Recent advances in production of bioenergy carrying molecules, microbial fuels, and fuel design - a review.

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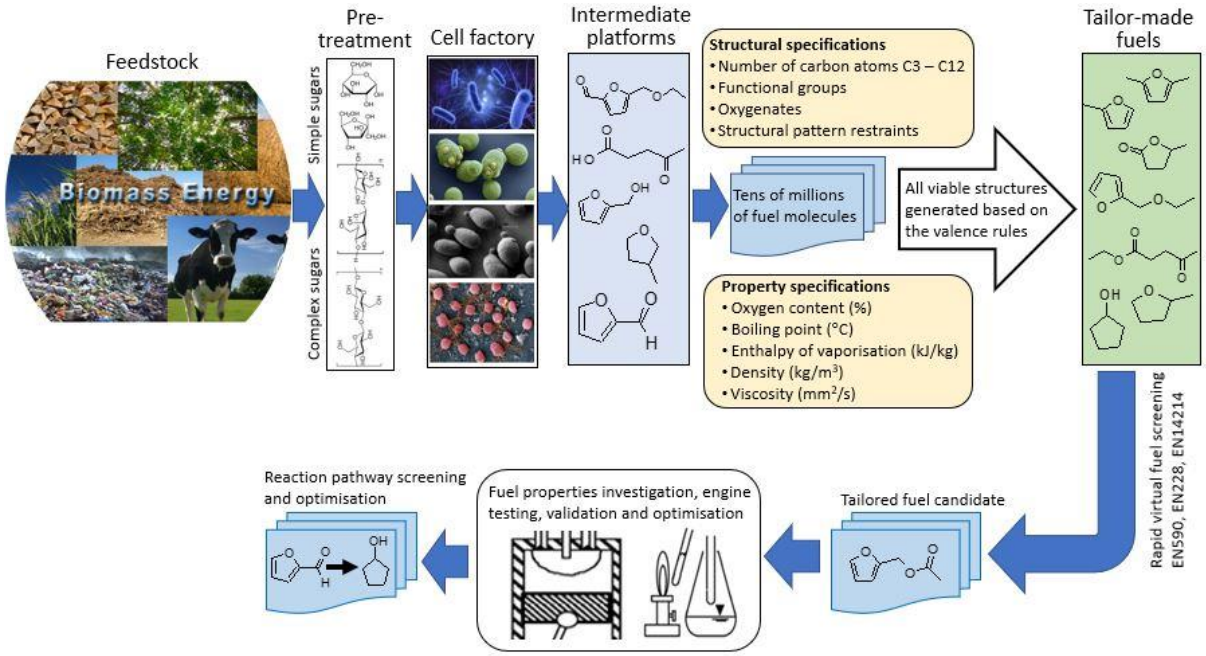
Graphical Abstract



Abstract: The need to replace fossil fuels with biofuels has become necessary due to increasing release of greenhouse gases and particulate matter by combusting fossil fuels. Biofuels are better options compared to fossil-based fuels due to the availability of cheap and abundant renewable feedstock. Due to large number of possible fuel structures clustered in databases, solo experimental search strategies cannot identify a clean and efficient fuel molecule, and application of computer-aided approaches are necessary with integration of product and production design for fuel molecules of single- and multi-species, mass- and energy-based production pathway screening for costs, and emission estimation models.

Optimization of biofuel production processes can surge fuel availability and development of surrogate fuel formulation and property modelling that would improve combustion efficiency of fuels in the engine infrastructure. In this review, we have taken more synergetic approach and analysed the microbial biofuel production processes utilizing 1) metabolic engineering tools on diverse microbes 2) chemo-catalytic pathways and 3) fuel design manoeuvre that is attracting much attention, and thus are extensively discussed in this review. The review emphasized that the utilization of new/modern chemo-catalytic refunctionalization of fuel molecules using new catalysts and enzymes have not only enhanced fuel yields but have led to the production of various novel advanced energy molecules from biomasses and microbes. The contribution of this review is that it highlights the current status of microbial fuels, metabolic engineering, fuel design and production of tailored made fuels, and potential future applications of microbial fuels in transport and energy sectors.

Keywords: Microbial fuels; Metabolic pathways; Energy molecules; Genetically engineered bacteria; Synthetic biology; Tailor-made energy molecules; Biological energy storage; Fuel design

Highlights:

- Production of bioenergy molecules needs metabolic pathway reconstruction
- Yield of 1-butanol with C. acetobutylicum using overexpression reached 130 g/L
- Yield of ethanol with 2,3-butanediol pathway by CRISPR remains low at 3.2 g/L
- Hydrogenation with carbon encapsulated Co catalyst yields 99% furfuryl alcohol
- Tailor-made fuels involve fuel synthesis through catalytic refunctionalization

1. Introduction

Maintaining a secure global energy supply while minimizing environmental impact of energy use is one of the most pressing challenges facing humanity. Replacing conventional fossil fuels with alternative fuels has become a necessity due to decreasing fossil fuels reserves, growing global energy demand, and ever-increasing emission of greenhouse gasses (GHGs), which have a negative environmental impact [1, 2]. Kumar et al. [1] conducted a comprehensive literature review as well as a novel approach on the application of microalgae for simultaneous cultivation and bioremediation of high nutrient containing wastewater. They also discussed the use of a tailor-made membrane in an appropriate module that can be used in upstream and downstream processes during algal-based biofuels production. Patel et al. [2] evaluated various pretreatment methods for efficient lipid extraction from the oleaginous cellular biomass available to date, and discussed their advantages and disadvantages, including their effect on the lipid yield. It is important to diversify alternative fuel feedstocks and design clean, efficient, and innovative fuel options in order to reduce reliance on petroleum-based fuels and subsequent GHGs emission.

To date, various technologies and renewable biomass sources have been utilized as feedstocks for biofuels production. They are categorized as first, second, third and fourth generations [3]. The first- and second-generation biomasses were sourced from food and nonfood products and are no longer used due to food vs. fuel related issues/debate. The use of microalgae, a third generation biofuels source, ameliorated the problems associated with first and second generations biofuels as well as reducing the concentration of CO_2 in the atmosphere; but were faced with low yields and high costs [1]. The emergence of fourth generation biofuels involves the application of metabolic and genetic engineering on

70 microbes to improve biofuels yields and production of energy carrying or new fuel molecules
71 (next generation fuels) from microalgae and other microbes such as bacteria and yeast [1, 4].
72 The use of microorganisms to produce renewable energy fuels can simultaneously resolve
73 energy and ecological concerns [4]. Microbial fuels are renewable fuel molecules produced
74 by microorganisms grown on different substrate and can supplement or/and in some
75 instances replace conventional fuels [4-6]. Ogunkunle et al. [5] reviewed global current
76 scenario of biodiesel adoption and combustion in vehicular diesel engines. Pater et al. [6]
77 studied de novo and ex novo lipid fermentation by oleaginous yeast using glucose and
78 sonicated waste cooking oil. To date, various feedstocks, metabolic engineering and
79 production technologies have been utilized for the production of microbial liquid fuels
80 through general metabolic routes as shown in Fig. 1. These pathways have failed to yield
81 commercial scale volume of biofuels due to microbe strain complexity, complex biological
82 processes and poor understanding of metabolic pathways. Increasing the production of
83 biofuels in order to compete with fossil fuels is a major challenge, which synthetic biology is
84 ameliorating.

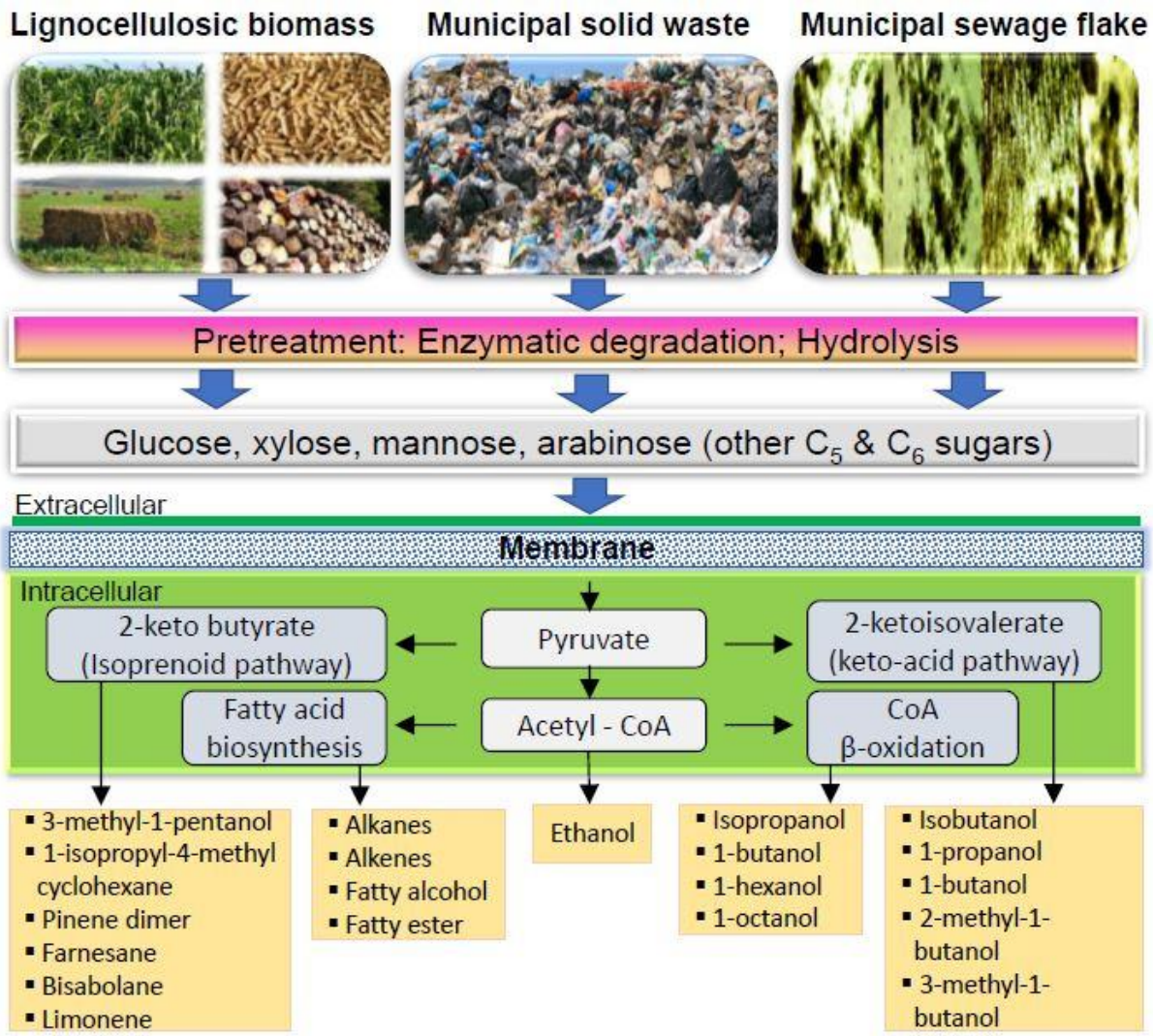


Fig. 1. Overview of pathways towards the production of microbial fuels. Extracellular: conventional lignocellulosic biomass, municipal solid waste and sewage flake substrates can be degraded enzymatically to produce C5 and C6 sugars that are utilized as carbon sources for microbial fuel production; Intracellular: four blocks in blue background show potential generic routes for biosynthesis of various fuels; The light red blocks show the various biofuel precursors that can be produced by each route.

Apart from abating food – fuel debate and land related issues, microorganisms can be utilized to design and produce specialized next generation fuels due to their metabolic diversity and flexibility [7]. They can be genetically manipulated to enhance fuel yields and the production of new fuels [8]. Microbial niches are ubiquitous in the environment offering several enticing traits, such as varied genetic and metabolic potential and strong ecological fitness. Microbial fuels use is not limited by seasons and time, has a lower production process cost when

compared with other renewable fuel production technologies such as diverse solids, and can utilize liquid and gaseous wastes streams [9, 10]. Peralta-Yahya et al. [9] reviewed recent developments in the engineering of metabolic pathways for the production of known and potential advanced biofuels by microorganisms. They focused on metabolic engineering of genetically tractable organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* for the production of these advanced biofuels. Festel et al. [10] conducted modelling of production cost scenarios for biofuels and fossil fuels in Europe. They showed that 2nd generation biofuels are most likely to achieve competitive production costs mid- to long-term when taking into account the effects from technological learning and production scale size as well as crude oil price scenarios between €50 and €200 per barrel.

