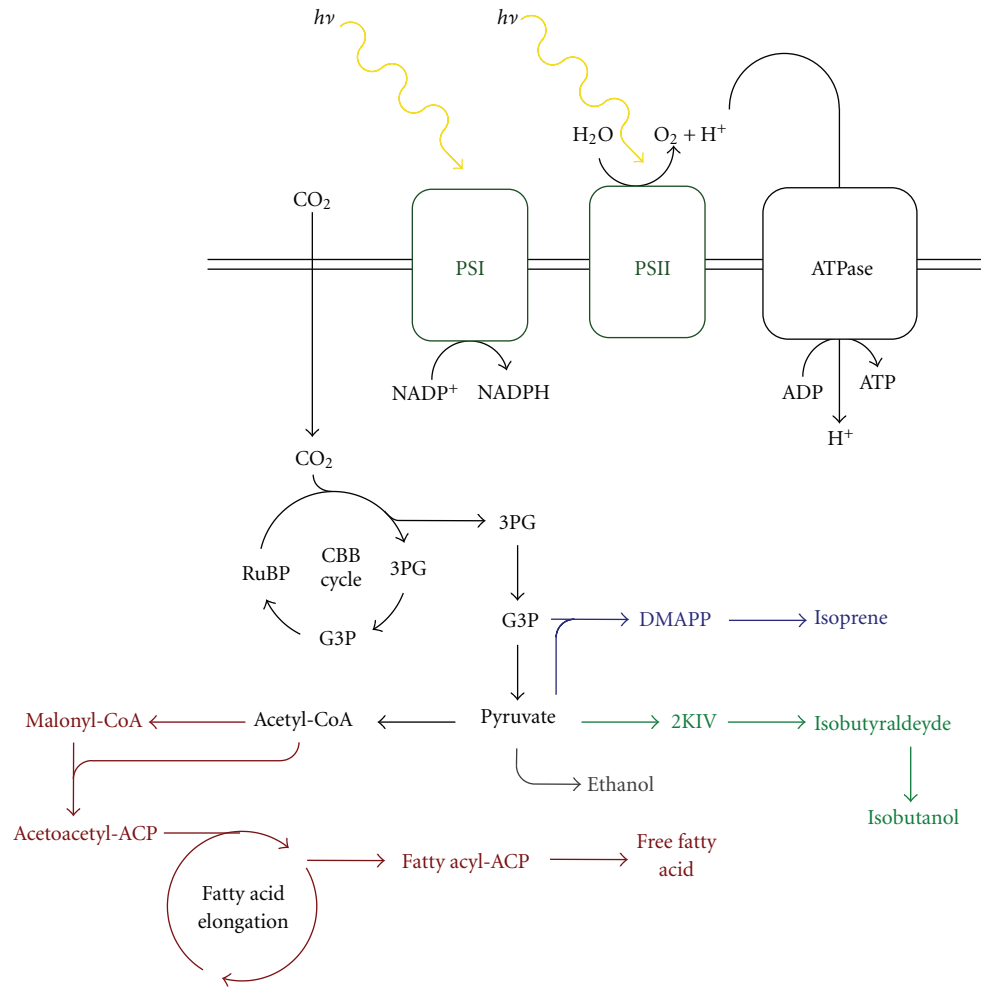

Figure 2: Metabolic schematic for direct photosynthetic biofuel production. Biofuel production pathways for traditional fermentative processes (grey), nonfermentative higher chain alcohols (green), isoprenoid fuels (blue), and fatty acid fuels (red) from CO_2 through photosynthesis. Abbreviations: 2 KIV (2-ketoisovalerate), 3PG (3-phosphoglycerate), ACP (acyl carrier protein), CoA (coenzyme A), DMAPP (dimethylallyl diphosphate), G3P (glyceraldehyde-3-phosphate), PSI (photosystem I), PSII (photosystem II), and RuBP (ribulose-1,5-bisphosphate).

The regulation of flux through divergent or branched pathways will also be critical, as the balance of carbon and electronic cofactors such as NADH must be considered to achieve an efficient process. These divergent pathways are especially common in the nonfermentative higher chain alcohol and isoprenoid pathways. To direct carbon flow in the desired manner, scaffolds can be engineered to connect the preferred branches of the pathway together. Regulatory RNA molecules can also be employed to minimize the expression of competing but essential pathways. Product yields may also be dramatically affected by the availability of NADH or NADPH. One possible solution is to engineer pathways with alternative cofactor specificity to increase their availability [66] or interconversion [67].