The production of conventional biofuels such as bioethanol and biodiesel using first, second and third generation feedstocks has been previously reported, highlighting the challenges of maintaining food security when faced with lower land availability and low product yields respectively [11]. More so, storage and direct use of these fuels in the existing transport infrastructure pose some major challenges. Bioethanol can form explosive vapor-air mixtures in the fuel tank under atmospheric conditions, is corrosive and hygroscopic, whereas biodiesel with high viscosity can clog injector systems and lower heat release [12, 13]. Costa et al. [12] presented a work aiming at filling some existing gaps in biofuel combustion modeling by performing investigations on two representative engine cases, for their characterization and performance enhancement. Two approaches followed, namely through reduced chemical kinetics coupled with turbulence within a coherent flame schematization, and through a turbulent species transport approach with detailed kinetics. With the increased use of ethanol as a fuel additive and as a main fuel, Setiyo et al. [13] investigated the effect of pure ethanol on fuel tank. The estimation of the fuel tank corrosion rate through the exponential regression

was obtained. From the assessment metrics, the fuel tank made of Fe_U_100 had a good corrosion resistance.

Substrate physicochemical properties and molecular structures of biofuels as compared to petroleum based fuels, limits the combustion and emission characteristics of the former (bioethanol and biodiesel) [14]. These shortcomings have attracted attention to fuel design, combining chemical and biological processes to produce fuels that will not only mimic conventional fossil-based fuels but enhanced engine performance and GHGs reduction [14].

There have been a few reviews and studies published on microbial fuels production. Keasling et al. [15] discussed engineering metabolic pathways to produce advanced biofuels, challenges with substrate and product toxicity with regard to host microorganisms and methods to engineer tolerance, and the use of functional genomics and machine learning approaches to produce advanced biofuels and prospects for reducing their costs. Shanmugam et al. [16] reviewed the advanced CRISPR/Cas-based genome editing tools for microbial biofuels production. They discussed the role of inducible on/off genetic circuits in response to environmental stimuli in the regulation of targeted genome editing (TGE) by minimizing metabolic burden and maximizing fermentation efficiency. The relevant stringent regulatory demands to ensure minimal off-target cleavage with maximum efficiency coupled with complete biosafety of this technology are considered. They concluded that the recent development of CRISPR-Cas technology should open a new avenue in creating microbial biorefineries for potentially enhanced biofuel production. Mahmood et al. [17] reviewed the advances in developing metabolically engineered microbial platforms to produce fourth-generation biofuels and high-value biochemicals. They covered the research efforts made during the previous decade to produce advanced biofuels and biochemicals through

146 engineered microbial platforms along with the engineering approaches employed. Das et al.
147 [18] reviewed metabolic engineering for enhancing microbial biosynthesis of advanced
148 biofuels. They provided a comprehensive outlook on the trends and developments in
149 metabolic engineering strategies for advanced biofuel production using different hosts. Choi
150 et al. [19] also reviewed metabolic engineering strategies toward production of biofuels. They
151 discussed metabolic engineering strategies recently exploited to enhance biofuel production
152 and facilitate utilization of non-edible low-value carbon sources. These strategies include
153 engineering enzymes, exploiting new pathways, and systematically optimizing metabolism
154 and fermentation processes, among others. They also discussed metabolic and bioprocess
155 engineering strategies to achieve competitiveness of current biofuel production systems
156 compared to those of fossil fuels. Carmona-Cabello et al. [20] investigated biodiesel
157 production using microbial lipids derived from food waste discarded by catering services.
158 Their study demonstrated that food industry waste from the hospitality sector can be used
159 for biodiesel production via microbial oil production. Suitability of food waste to produce an
160 alternative fermentation medium for microbial oil production in two steps (solid-state
161 fermentation using potato peels and food waste hydrolysis) has been demonstrated. All these
162 studies have focused mainly on biological production of microbial fuels and never considered
163 challenges associated with application. The application of synthetic and system biology
164 appears to have improved biofuels production, but the demand and supply gap is still wide.
165 Consequently, the need to utilize other means such as fuel design to produce clean and
166 efficient fuel molecules capable of mimicking physicochemical property of real fuels has been
167 conceived. This tends to alleviate operational challenges such as technical incompatibility and
168 irregularities in combustion environment of the existing infrastructure.

169 Therefore, in this review, we have taken more synergetic approach and analysed the microbial

biofuel production processes utilizing 1) metabolic engineering tools on diverse microbes 2) chemo-catalytic pathways and 3) fuel design manoeuvre that is attracting much attention, and thus are extensively discussed in this review. The review emphasized that the utilization of new/modern chemo-catalytic refunctionalization of fuel molecules using new catalysts and enzymes have not only enhanced fuel yields but have led to the production of various novel advanced biofuels from biomasses and microbes. Advantages, disadvantages, challenges and applications/potentials of microbial fuel developments were outlined. The value of this review is that it highlights the current status of microbial fuels, metabolic engineering, fuel design and production of tailored made fuels, and potential future applications of microbial fuels in energy and transport sectors. An overview of the structure of this review article is presented in Fig. 2.

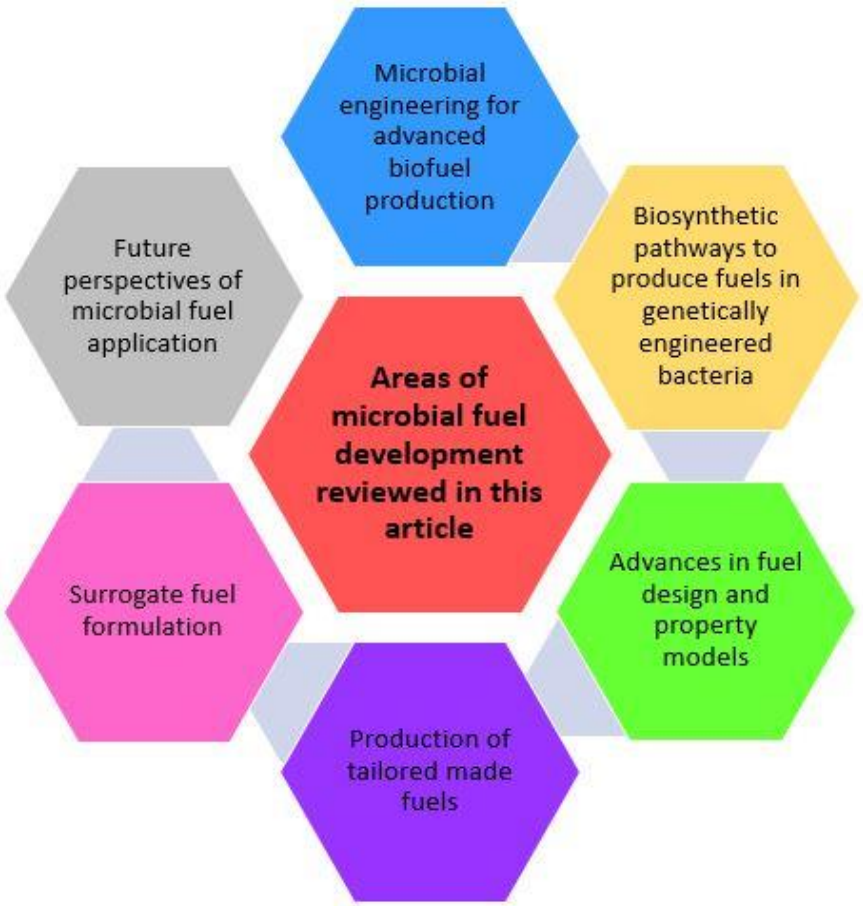


Fig. 2. Overview structure of this review article

2. Advances in bioenergy carrying molecules and microbial fuels

2.1 Microbial engineering for advanced biofuels production

Microbial bioengineering involves the manipulation of microbial metabolic pathways for the construction of enhanced strains engineered to improve microbial biofuels yield and other value added chemicals [21]. This approach is key to addressing low biofuels production rate, high costs and production of biofuels precursors incompatible with the existing transport infrastructure [21, 22]. Kumar et al. [21] investigated future microbial applications for bioenergy production. They concluded that the most challenging hurdle of producing biofuels using "microbial factories" is to generate a large amount of fuel on a comparatively lower budget and greater efficiency as compared to the conventional fossil fuels. In other words, for replacing petrol with bioethanol, the latter should be cheaper, which could be a highly challenging task in terms of meeting the daily quantities. Peralta-Yahya et al. [22] stated that producing microbial biofuels in yields high enough to be useful requires the engineering of the microorganism's metabolism. They concluded that data-driven and synthetic-biology approaches can be used to optimize both the host and pathways to maximize fuel production. Also, microbial metabolic networks have not been characterized, hence, the gap between laboratory and commercial market. The deployment of microbial engineering (metabolic engineering, genetic engineering, synthetic and systems biology) on selected microbes can help produce renewable energy fuels, capable of bridging the gap existing between energy supply and demand, while preserving the environment [23, 24]. Chubukov et al. [23] reviewed synthetic and systems biology for microbial production of commodity chemicals. They concluded that often underestimated challenge is the successful scale up of processes to commercial volumes. Sustained efforts in improving reproducibility and predictability are

needed for further development of microbial biofuels. Yuan et al. [24] reviewed current strategies of metabolic engineering employed for the production of a few key nutraceuticals with selecting polyunsaturated fatty acids, polyphenolic compounds, carotenoids and non-proteinogenic amino acids as exemplary molecules. They concluded that metabolic engineering efforts are enabling rapid production of these molecules.

The goal of microbial bioengineering is to generate microbe strains that can convert GHGs into valuable chemicals and biofuels [25]. To achieve this goal, we need to employ a multifaceted approach that utilized 'omics' and systems modeling approaches along with metabolic and genetic engineering tools, to microorganisms with the potential to produce advanced biofuels with similar properties as those of petroleum-based fuels. Increased fuel and production of new and efficient fuels through this reiterative process can alleviate the numerous biofuel production challenges. To achieve this, there is a need to coherently implement the four microbial steps outlined in Fig 3.

Choosing native host strains that exhibit high metabolic fluxes of the desired fuel precursors [26]. The use of metabolic engineering to expand the available model microorganisms have eased the overdependence on *E. coli* and *S. cerevisiae*. These two microorganisms have been predominantly used due to the availability of engineering tools and good understanding of their metabolism [27-29]. Ostergaard et al. [27] discussed the recent examples that illustrate the possibilities of designing strains of *S. cerevisiae* with new or improved properties through pathway engineering and protein engineering. They stated that since the sequence of the complete yeast genome is available, targeted genetic changes are easily obtained by recombinant DNA technology, which facilitates and accelerates metabolic engineering. Nevoigt [28] also discussed the same bacterium and emphasized that biotech industry would definitely benefit from utilizing engineered organisms. This would, however, require a greater

231 acceptance by consumers. To achieve this, it will be necessary to provide more effective
232 community education and to ensure greater transparency regarding the legal provisions
233 governing approval of GMOs for biotech applications. Cravens et al. [29] described recent
234 developments in metabolic engineering at the level of host, pathway, and enzyme, and
235 discussed how the field is approaching ever more complex biosynthetic opportunities.
236 Two reasons to employ synthetic biology for metabolic engineering in microorganisms are
237 specificity and ease of genetic manipulations. In metabolic engineering, the host's metabolic
238 pathways are modified to improving endogenous metabolic pathways or to initiate
239 exogenous pathways to enhance the production of desired microbial fuel precursors. In
240 addition, synthetic biology can also be used for microbe strain development for enhanced cell
241 factories, metabolic pathway reconstruction and process regulation and optimization [24,
242 30].
243 The challenges incurred in building cells into resourceful factories are due to incomplete
244 annotated microbe metabolic networks, lack of understanding of genomic regulatory
245 networks and complex interaction between molecular pathways that impede attempts to
246 divert resources [21, 31].

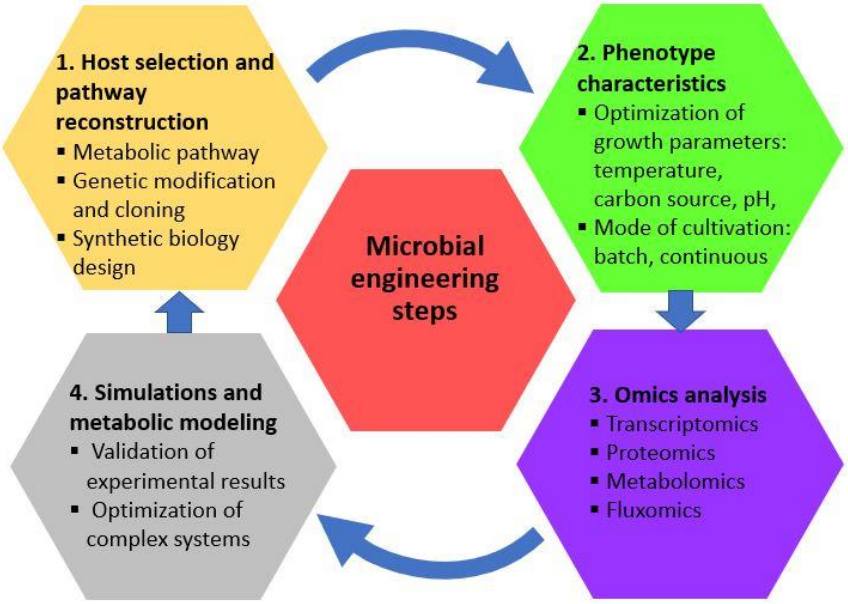


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Fig. 3. Schematic display of microbial engineering steps: 1. Host selection and development of engineered or improved host towards yielding targeted biofuel; 2. Culturing the engineered host by simple optimization of growth parameters and conditions; 3. Analysis of complex and modified pathways generated by the application of synthetic biology; 4. Simulating the output of the analysis in 3 and generating a model that could aid the optimization of more complex case and validation of experimental results.

The manipulation of microbe metabolic pathways using modern genetic tools, has revolutionized the process of microbial biofuels production, allowing for the design and construction of new biological systems for the biosynthesis of new fuels (synthetic biology), process optimization and systems models to ensure efficiency (systems biology).

Synthetic biology is used for the design and development of new biological units, such as enzymes, genetic regulatory circuits and cells, or for the reconstruction of existing biological systems for the highly effective and regulated modulation of target gene expression for high-performance strain building [32, 33]. These units can be modeled and experimentally tested through a reiterative process to achieve precise performance standard.

Systems biology is the study and simulation of complex biological systems in numerical and mathematical terms. It can accelerate new pathway designs and process optimization by integrating biological parts and design developed from 'omics' data, which can be used to construct and validate models [33]. Unlike other engineering fields, biology is extremely non-linear and unpredictable, with less knowledge of the system components and how they might interact under specific conditions. Challenges facing microbial engineering processes are shown in Table 1. These challenges are exacerbated due to the fact that regulatory elements for each reaction is not known, incomplete metabolic pathways and lack of understanding of the over systems complexity [34, 35].

It is only when these are addressed that systems of significant scale will be possible. Recently, a lot of efforts have been geared towards developing new and modern metabolic engineering

274 tools that can be used to improve stability, reduce metabolic burden and toxic intermediate,
275 and novel enzymes and pathways for maximizing the production of biofuels and other valued
276 chemicals [24].
277

278 Table 1. Microbial engineering: steps and notable challenges [36-40].

Microbial engineering steps	Challenges
1. Host selection and pathway reconstruction	Limited number of natural hosts. Some microbes cannot be genetically manipulated; Unavailability of genes and genetic tools; Unavailability, low efficiency and poor understanding of active enzymes; Low yield and titer due to long and complex pathways; Unavailability of metabolic pathways for some target biofuels; Loss of carbon by unwanted metabolite may occur; Limited information of the genetic and metabolic characteristics of some microbes.
2. Phenotype characterization	High energy demand for product recovery; Culture can be contaminated; Product toxicity; Growth can be affected by harsh growth conditions such as media, aeration, temperature, mode of cultivation; Metabolic stresses and genetic instability due to micro-environmental fluctuations such as oxygen and pH levels; Pretreatment of substrates into useful carbon source.
3. Analysis	Separating protein of interest can be difficult as proteins have similar physical attributes; There is no clear correlation between genes and cellular metabolites; Loss of carbon by unwanted metabolite may occur; Leakages may occur when terminating cell activity and extracting metabolites; Metabolic burden; Toxic intermediate may arise; Report about the combination of various omics technology is scanty.
4. Simulation and metabolic modelling	Inaccurate predictions may result due to non-linearity and unpredictability of biological systems; It is difficult to predict the effect of changes in gene expression on metabolic fluxes using stoichiometric models; Most models like kinetic models, ensemble models, and metabolic control analysis can only be applied to a small number of reactions and pathways; Low titer and productivity problems still remain unabated.

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2.2. Biosynthetic pathways to produce fuels in genetically engineered bacteria.

2.2.1. Host selection

Host selection is one of the most consequential choices made when designing and constructing biological systems. We have the option to select either native or non-native microorganisms. Native host are defined as microorganism where the desired metabolic pathway of interest is found in the microorganisms. Non-native host, including *E. coli, S. cerevisiae, Bacillus subtilis* are considered "laboratory domesticated" microorganisms [41]. These non-native hosts offer several advantages because they grow quickly in the lab, there are plenty of genetic and molecular biology tools to introduce or modify genes into their genomes and the there is a large body of knowledge regarding their physiology and biochemistry [42]. However, they face challenges when compared with the native hosts since they might not have the complete metabolic pathway, and they are limited in their use of alternative carbon sources [43].

On other hand, native host can utilize a wider range of carbon sources, offering an opportunity to use alternative inexpensive feedstocks, such as cellulose or food by-products. They usually exhibit resistant to solvents and chemicals since they live with high concentration of these compounds in their environments. Examples of extreme environments could be the high temperature, a strategy sometimes employed to minimize the production of other undesirable products, which will reduce the yield of the product of interest. In this sense, thermophilic bacteria, such as *Pyrococcus furiosus* and *Thermoanaerobacterium saccharolyticum*, have been used to produce 1-butanol obtaining good yields [44]. *P. furiosus* has been studied to elucidate the modulation and balance of both the engineered pathway and the host's metabolism using a temperature-dependent product formation.

Advantages and disadvantages have been defined for both native and non-native host and,

304 depending of the product generated by the metabolic pathway, we could choose between
305 several examples in both types [45]. When selecting a host, we need to consider the
306 product(s) we would like to produce, the feedstocks available, and the inherent metabolic
307 pathways of the host; we could use a native host, when the natural metabolic pathway is
308 present but the molecular tolls have not been developed, and non-native host, when we have
309 the genetic tools available to engineer the above metabolic pathway but sometimes they
310 need to overlap with stress problems.

311 *2.2.2 Genetic tools developed for biofuel production*
312 Several methods have been described for the manipulation of the genomes in the selected
313 host, but they could be further classified into DNA assembly and Genome-editing techniques
314 [46]. A cornerstone of synthetic biology, DNA assembly uses multiple DNA fragments and
315 physically linking them together. We could include into this section on-step PCR, Gibson
316 assembly, BioBricks or golden gate [47-49]. On-step PCR would be the ligation of several genes
317 into one genetic construct by overlap extension PCR.
318 Gibson Assembly was first used by Gibson et al. in 2009 to assemble the artificial genome of
319 *Mycoplasma genitalium* to overcome the difficulties in assembling the 600,000 bp genome
320 [48]. This method uses three different enzymes; exonuclease, DNA polymerase, and DNA
321 ligase, to join multiple DNA fragments into a single DNA strand [49, 50]. Gibson Assembly is a
322 single reaction technique that facilitates the assembly or deletion of several parts into the
323 genome. Gibson Assembly has several advantages over other DNA assembly methods in that
324 there is no need for a restriction digest site in the sequence of interest, it uses fewer reagents
325 and steps, and has no remaining unwanted DNA, or scars, between the ligated fragments.
326 This strategy was successfully used by Gao et al. [51] to assemble the ß-carotene pathway
327 into *Yarrowia lipolytica*.

Although Gibson Assembly and Golden Gate cloning are two of the most commonly used DNA assembly methods, they have several drawbacks due to their reliance on sequence specificity, insert size restrictions, decrease in efficiency as more DNA fragments are added to the reaction mixture in Gibson Assembly, and the use of restriction enzymes Golden Gate cloning [50]. These shortcomings limit the ability to build combinatorial libraries, as they require specific primers for each assembled construct, which must be confirmed using DNA sequencing to be sure that there are no sequence errors introduced via the polymerase amplification reaction. Golden Gate cloning is similar to Gibson Assembly in that it can join multiple DNA fragments in a scarless assembly, but has additional specificity elements due to the use of Type IIS enzymes [52]. This is a highly specific and efficient cloning method that allows the ligation of multiple DNA components to be inserted in sequential order into a single plasmid in a single-step; one-pot reaction [47].

The BioBricks Assembly Standard was developed at the Massachusetts Institute of Technology by Tom Knight in 2003. The BioBrick Assembly Standard applied engineering design principles to create a modular synthetic biological circuits parts library [53, 54]. Since then, the Knight group and the International Genetically Engineered Machines (iGEM) competition have created The Registry of Standard Biological Parts that currently contains engineering description of over 20,000 BioBricks parts [55]. These genomes editing and assembly techniques have been successfully used to produce transgenic microorganisms engineered to produce biofuel manipulating the genome of native and non-native host. The Gibson Assembly method was used to introduce a multi-enzyme pathway into *P. furiosus*, a microorganism that lives at 100 C for butanol production [56, 57]. The ability to bioengineer such extremophiles is of industrial value to reduced contamination risk, ease of removal of volatile compounds, and the potential to use temperature as a controlling factor in the

352 production of desired high value products.

353 Park et al. [58] used Golden Gate cloning to engineer *Y. lipolytica* for production of odd-chain
354 fatty acids. They presented a method where they constructed a seven-enzyme plasmid to
355 favor the use of acetyl-CoA instead of propionyl-CoA to produce an odd-chain fatty acid. This
356 change allowed for the use glucose as a carbon source to produce the odd-chain fatty acid.

357 One of the most promising entries into the genome editing toolbox is the RNA-guided DNA
358 recognition CRISPR (clustered regularly interspaced short palindromic repeats) Cas (CRISPR-
359 associated) (CRISPR-Cas) system. CRISPR-Cas systems are prokaryote adaptive immunity
360 response systems that protect them from viruses and other foreign elements [58, 59].
361 Researchers have taken advantage of the specificity of this system to introduce sequence
362 specific changes in the genome of interest. This technology has been used to engineer
363 bacteria, yeast and microalgae to generate biofuels and other targeted molecules of interest.
364 For example, Li et al. [60] engineered *Synechococcus elongatus* PCC 7942 as microbial cell
365 factory for the production of succinate using CRISPR/Cas 9 system. In addition, Lee and Seo
366 [61] successfully used the same technology to engineer *S. cerevisiae* and produce 2,3-
367 butandiol from glucose and cassava hydrolysates.

368 There is an increasing set of genomes editing tools to choose from for designing and
369 introducing new metabolic pathways into microbial organisms. A few number of the
370 applications and yields of these methods/tools are outlines in Table 2. These technologies
371 provide a road map on the way forward in genetic engineering, facilitating the development
372 of biotechnological applications to address energy concerns in the future.

373 Table 2. Products of metabolically engineered microbes for advanced fuel production

Fuel molecules	Host	Substrate	Metabolic engineering strategies	Yields g/l	Ref.
Alkane	*Cupriavidus necator*	20 g/l of fructose	Overexpression of heterologous ferredoxin	1.48	[61]
Fatty acid methyl ester	*Y. lipolytica*	20 g/l of glucose	Enhance NADH supply by gene editing	98.9	[62]
Fatty acid ethyl ester	*R. opacus*	10 g/l of glucose	Gene edition deleting 7 genes	21.3	[63]
Ethanol/butanol	*C. acetobutylicum*	10 g/l of glucose	Introduce 2,3-butanediol pathway by CRISPR	3.2 ethanol; 12.1 butanol	[64]
1-butanol	*C. acetobutylicum*	80 g/l of glucose	Site-directed mutagenesis engineering aldehyde/alcohol dehydrogenase	10.31	[65]
1-butanol	*C. acetobutylicum*	20 g/l of glucose	Overexpression (improved direct 1-butanol forming flux, *in situ* recovery)	130	[66]
Isobutanol	*S. cerevisiae*	4 % w/v of glucose	Overexpress L-valine pathway	2.09	[67]
3-methyl-1-butanol	*Corynebacterium glutamicum*	5 g/l of glucose	Random mutagenesis with gene editing	0.7	[68]
Isopropanol	*Clostridium beijerinckii*	60 g/l of glucose	Genome shuffling	50	[69]
Isopentenol	*E. coli*	1 % glucose	Ribosome binding site engineering	2.23	[69]

374

2.2.3. Pathway reconstruction

Bioethanol has traditionally represented the largest produced renewables biofuel, but the future, it will be replaced by more advanced biofuels and higher alcohols due to their greater fuel properties, which include higher density, less volatility, and less corrosiveness [70]. Exploring pathway reconstruction through metabolic engineering to produced higher alcohols such as 1-butanol, 1-propanol or 2-methyl-1-propanol has been extensive studied due to the ease of substitution of biofuels with blended degrees. For instance, 1-butanol has been produced in several strains of *Clostridium*, like *Clostridium acetobutylicum*, because their natural ability to produce this alcohol through the acetone-butanol-ethanol fermentation pathway [71]. Fig. 4 shows the three main metabolic pathways used to design and understand: (A) the type of biofuel produced, (B) 2-ketoacid, which is the main pathway for the production of branched-chain alcohols, and isoprenoid pathways, which is used for the production of branched and cyclic hydrocarbons, (C) reversed β-oxidation and fatty acid synthesis for the production of branched-chain alcohols, short and long-chain alkanes and fatty acid methyl esters [72-74].