Synthetic biology will also be key in rewiring existing regulation. The design of new regulatory pathways from synthetic genetic elements will be important in sensing the extracellular or intracellular environment and producing a programmed cellular response. For biofuel production from lignocellulosic biomass, this is of great importance as the efficient uptake of a mixture of hexose and pentose sugars simultaneously is desirable. Synthetic biology can provide tools to construct new circuits devoid of unwanted regulation from the bottom up [68] to sense the extracellular environment and produce the necessary response to digest the sugars. Ultimately, the bottom-up construction of biological circuits may extend beyond individual pathways toward all of metabolism, as the synthesis of entirely synthetic, replicable, and functional genomes has recently been accomplished [69]. In the future, this will allow synthetic biologists to build heterologous pathways into organisms devoid of unwanted pathways or properties, therefore, increasing the selectivity and yield of the process.

The development and optimization of many aspects of biofuel production technology can benefit from the work already accomplished through synthetic biology. As the

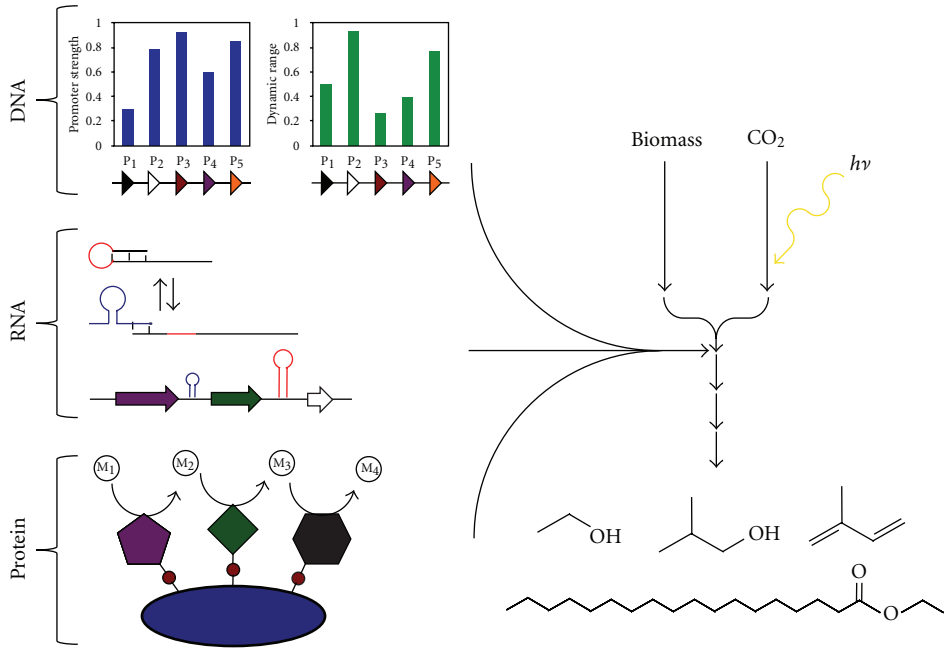

FIGURE 3: Synthetic biology for biofuels. Synthetic biology provides tools at the DNA, RNA, and protein levels that can guide the development of biofuel production processes.

number of available tools increases, the standardization of these parts will become increasingly important. One of the current challenges facing synthetic biology is the reproducibility of the developed tools in different systems, as the variability in cellular regulation and physiology can vary greatly from host to host. Proposals for standardization have been made [70], although as the complexity of these systems increase so will their variability. However, current technology developed by synthetic biology has already allowed for the successful design of many heterologously expressed biofuel production and substrate utilization pathways extending beyond the most user-friendly organisms. The advancement of synthetic biology toward new diagnostic tools and high throughput screening systems will aid in the further development of these biofuel processes for pathway optimization and enzyme discovery or improvement. The success that synthetic biology has already afforded biofuel production technology lends confidence to future synergistic developments and breakthroughs.

4. Conclusions

The desire for renewable liquid fuel replacements to petroleum has steadily increased with concerns about the current fuel economy's stability and environmental impact. The development of new biofuel production processes has sought to mitigate some of these issues. These fuels, whether designed for motor gasoline, diesel fuel, or jet fuel, will face challenges in strain development and productivity in a cost-sensitive market. The integration of synthetic biology with the development of these processes will be significant in bringing the biofuels industry from its infancy to a commercially viable alternative to petroleum.

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