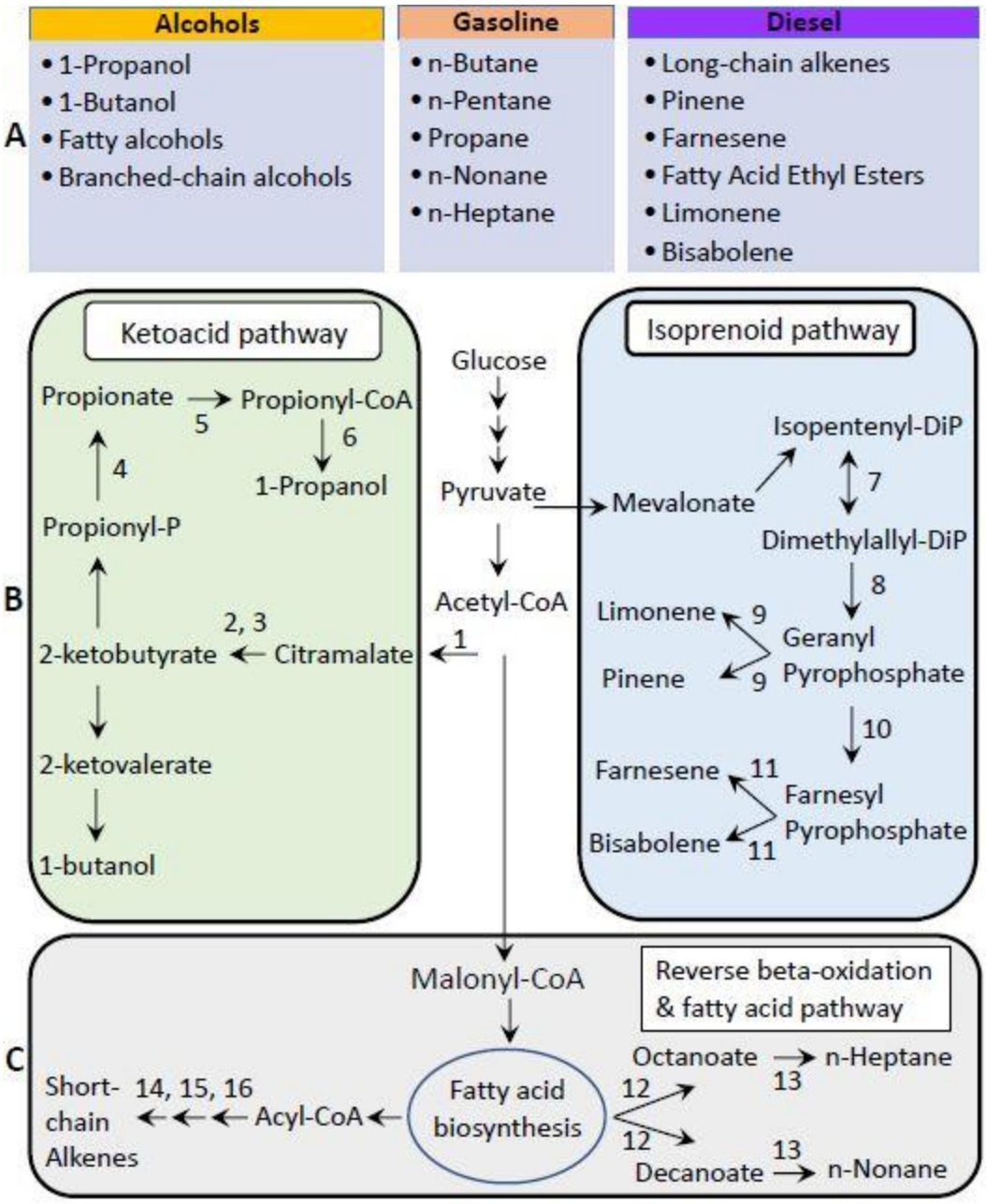


Fig. 4. Main bioenergy carrying molecules and metabolic pathways generated in biosynthetic fuels. This figure was made by authors using information from [74-76]. **A**. Molecules according to the fuel category; orange-alcohols; pink-gasoline, and purple-diesel. **B**. Metabolic pathways involved in the generation of the above molecules. **C.** Reversed β-oxidation and fatty acid synthesis for the production of branched-chain alcohols, long-chain alkanes, and fatty acid methyl esters. Abbreviations for the enzymes are: **1**, citramalate synthase; **2**, 3-isopropylmalate isomerase; **3**, 3-isopropylmalate dehydrogenase; **4**, acetate kinase; **5**, acetyl-CoA:acetoacetyl-CoA transferase; **6**, alcohol dehydrogenase; **7**, isopentenyl pyrophosphate isomerase; **8**, geranyl pyrophosphate synthase; **9**, monoterpene synthases; **10**, farnesyl pyrophosphate synthase; **11**, sesquiterpene synthases; **12**, carboxylic acid reductase; **13**, a fatty aldehyde decarbonylase; **14**,

418 acyl-ACP thioesterase I; **15**, acyl-CoA synthetase and **16**, aldehyde deformylating oxygenase (or
419 aldehyde decarbonylase

420 The 2-ketoacid pathway to higher alcohols has been used due to the fact they are intermediates
421 in amino acid biosynthesis pathways. The 2-ketoacids undergo decarboxylation, forming
422 aldehydes or ketones, which are further metabolized to alcohols by alcohol dehydrogenases.
423 Atsumi et al. [77] took advantage of this native pathway and introduced 2 non-native enzymes
424 into *E. coli* to produce alcohols. The authors used six different 2-ketoacids, produced their
425 respective alcohol derivatives. Another strategy to produce higher alcohols has been the
426 elimination of competitive metabolic pathways [78]. This has been used to increase the titers of
427 1-butanol in microbes by preventing the key precursor, acetyl-CoA, from being shunted into
428 competitive pathways. This approach was used to delete the enzyme acetate kinase in
429 *Clostridium tyrobutylicum* to decrease the acetyl-CoA conversion into acetate, which led to a
430 nine-fold increase in 1-butanol titer [78, 79]. In a separate work, Saini et al. deleted the native
431 *frdA* subunit of fumarate reductase, lactate dehydrogenase, aldehyde-alcohol dehydrogenase
432 and phosphate acetyltransferase genes in *E. coli* and introduced the acetoacetyl-CoA transferase
433 and butyraldehyde-butanol dehydrogenase genes from *Clostridium spp*. to produce 1-butanol
434 using glucose and butyrate as carbon sources [79, 80]. A strategy of upscaling the production of
435 higher alcohols is the reducing cofactors imbalance. Matsuda et al. [81] increased the production
436 of isobutanol in an isobutanol producing *S. cerevisiae* strain from 0.23 g/l to 1.62 g/l by
437 introducing a shunt pathway to maintain the NADP+ to NADPH ratio in the cell as well as deletion
438 of contending pathways. Combining these techniques clearly improved product yield but titer is
439 still low. Hence, optimizing the process could lead to commercial production of isobutanol, as
440 compared to 1-butanol, isobutanol has a comparable energy content and a higher-octane

441 number.

442 Other non-clostridial host, such as *E. coli, S. cerevisiae* and *S. elongatum* have recently been used
443 to produce 1-butanol [73, 81]. Shen et al. [82] constructed a modified clostridial 1-butanol
444 pathway in *Escherichia coli* to provide an irreversible reaction catalyzed by trans-enoyl-coenzyme
445 A (CoA) reductase (Ter) and created NADH and acetyl-CoA driving forces to direct the flux. They
446 achieved high-titer (30 g/liter) and high-yield (70 to 88% of the theoretical) production of 1
447 butanol anaerobically, comparable to or exceeding the levels demonstrated by native producers.
448 Steen et al. [74] engineered *Saccharomyces cerevisiae* with an n-butanol biosynthetic pathway,
449 in which isozymes from a number of different organisms (*S. cerevisiae*, *Escherichia coli*,
450 *Clostridium beijerinckii*, and *Ralstonia eutropha*) were substituted for the *Clostridial enzymes* and
451 their effect on n-butanol production was compared. By choosing the appropriate isozymes, they
452 were able to improve production of n-butanol ten-fold to 2.5 mg/L. Dekishima et al. [84]
453 investigated metabolic engineering of cyanobacteria for 1-butanol production from carbon
454 dioxide. To produce 1-butanol from CO2, they transferred a modified CoA-dependent 1-butanol
455 production pathway into a cyanobacterium, *Synechococcus elongatus* PCC 7942, and
456 demonstrated the activity of each enzyme in the pathway by chromosomal integration and
457 expression of the genes.

458 Production of 1-butanol was also accomplished by introducing the clostridial CoA-dependent
459 reversed oxidation pathways into these microorganisms. Dekishima et al. [84] produced 1-
460 hexanol from 1-butanol by heterologous expression of the β-ketothiolase (BktB) from *Ralstonia*
461 *eutropha* and the 3-hydroxybutyryl-CoA dehydrogenase and crotonase *C. acetobutylicum* in *E.*
462 *coli*. The isoprenoid pathway is the typical platform to produce diesel-like fuels. In this pathway,

463 monoterpenes or sesquiterpenes are converted into limonene or farnesene through the
464 mevalonate or the D-xylulose-5- phosphate pathways [85]. These biodiesel-like fuels are close to
465 be commercialized into the jet fueling. Therefore, these isoprenoids-type biofuels are an
466 excellent biosource for the diesel and jet fuel substitute because their chemical properties are
467 very similar to the traditional fossil fuels.

468 *2.2.4. Omics analysis*
469 Recent advances in multiple omics tools, such as genomics, transcriptomics or proteomics, has
470 facilitated the global analysis of the genotype to phenotype association in engineered and non-
471 engineered microorganism [86]. The new "omics" era is driving our understanding of the
472 regulation and interconnectedness of biological processes through the ability to simultaneously
473 analyze DNA, RNA, proteins and metabolites extracted from the same experimental samples. In
474 this sense, next generation sequencing of nucleic acids has facilitated the identification of cryptic
475 metabolic pathways in new improved strains decreasing dramatically the cost of engineered
476 microorganisms. RNA microarrays can detect the transcriptome expression and mass
477 spectroscopy can obtain the metabolite profile of strains of interest. Finally, we can obtain
478 glycome and lipidome through LC/MS, GC/MS, NMR and flux analysis [86].
479 These techniques are being used more frequently due to the lower cost for analysis and more
480 robust analysis models. For example, *Synechocystis sp.* PCC6803 has been analyzed using high
481 throughput transcriptomics to detect small RNA molecules in response to light or substrate
482 depletion stress [87, 88]. In addition, the same strain was analyzed with proteomics to
483 understand the proteins involved in the redirection of carbon flow *Tricoderma reseei*, a well-
484 characterized fungi used in the cellulose conversion into biofuel, has been fully explored and

485 characterized with the help of advanced proteomics to correlate the regulation of gene
486 transcripts (transcriptomics) with protein expression (proteomics) and generate novel strategies
487 to maximize this conversion with a most cost-effective way [89, 90].

488

489 *2.2.5. Simulation and metabolic modeling*

490 Omics techniques have produced a large set of metadata on the microorganism genomes and
491 their potential engineering for industrial use. This has led to the development of biological
492 databases, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) [91], Biochemical Genetic
493 and Genomic (BiGG) [92] and ENZYME [93], which act as repositories for use with bioinformatics
494 tools. The mining of these databases by bioinformatics tools has proven indispensable to
495 predicting potential metabolic routes toward production of novel biofuels. For example, The *Y.*
496 *lipolytica*'s genome has been mined in order to understand potential metabolic pathways
497 involved in this lipid synthesis [94].

498 The construction of genome-scale models (GEM) is vital to simulating *in vitro* the cellular behavior
499 under physiological and stress environments. Pan and Hua [95] reconstructed a metabolic
500 network in this yeast using genome annotation and the biochemical databases previously listed
501 to predict the growth in minimal media with some carbon sources. This helped in the knowledge
502 of new carbon uses degradation by *Y. lipolytica*.

503 All this data provides GEMs with the necessary data for the evolution of metabolic prediction
504 algorithms, with the recent introduction of enzymatic constraints. These new model algorithms
505 increase the accuracy of current models by taking into consideration the kinetic parameters of
506 enzymes involved in the metabolic pathways along with the relative concentration of secondary
507 metabolites observed in the cellular environment [96].

Recently, bioinformatics tools have been developed to assist in the design of genome wide metabolic engineering using CRISPR/Cas9 systems. Tools such as CCTop or CRISPR aid in genome design for use in eukaryotic cells and CRISPR-era or CRISPy-web are available for bacteria and other non-model microorganisms [97]. CRISPy-web allows scanning possible sgRNA from the target genome of interest in non-model microorganisms and CRISPR-era allows designing sgRNA for or gene activation or repression by CRISPRi [98-100].

2.3 Using cell-free enzymes / enzymatic cocktails for conversion of precursors to energy rich molecules.

Lignocellulosic materials are the most abundant and inexpensive feedstock for biofuel production. It contains high concentration of polysaccharides and lignin, which makes a highly complex structure, often considered as recalcitrant to enzyme treatment because of their high crystallinity and water insolubility [101]. Lignocellulose is composed of cellulose and hemicellulose as carbohydrate component, tightly packed by phenol aldehyde lignin polymer [102]. The pre-digestion of this recalcitrant material has been identified as key for the release of sugars and use by biofuel-producing microorganisms through fermentation or anaerobic digestion. This pretreatment breaks down lignin and glycosidic bonds, reducing the crystallization and increasing the sugar accessibility to the microbes. The pretreatment could be divided into biological, chemical, physical, or a combination of these methods. In this section, we will focus on the biological pretreatment with the help of efficient enzymes and enzymatic cocktails.

Lignin can be enzymatically modified mainly through laccase, lignin peroxidase and manganese peroxidase enzymes. These enzymes have been shown to be efficient pretreatments of lignocellulose for downstream bioethanol conversion. Rencoret et al. [103] used laccase from *Picnoporus cinnabarinus* to pretreat wheat straw obtaining a 60% increase in glucose yield. In

addition, Asgher et al. [104] treated sugarcane bagasse with a cocktail of laccase, lignin and manganese peroxidase enzymes from *Pleutorus ostreatus IBL-02*, reporting an ethanol production of 16.2 g/l after 34 % delignification. Although this titer is lower than 18.2 g/l produced when treated with alkali, optimizing various parameters such as temperature, pH and reaction time increased ethanol yield to 28.15 g/l. Table 3 shows the list of enzymes and enzymatic cocktails used to increase the lignocellulosic material conversion.

Cellulases, α-glucosidases and hemicellulases have been extensive reported as key enzymes involved in the enzymatic degradation of cellulose and hemicellulose [105]. However, end products of these enzymatic reactions have been described as inhibitors of the process, decreasing the reaction rate and reducing the yield in the fermentation process. In this sense, cellobiose has shown to inhibit cellobiohydrolases and endoglucanases by binding to the active site or the attached catalytic binding module (CBM) [106]. While glucose has been reported to bind to the active site of β-glucosidases and, in a lesser extent, to cellobiohydrolases and endoglucanases. Moreover, monosaccharides and small oligosaccharides derived from hemicelluloses, mainly xylose and xylo-oligosaccharides, have been documented to bind to celluloses and preventing the binding of the enzyme to the cellulose chain [107]. End product inhibition is the key driver in the reduction of yields for the final biofuel conversion. In fact, the external addition of 60-200 g/l of glucose to the fermentative process resulted in the 20% inhibition regardless the biomass, type of pretreatment and enzyme preparations [108].

Because of this inhibition process, effort has been made to search for new and more efficient enzymes to reduce this problem. Optimization of enzyme loading, new enzyme formulations/cocktails and protein engineering to reduce inhibition have been shown as

promising strategies to improve biofuel yields. Supplementing enzymes blends with accessory enzymes, such as pectinases, laccases or lytic polysaccharide monooxygenases (LPMO) was sufficient to achieve a glucose titer and yield of 122 g/l and 80 % respectively on the sugarcane bagasse treatment, indicating the potentials of industrial application of the process [109]. Finally, LPMOs has been shown to increase the reaction rate of cellulases by acting in a synergistic way driving efficient lignocellulose conversion. These oxidative enzymes are of great importance because they enhance the cellulose activity and they are supplemented in commercial enzymatic blends by Dupont® or Novozymes®.

Another strategy used for the improvement of endoglucanases is protein engineering. Reyes-Ortiz et al. [110] constructed chimeric enzymes fusing two thermostable glucanases with a family 2a carbohydrate-bonding module (CBM). This process enhanced the activity of endoglucanases in three-fold. Other examples of protein engineering are modification to cellulosomes, the natural extracellular complex that some microorganisms employ to fully degrade cellulose. This machinery is a multidalton protein complex with several glycoside hydrolase and CBM modules anchored in the surface of the bacteria. This complex machine is highly efficient at cellulose degradation due to it incorporating proximity strategy. It is composed of several glycoside hydrolase family enzymes that act synergistically to depolymerize carbohydrates. Examples of this cellulosome has been extensively shown in natural bacteria, like the simpler example in cellulolytic *Clostridium thermocellum* [111] or the more complex in *Ruminococcus flavefaciens* [112] or *Pseudo)Bacteroides cellulosolvens* [113]. Recent efforts have been made to synthetize recombinant cellulosome composed of modules from different organism, including thermostable or salt tolerant bacteria or fungi [114, 115]. This proximity strategy naturally employed by

576 bacteria has been employed as a cellulosome design approach using a combinatorial set of

577 enzymes, including auxiliary LPMOs, as biotechnological nanomachines [116].

578 Table 3. Enzyme and cocktails used to increase the lignocellulosic material conversion.

	Target substrate	Process enhanced	Reference
Enzymes			
Laccase	Lignin	60% increase for glucose yield	[117]
Cellulases	Cellulose	Cellobiose inhibition decrease	[118, 119]
β-glucosidases	Cellulose	Glucose inhibition decrease	[118, 119]
Xylosidases	hemicellulose	Xylose inhibition decrease	[118, 119]
cellobiohydrolases	Cellulose	Cellobiose inhibition decrease	[120]
endoglucanases	Cellulose	Cellobiose inhibition decrease	[120]
CBM-endoglucanase	Cellulose	Higher cellulose affinity	[109]
Enzymatic cocktails			
Laccase, Lignin and Manganese Peroxidase mix	Lignin	34% delignification obtaining 16g/l ethanol	[121]
Pectinases, Laccases LPMOs mix	Cellulose and chitin	122 g/l and 80% glucose release from cellulose	[122]
Multicomplex cellulosome	Cellulose	Higher cellulose affinity and catalytic efficiency	[115, 123, 124]

579

580

Summary of section 2: In this section we have reviewed biosynthetic pathways in engineered bacteria for microbial fuel production. It includes microbial engineering steps and techniques such as host selection, development of genetic tools, pathway reconstruction, omics analysis, and metabolic simulation/modeling. This section also covered types of enzymes and enzyme cocktails for conversion of precursors to energy rich molecules. The section provides a list of energy molecules produced by host bacteria, substrates, and metabolic engineering strategies employed, along with product yield.

A few notable challenges in microbial engineering have been also highlighted. These include:

- Limited number of natural hosts;
- Low yield and titer due to long and complex pathways;
- Loss of carbon by unwanted metabolite may occur;
- High energy demand for product recovery;

- Metabolic stresses and genetic instability due to micro-environmental fluctuations such as oxygen and pH levels;
- There is no clear correlation between genes and cellular metabolites;
- Inaccurate predictions may result due to non-linearity and unpredictability of biological systems.

3. Advances in fuel design and property models

3.1 Overview

The utilization of substrates and some mono carbon gases mentioned earlier in this review as carbon sources required to produce eco-friendly oxygenated microbial liquid fuels through have been studied. The application of synthetic and system biology appears to have improved biofuels production, but the demand and supply gap is still wide. Consequently, the need to utilize other means such as fuel design to produce clean and efficient fuel molecules capable of mimicking physicochemical property of real fuels has been conceived. This tends to alleviate operational challenges such as technical incompatibility and irregularities in combustion environment of the existing infrastructure.

Through fuel design, biofuels produced from lignocellulosic biomass or other chemical compounds/reagents can be tailored to display physicochemical properties like those of fossil-fuels, allowing them run in the existing transport platforms. The aim of fuel property design is to produce fuels that will not only mimic conventional fossil-based fuels but enhanced engine performance and reduce GHGs and PM. This could be achieved by 1) redesigning biofuels through refunctionalization of their molecular structures and functional groups towards having same physicochemical properties and with those of real fuels; this is regarded as 'tailored–made fuels from biomass' (TMFB) and 2) identifying chemical compounds/reagents of desired properties, which could be blended as surrogate fuels with similar properties as those of real fuels; regarded

602 as ‘surrogate fuel formulation’ [125, 126]. Villeda et al. [125] developed a model-based fuel
603 design methodology which is based on an integrated product and process design approach,
604 considering aspects of both fuel combustion and fuel production. They aimed at identifying
605 possible fossil fuel surrogates from a database of rigorously generated molecular structures. Li et
606 al. [126] developed surrogate formulation methodology for biodiesel based on chemical
607 deconstruction in consideration of molecular structure and engine combustion factors. Tailored-
608 made biofuels are fuels made from biomasses that are upgraded to exhibit certain desired
609 properties for high efficiency and clean combustion [127]. It has been reported that the
610 production and utilization of biofuels/oxygenates have several advantages such as carbon
611 neutrality, renewability, clean and high combustion efficiency, low sulfur and aromatic content
612 [128, 129]. The presence of 10-45% oxygen distinguishes biofuels from fossil fuels [130].
613 However, some drawbacks like emission of toxic nitrogen oxides and PM, limited infrastructures,
614 high fuel consumption and less energy content have been observed from various literature [131,
615 132]. Attempts have been made to enhance the efficiency and emission related drawbacks
616 associated with the use of biofuels through model-based design of TMFB [133]. Reports of
617 advances in model-based design of some novel fuels are scanty.
618 In this section, we will identify various pathways for the production of different oxygenated fuel
619 species, advances in model-based fuel design and demonstrate the model-base formulation of
620 single fuel species, multi-species and various blends (multiple fuel species) of four new fuel
621 species such as 2-pentanol, cyclopentanol, ethyl furfuryl ether and 5-(ethoxymethly)furfuryl
622 amongst others. The effects of the properties of these fuels on engine performance will be
623 compared with those of conventional fossil fuels.

3.2 Production of tailored made fuels from biomass (TMFB)

TMFB involves selective fuel synthesis through catalytic refunctionalization of biomasses to produce biofuel molecules, and upgrade of fuel intermediate into possessing some desired physicochemical properties required for clean combustion and high efficiency [127]. Converse to refunctionalization, a process regarded as defunctionalization has been developed. This process allows for the design of optimum reaction pathways starting from the biofuels back through intermediates selected from a range of available chemical platforms (building blocks) from the carbohydrate feedstock of biomass and transformed these molecules into biomasses, in analogy to a retrosynthetic analysis in traditional organic synthesis [134, 135]. vom Stein et al [134] discussed conceptual approach to tailor-made fuels via combined product and process design. They concluded that the selective conversion of lignocellulosic biomass provides a possible approach to the sustainable production of fuels and chemical products. Palkovits et al. [135] discussed the challenges of catalytic deoxygenation, novel strategies for separation, and opportunities provided at the interface to biotechnology. They emphasized that biomass as highly functionalized feedstock can provide manifold opportunities for the transformation into attractive platform chemicals. While biofuels production from lignocellulosic biomass through gasification or pyrolysis seems to always produce fuels of multiple compositions such as hydrocarbons, hydrolysis of lignocellulosic biomass can potentially lead to selective synthesis of a pure biofuel component like bio-ethanol, 2-methyl furan or biodiesel [136]. Den et al. [137] critically reviewed the research on chemical oxidative techniques for the pretreatment of lignocellulosics with the explicit aim to rationalize the objectives of the biomass pretreatment step and the problems associated with the conventional processes. Guczi et al. [138] discussed

the use of biomass or biomass derived materials as energy sources, hydrogen formation in methanol and ethanol reforming, biodiesel production, and the utilization of biogases, which are all promising processes for energy production that depend heavily on catalysts [139].

These biofuels are tailored either as a single fuel species or intentionally blended with other fuels to maximize internal combustion (IC) engine performance and reduced toxic gas emissions [140]. For instance, two pure compounds produced from biomass, suspected to be potential biofuels; 2-butanone or methyl ethyl ketone (MEK) and 2-methylfuran were acknowledged within the Cluster of Excellence ‘‘TMFB”, and were examined using in spark ignition (SI) engine. The results obtained were compare with those of PMS and bench biofuel (bioethanol). It was observed that both selected bio-products showed superior combustion characteristics, significant reductions in PM, with increased emissions of nitrogen oxides when compared using engine test devices.

Apart from known non-food lignocellulosic biomasses, organic municipal sewage sludge (flakes) and organic municipal solid waste are potential sources of readily available feedstock which can either be directly converted to biofuel or biofuel precursor. Dornau et al. [141] showed that the organic portion of municipal solid wastes consist of over 50% lignocellulosic biomass by mass of municipal waste, which could be utilized as feedstock for the production of intermediates/platform molecules such as itaconic acid and furfuryl, which are precursors for subsequent production of biofuels. Also, Bharathiraja et al. [142] stated that the organic proportion of municipal sewage flakes contain about 15 % carbohydrate and 35 % fat, which can be utilized to produce C5 and C6 sugars and biodiesel precursor(lipids) respectively. Firstly, the pretreatment and enzymatic degradation of these lignocellulosic biomasses to produce simple C5 and C6 sugars have been outlined earlier in this review. The simple sugar produced from

669 various biomasses can either be used as carbon source for microbial cell factories, as mentioned
670 earlier, or catalytically reconfigured to yield biofuels [143].
671 Many enhanced clean combustible tailor-made fuels and fuel intermediates could be produced
672 from C5 and C6 sugars sourced from diverse biomasses/feedstock by simple bond-breaking and
673 bond-formation processes through selective hydrogenation, dehydration, hydrolysis,
674 esterification and other new processes like ethanolysis and use of novel catalysts for catalytic
675 transformations [144, 145]. For the sake of simplicity, the routes of catalytic production of various
676 platform chemicals, leading to the formation of novel oxygenated fuels utilizing C6 and C5, and
677 each chemical process/reaction and corresponding chemical structure have been outlined in
678 Fig.5 and Table 4 respectively. The processes leading to the production of hydrocarbon fuels,
679 especially from ϒ-valerolactone were excluded since our focus lies strictly on chemical processes
680 to produce oxygenated fuels, and identifying the physical, chemical and combustion
681 characteristics of these new fuels. The overview of various biomasses that can be converted into
682 various oxygenated fuels and fuel platforms is shown in Fig 5.

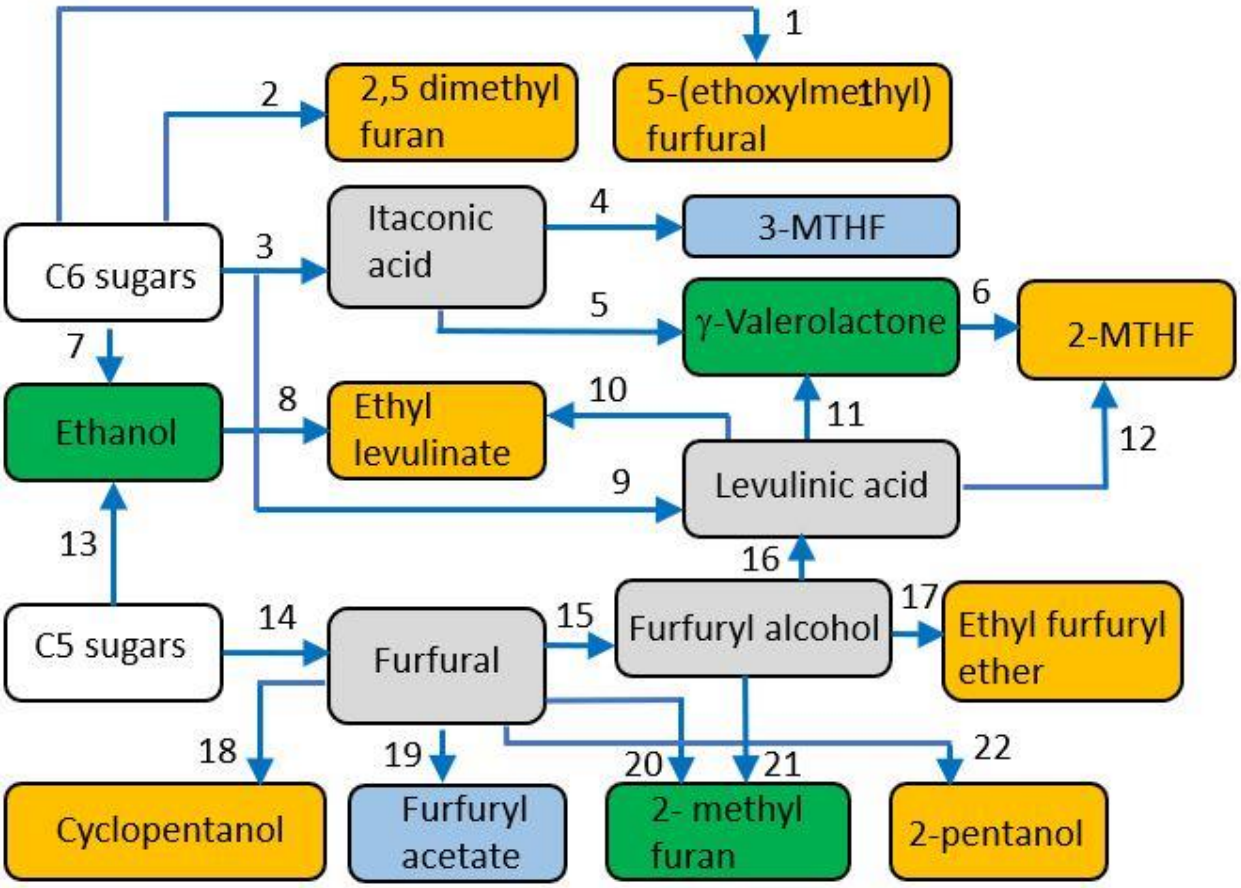


Fig. 5. Pathways leading to the production of new fuel molecules using C6 and C5 sugar sources. This figure was made by authors using information from [140, 146-148]. For convenience, only pathways/routes are outlined in Fig.5 and chemical processes with references are outlined in Table 4. Each color denotes: white - monosaccharide; gray - chemical platforms (intermediates) for fuel production; green - serves dual purposes 1) as fuel molecules and 2) chemical platform for fuel production; orange - new fuel molecules; blue - potential fuel molecules and fuel additives.

Briefly, furfural and itaconic acid produced by enzymatic hydrolysis of C5 and C6 sugars respectively are key platforms to produce biofuels and other fuel precursors [148]. Apart from converting C6 sugar into intermediate fuel precursors such as itaconic and levulinic acids, the direct conversion of C6 sugar to biofuels such as 2, 5 dimethyl furan and ethoxy methyl furfural, bypassing intermediate reactions is noteworthy [149, 150]. Furfural which exists in large quantity

697 in hemicelluloses, and mainly produced by hydrogenation and dehydration of xylose can be
698 converted into novel biofuels such as cyclopentanol, 2-pentanol and ethyl furfuryl ethers, and
699 other biofuel producing chemical platforms. Li et al. [148] reviewed the conversion of furfural to
700 C4 and C5 chemicals by various catalytic processes. They divided the chemical products from
701 furfural into several groups according to their carbon numbers and synthesis routes, with
702 emphasis on the catalysts and reaction mechanisms. Ma et al. [151] prepared series of cobalt
703 catalysts with different supports for the selective conversion of biomass-derived furfural to
704 cyclopentanol in one step. Zhao et al. [152] studied the conversion of furfuryl alcohol into ethyl
705 levulinate over glucose-derived carbon-based solid acid in ethanol.
706 Most of these fuels are yet to be tested in IC engines. Apart from chemo-catalyst process,
707 pathways for direct production of these biofuels, bypassing the intermediate products can
708 engender faster and easier production process. Fig 6 shows various steps/pathways required to
709 produce tailored-made biofuels from various feedstocks. Notably, intermediate fuel molecules
710 are hardly chosen from bulky database of physiochemical properties and are therefore prone to
711 be neglected. These intermediates can also be upgraded into incredible variety of molecular
712 structures which could be tested as fuel species. Produced tailored biofuels and their blends with
713 other fuels are investigated and validated through experimentations in IC engines [153].
714

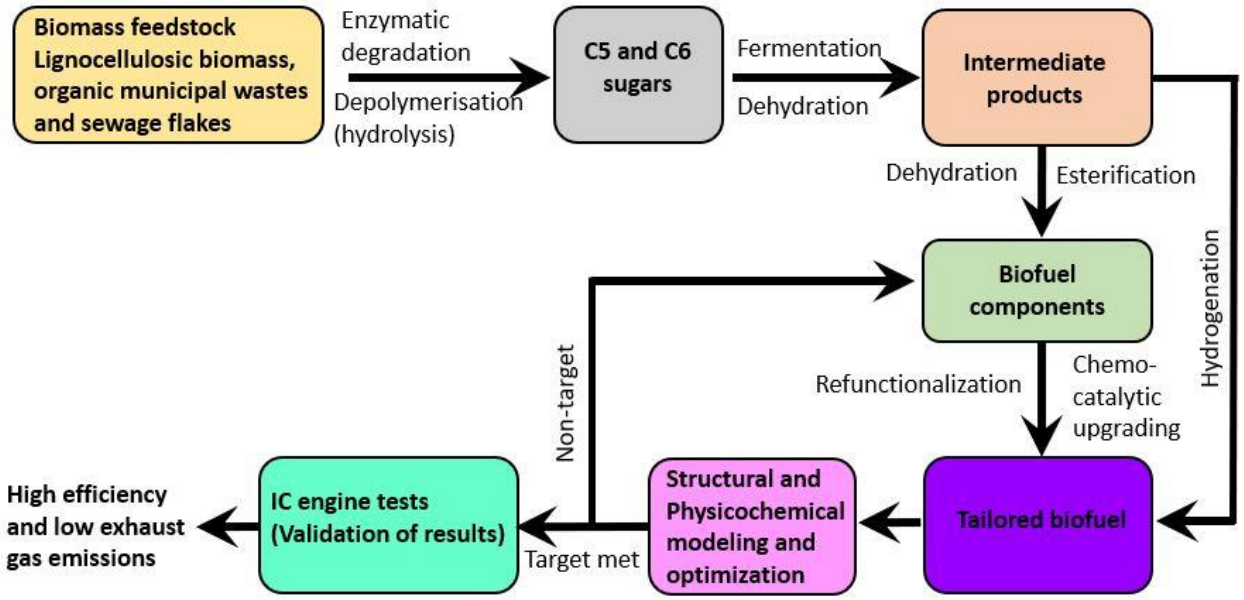
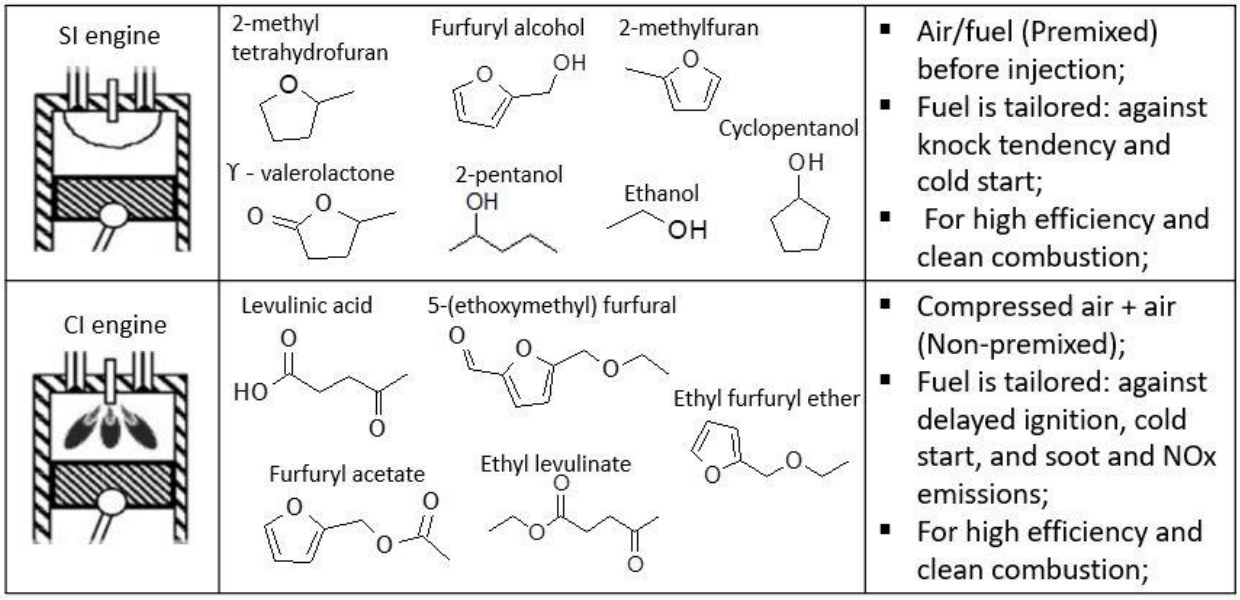


Fig. 6. Overview of biofuel production pathway from biomass and municipal wastes tailored for clean combustion. This figure was made by authors using information from [127].

The increasing research into this topic has led to the synthesis and identification of many chemical and fuel molecules and will continue to aid in identifying new fuel molecules through further research advances. The engine performance of any new fuel species in single/pure or blended form identified by model-base fuel design will be determined through experimentation in either a SI or CI engine, depending on their functional groups, molecular structures octane/cetane number. For instance, models and experimental results have always shown an inverse proportion of ignition delay and alkyl chain length and cetane number, which implies that decrease in ignition delay occurs in increasing alkyl chain length and cetane number. Hellier et al. [153] conducted experiments on combustion and emissions undertaken on a single cylinder diesel engine supplied with 18 different fuels each comprising a single, acyclic, non-oxygenated hydrocarbon molecule. These molecules were chosen to highlight the effect of straight carbon chain length, degree of saturation and the addition of methyl groups as branches to a straight

carbon chain. Also, Hellier et al. [154] reviewed biodiesel composition from various sources, and the effects of differing composition on combustion phasing and the emissions of regulated pollutants, NOx and particulate matter, in compression ignition engines. They concluded that primary influence of biodiesel composition on fuel ignition delay is through the fatty acid alkyl moiety, with either an increase in alkyl chain length or degree of saturation reducing the duration of ignition delay. Heuser et al. [155] investigated the utilization of octanol and DNBE in a state-of-the-art single cylinder diesel research engine. They focused on engine emissions compared to those from conventional diesel fuel. The results showed that soot emissions can almost be avoided completely with octanol, but due to its longer ignition delay, an increase of HC- and CO-emissions was observed.

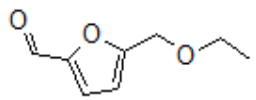
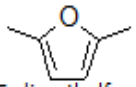
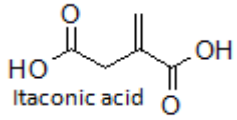
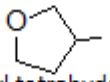
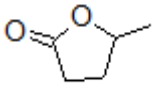
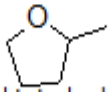
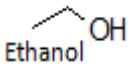
Thus, most fuel molecules of lengthy alkyl can be compatible with CI engines and can be tailored against ignition delay and cold start as shown in Fig. 7. This understanding can be explored in the design of fuel molecules where the reduction of ignition delay is of concern. Overall, the compatibility and performance of fuel molecules in SI or CI engine depends on fuel structure, volatility and octane/cetane number [156, 157], which are also considered for the identification of fuel molecules from a database of millions of fuel chemicals through computational and experimental approaches.

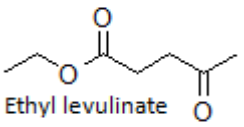
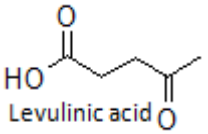
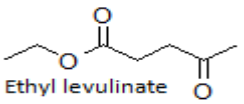
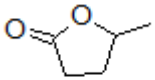


749

750 Fig. 7. Promising pure/single fuel species combustible in spark ignition (SI) and compression
751 ignition (CI) engines, depending on molecular structure, functional group, and physicochemical
752 properties such as cetane/octane numbers and volatility. Fuels of lengthy alkyl seemed to
753 perform better in CI engines. This figure was made by authors using information from [14, 127].

754 Table 4: Chemical processes to produce intermediate and fuel molecules

Process number	Process type	Description (catalysts, reaction temperature, pressure & time (Class of product))	Structure of product	Max. Yield (%)	Ref.
1	Acid catalysis and etherification	H-form zeolite and Amberlyst-15, 96^{O}C, 11hrs. (biofuel)	 5-(ethoxymethyl)furfural	46	[158]
2	Dehydration and Hydrogenolysis	Ru/15CuZr catalysts, 200^{O}C, 15 bar of hydrogen, 5.5 hrs (biofuel)	 2,5-dimethylfuran	45.6	[159]
3	Fermentation	Produced from *Aspergillus terreus* fungal strain from glucose (intermediate)	 Itaconic acid	26.3[a]	[160]
4	Hydrogenation	Pd−Re/C catalysts, 200^{O}C, 1000 psig H_2, 24 hrs (potential biofuel)	 3-methyl tetrahydrofuran	80	[161]
5	Hydrogenation	(Nanoparticle catalysis): $Ru_3(CO)_{12}$, 190 °C, syngas ($2H_2$/CO)100 bar, 20hrs (intermediate and biofuel)	 ϒ- valerolactone	70	[162]
6	Hydrogenation and dehydration	Ru/C + zeolite HY, 200^{O}C, 3 MPa H_2, 46 hrs (biofuel)	 2-methyl tetrahydrofuran	88.8	[163]
7	Fermentation	Electrostatic fermentation of *S. cerevisiae* from glucose (intermediate and biofuel)	 Ethanol	12.3	[164]

8	Esterification	Amberlyst-15 and sulfated SnO_2 catalysts, 70^{O}C, 5 hrs (biofuel)	 Ethyl levulinate	56	[165]
9	Dehydration and Hydrolysis	Fe/HY zeolite catalysts, agitation speed 200 rpm, 180^{O}C, 240 mins (intermediate)	 Levulinic acid	66	[166]
10	Esterification	Amberlyst-15 and sulfated SnO_2 catalysts, 70^{O}C, 5 hrs (biofuel)	 Ethyl levulinate	56	[167]
11	Hydrogenation	Ni/NiO catalyst, 110^{O}C, 40 bar pressure (intermediate and biofuel)	 ϒ- valerolactone	94	[168]

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756
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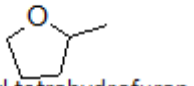
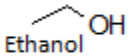
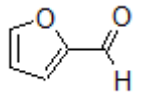
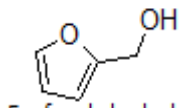
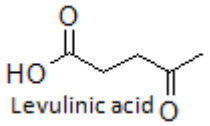
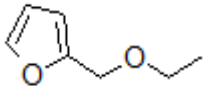
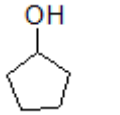
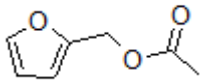
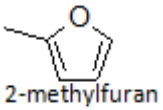
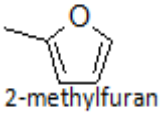
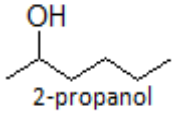
Process number	Process type	Description	Structure of product	Max. Yield	Ref.
12	Hydrogenation	Cu–Ni/Al_2O_3–ZrO_2 catalysts, 220 °C, 3 MPa H_2, 30 mins (biofuel)	 2-methyl tetrahydrofuran	99.8	[169]
13	Fermentation	*S. passalidarum* CMUWF1–2, 30^{O}C (intermediate and biofuel)	 Ethanol	43	[170]
14	Dehydration	Arenesulfonic SBA-15 catalysts, 160^{O}C, 15 bar, 200 rpm, 20 hrs (intermediate)	 Furfuryl	80	[171]

15	Hydrogenation	N-doped carbon encapsulated Co catalysts (Co-N-C), 150 °C, 6 hrs (intermediate)	 Furfuryl alcohol	99	[172]
16	Hydrolysis	$ArSO_3H$-Et-HNS catalyst, 120^{O}C, 120 min. (intermediate)	 Levulinic acid	81.3	[173]
17	Etherification	$H_1Cs_2PW_{12}O_{40}$ catalyst, 130^{O}C, stirring speed 800 rpm, 2.5 hrs (biofuel)	 Ethyl furfuryl ether	65	[174]
18	Hydrogenation and Hydrolysis	Cobalt, supported on ZrO_2-La_2O_3, 160^{O}C, 2 MPa H_2, (biofuel)	 Cyclopentanol	82	[175]
19	Hydrogenation and Esterification	$RHSiO_2$-Cu-Al-Mg catalyst, 150^{O}C, at 40 bar (potential biofuel)	 Furfuryl acetate	24.5	[176]
20	Hydrogenation	Ni-Cu/Al_2O_3 catalyst, 210^{O}C,4 hrs (intermediate and biofuel)	 2-methylfuran	75.6	[177]
21	Hydrogenation	Au:Pd/SiO_2 Nanopowder catalyst, 25^{O}C, 1000 rpm, 1 bar H_2, 3 hrs (intermediate and biofuel)	 2-methylfuran	92	[178]
22	Hydrogenation	Co–Cu/Al_2O_3 catalyst, 240 °C, 45 bar H_2 pressure, 12 hrs (biofuel)	 2-propanol	71.1	[179]

758 Max yield (%) is the ratio of the real yield (g/g of sugar) to theoretical yield (g/g sugar)

3.3 Effects of physicochemical properties of oxygenated fuels on IC engine performance

The chemical structures of fuel were shown to have direct influence on fuel physicochemical properties and combustion efficiency [14]. However, the relationships between fuel properties and engine performance and emissions are intricate and difficult to understand due to diverse engine technologies and operating conditions, different structures and functional groups of individual fuel components, and inconsistent and varying fuel properties during engine operations [180-182]. Overall, fine-tuning the physicochemical/combustion properties of fuels with respect to ASTM or other established standards to enhance combustion efficiencies and reduce GHGs, NOx and PM emissions is the essence of fuel design. The predominant use of fossil fuels today can be traced to easy storage, high net heat value [183] and availability of available infrastructure. But we cannot completely the effects of continued fossil fuel use and trade-off these to the environmental degradation caused by combusting these fuels.

Table 5 shows various oxygenated fuels and their physicochemical properties, which play key roles in the combustion and emission level of each fuel as compared to fossil fuels. The potential of these oxygenated fuels has been under-tapped due to operational and storage challenges. For instance, 2-methylfuran, dimethylfuran and alcohols can form explosive vapor-air mixtures in the fuel tank under atmospheric condition due to low flash point, are corrosive and hygroscopic, whereas biodiesel, ethyl levulinate, cyclopentanol and 2-pentanol have been identified with high viscosity, leading to gelling, injector system damage and lower heat release [184]. In addition, incompatibility of biofuels with existing transport infrastructure due to disparity in these properties has been acknowledged in the literature as a hurdle to the utilization of biofuels in transportation (internal combustion engines), power

783 generation systems and heating sectors, as it devalues the efficiency of biofuels and promotes
784 the dominance of the existing diesel and gasoline transport infrastructures [185, 186].
785 Ideally, the properties of future fuels may differ from those of fossil fuels, thus the established
786 fossil-based fuels standards should not be a measure for defining the performance of future
787 fuels. Therefore, to maximize the full potential of existing and emerging future fuels, the
788 effects of fuel properties such as density, viscosity, oxygen content, boiling point,
789 cetane/octane numbers and enthalpy of vaporization of these fuels and their blends in premix
790 and non-premix engine modes of combustion need to be exhaustive studied.

Table 5. Physicochemical properties of various oxygenates compared to AGO and PMS. Note: **AGO**: diesel fuel ($C_{14}H_{24}$) [184-186]; **PMS**: gasoline (C_8H_{18}) [184, 187-189]; **ETH**: ethanol (C_2H_6O) [184, 186, 188, 190]; **VAL**: ϒ-valerolactone ($C_5H_8O_2$) [191, 192]; **2MF**: 2-methylfuran (C_5H_6O) [188, 191, 193, 194]; **ELE**: ethyl levulinate ($C_7H_{12}O_3$) [195-197]; **2,5 DMF**: 2,5-dimethylfuran (C_6H_8O) [188, 198, 199]; **2 MTHF**: 2-methyl tetrahydrofuran ($C_5H_{10}O$) [193, 200]; **EFE**: ethyl furfuryl ether ($C_7H_{10}O_2$) [194, 197]; **EMF**: ethoxy methyl furfural ($C_8H_{10}O_3$)[201, 202]; **CPNTL**: cyclopentanol ($C_5H_{10}O$) [194, 203, 204]; **PNTL**: 2-pentanol ($C_5H_{12}O$)[204-207]; t=20^{O}C; x=25^{O}C; s and f = Sigma-Aldrich &Thermo Fisher safety data sheet respectively.

Properties	**A GO**	**PMS**	**ETH**	**VAL**	**2MF**	**ELE**	**2,5 DMF**	**2MTHF**	**EFE**	**EMF**	**CPNTL**	**PNTL**
Molecular weight	192	114	46	100	82	144	96	86	126	154	86	88
Specific gravity	0.85	0.7-0.76	0.79	1.05	0.91	1.01	0.99	0.854	0.98	1.1	0.94	0.81
Boiling point (^{O}C)	190-280	27-225	78	207	64	206	94	80.3	137	253	139^{f}	118^{s}
Flash point (^{O}C)	65-88	-45 to -13	13	96	-22	91	-1^{s}	-12	42	110^{s}	51^{f}	34^{s}
Pour point (^{O}C)	-35 to -15	-	<-50	-	-	-13	-	-	-	-	-	-
Cloud point (^{O}C)	-15 to -5	-50	-	-	-	-79	-	-	-	-	-	-
Freezing point (^{O}C)	-30 to -40	-40	-114	-31	-89	-60	-	-136	-	-	-19^{f}	-73^{s}
Vapor pressure(kPa) at 20^{O}C	-	32.8	16	3.5	18.5	4.5	13.4	-	-	-	-	0.8
Viscosity (mm^2/s) at 40^{O}C	2.69	0.5-0.8^{t}	1.2	2.1	4.4^{xf}	1.5^{x}	0.53^{t}	4.7^{xf}	0.95^{x}	-	5.9	4.2^{x}
Surface tension (N/m)	-	0.024^{t}	0.022^{t}	-	0.025	-		0.026	-	-	-	-
Auto-ignition (^{O}C)	210	257	362^{f}	-	-	425^{f}	286	260^{s}	-	-	375^{f}	343^{f}
Flame speed rate (cm/s)	-	33-44	39-42	-	-	-	-	-	-	-	65	-
Flammability limit (vol.% in air)	0.6-7.5	1.4-7.6	4.3-19	-	-	-	-	-	-	-	-	-
Air/fuel ratio	14.6	14.8	9	-	10.1	-	10.8	-	-	-	11.2	11.7
Octane number (research)	20-30	86-100	109	-	103	-	101	86	96	-	-	99.4
Cetane number	45	8	8	<10	9.6	6	9.8	20.5	18.4	-	-	14.6
Enthalpy of vaporization(kJ/kg)	600	360	900	442	358	307	332	364.4	277	-	511	608
Net heat value (MJ/kg)	42.6	46	27	29.7	31.2	25	33.3	32	29	28.5	-	35
Carbon mass fraction (%)	87.5	84.2	52.2	60	73.2	58.3	75	69.8	66.7	62.3	69.8	68.2
Hydrogen mass fraction (%)	12.5	15.8	13	8	7.3	8.4	8.3	11.6	7.9	6.5	11.6	13.6
Oxygen mass fraction (%)	0	0	34.8	32	19.5	33.3	16.7	18.6	25.4	31.2	18.6	18.2

798 As can be seen in Table 5, oxygenated fuels are denser (higher specific gravity) than
799 conventional hydrocarbon fuels. Density is the mass per unit volume of fuel injected into the
800 cylinder for combustion. It varies inversely to volume; hence the density of injected fuel into
801 the cylinder could be regulated by the injector control system through fuel volume regulation
802 and not by mass [208]. Density, viscosity and surface tension are related in physical nature,
803 and can be affected by temperature. At the same temperature, fuel of higher density tends
804 to have higher viscosity and surface tension, and these properties greatly influence fuel
805 injection and tribology characteristics, atomisation and efficiency [209]. Some authors have
806 reported that lowering the values of these fuel parameters could lead to the production of
807 fuel of better spray characteristics and atomization, while others attributed better fuel
808 atomization, evaporation and spray characteristics to the integration of high-pressure
809 injection nozzle as part of engine accessory. However, the later attribute is key to the
810 investigation of spray characteristics of oxygenates [210-213]. In general, increases in fuel
811 density increases viscosity, leading to the injection of larger fuel droplets that can result in
812 incomplete combustion. Consequently, efficiency decreases as NOx and PM increase [214].

813 Fuel cetane/octane number and ignition temperature plays key roles in the smooth and
814 efficient running of IC engines. Cetane number is an index used in measuring the combustion
815 quality of fuel in a diesel engine. Higher cetane number can cause quick ignition tendency of
816 injected fuel [184]. Injection of high cetane number fuel into the cylinder of a CI engine (non-
817 premixed) requires instant auto-ignition before the end of compression stroke to maximize
818 power output. Thus, high cetane number and lower ignition temperature are key conditions
819 for enhanced fuel combustion efficiency in CI engines. It is worth noting that fuels of higher
820 cetane number can cause quick ignition tendency leading to high power output, but fuel of
821 lower cetane number could increase ignition delay, leading to a more homogenous air/fuel

822 mixture, which will in turn reduce soot formation (by reducing fuel rich mixture zone) and
823 particulate matter PM [215, 216]. This trade-off needs to be balanced through fuel design
824 process and optimization. There exists an ignition delay database of pure single fuel molecules
825 generated from automated ignition quality tester (IQT) and structural group contribution
826 model used in predicting ignition delay of new fuel species [217].
827 Conversely, in SI engines, premixed air/fuel mixtures do not need to auto-ignite, even at the
828 end of the compression stroke, as the spark plug ignites the compressed mixture [218].
829 Setting the correct ignition timing is necessary to prevent engine knock, backfiring, wasted
830 spark and excessive vibration [219]. Also, the availability of end gas (air/fuel mixture region
831 not ignited) which can later ignite due to high temperature and pressure of the cylinder
832 environs can lead to the occurrence of knock. Octane number measures the ability of fuel to
833 resist knocking. Fuel of higher-octane number has a better anti-knock quality [220, 221].
834 Octane number and cetane number vary inversely even though the interplay between octane
835 number and auto-ignition is still unclear [222]. Fuel of high-octane number and low cetane
836 will have a high knock resistance characteristic when run in SI engine and hardly auto-ignite
837 as in the case of gasoline [223]. Fuel with high cetane number and low octane number is prone
838 to quick auto-ignition. Looking at Table 5, most oxygenated fuels therein have higher-octane
839 numbers when compared with gasoline and appreciable values of cetane number. As a result,
840 oxygenated fuels can be run in SI, CI and other engines of various combustion mode, as well
841 as serve as better additives for fuel blending.

842 Other properties such as oxygen content and boiling point are also critical to engine
843 performance and exhaust gas emissions [224]. The presence of oxygen content differentiates
844 oxygenated biofuels from fossil fuels. In general, blending non-oxygenated fuels with

oxygenated fuel provides the mixture with oxygen atoms, thereby decreases the number of active carbon responsible for soots and GHG emissions [225]. By simple percentage by mass calculations, oxygenated fuels contain 15–40% oxygen whereas fossil fuels have none, leading to differences in the chemical properties of biofuels with respect to fossil fuels. If novel eco-friendly fuels are to be produced, the effects of oxygen contents need to be exhaustively studied and integrated into fuel design and property predictive models.

Fuels with little or no presence of oxygen lead to soot formation and PM due to incomplete oxidation of the rich region of fuel molecules in the cylinder environs [226, 227]. Oxygen content of 30% can completely remove soot and reduce PM to the minimum, as the presence of oxygen seems to improve combustion phase diffusivity and post-flame oxidation of soot [228]. Although, the relationship between oxygen content and cetane/octane numbers has not been established, the presence of oxygen in biofuels and fuel blends has improved fuels anti-knock resistance in SI and reduced ignition delay in CI engines [14, 224], leading to more efficient combustion, thereby cutting-down toxic exhaust gas emissions in IC engines. Donahue et al. [229] stated that increasing the percentage of oxygen in fuels could reduce ignition delay and smoke level, increase the release of NOx gas but does not affect the spray characteristics.

It has been observed that soot and PM could be significantly reduced with a minimal effect on NOx emissions by increasing the oxygen content of fuel. Also, at a constant NOx emission level, soot, PM and unburnt hydrocarbon could be drastically reduced by increasing the oxygen content of fuel [229]. More so, at low oxygen content, exhaust gas emissions can be reduced by timely ignition and moderated injection pressure. The only challenge is that of lower energy content inherent in oxygenated fuels. But, from technical perspective, lower

868 energy content observed in oxygenated biofuels ought not to be a major concern because
869 oxygenated biofuels have lower air-fuel ratio than fossil fuels. Consequently, with increased
870 air volume, the resulting in-cylinder energy could be higher using ethanol vs. PMS in SI engines
871 [230]. More so, the effects of the number of carbons to oxygen bond (whether single or
872 double) has been studied. They show that biofuels of single carbon to oxygen ratio such as
873 alkanols are more ignitable and effective in reducing soot emission than those of double
874 bonds like alkanones or ketones [224, 226, 231]. The effects of having the presence of both
875 single and double carbon to oxygen bonds in biofuels such as biodiesel and ethyl levulinate
876 have not been studied.
877 The boiling point of fuel is one of the key properties that determines fuel storage feasibility,
878 IC engine operation (vaporization and combustion) and the extent of particulate deposits
879 [232]. In SI engines, fuel of low boiling point tends to have shorter liquid penetration length,
880 diminishing problems associated with wall wetting and fuel-in-oil dilution. It has been
881 reported that fuel of low boiling point can reduce particulate matter emissions with increased
882 NOx [233] . High boiling point and enthalpy of vaporization of oxygenated fuels can lead to
883 engine operation difficulty in low ambient temperature, but after the engine warm-up phase,
884 high enthalpy of vaporization, filling efficiency and knock resistance are enhanced [227].
885 Comparatively, the CI engines burn less volatile fuel when compared with SI. Apparently, wall
886 wetting, and fuel-in-oil dilution abound if fuel particles are large. To alleviate these concerns,
887 modern fuel injection methods are employed to achieve fuel combustion at low temperature
888 and reduced soot and NO_X emissions [234, 235]. Fisher et al. [234] emphasized that a
889 quantitative understanding of liquid-phase penetration for biodiesel fuels is needed to help
890 mitigate the accelerated dilution of engine lubrication oil with unburned fuel. They reported
891 liquid penetration lengths measured in an optical engine under time-varying in-cylinder

892 conditions for soy- and cuphea-derived biodiesel fuels. Dec [235] emphasized the importance
893 of achieving highly diluted and well-premixed charge to achieve low emissions and reviewed
894 the principles of HCCI and diesel LTC engines along with the effects of in-cylinder fuel injection
895 processes. The physicochemical properties of single pure oxygenated fuels may not resolve
896 all energy and ecological concerns linked with the use of conventional fossil fuels. At times,
897 single species fuels fail to exhibit all the necessary properties, thus, the model could also be
898 used to predict the properties of a mixture of multiple fuel blend species [236]. Also, public
899 demand may not be met due to insufficient fuel production volume. Going forward, these
900 pressing issues could be alleviated through target-oriented fuel design, which involves
901 blending different categories of liquid fuels and fuel molecules to produce blended fuels of
902 unique and desired properties through the application of fuel design concepts. By this,
903 biofuels and blends could be modified to desired physicochemical properties that could
904 enhance efficiency and clean combustion which is the remote essence of fuel design.

Summary of section 3: In this section we have reviewed advances in fuel design and property models. Different chemical processes to produce intermediate and fuel molecules were discussed. General overview of biofuel production pathways from biomass and municipal solid wastes tailored for clean combustion was given and the production of tailor-made fuels from biomass has been elaborated and discussed along with the effects of physicochemical properties of oxygenated fuels on IC engine performance. Promising pure/single fuel species combustible in spark ignition (SI) and compression ignition (CI) engines were discussed. This section has also provided a list of pathways leading to the production of new fuel molecules using C6 and C5 sugar sources**,** and a list of physicochemical properties of various oxygenates compared to conventional diesel and gasoline fuels.

905

906 ## 4. Surrogate fuel formulation

907 Real fuels such as gasoline, diesel and jet aviation are composed of variable, complex mixtures
908 of chemical components. Thus, numerical simulations of their combustion remain
909 challenging. The use of surrogate fuel as an attractive option to circumvent this problem has
910 been reviewed in various literature [237-239]. A surrogate fuel ought to be a blend of a limited

number of chemical components that exhibits some physicochemical properties of interest [240]. By choosing a limited number of fuel components minimizes unknows in reaction and presents a clearer picture of the effects of surrogate fuel properties on combustion process/kinetics, allowing for a more exact inference about engine performance and exhaust gas emissions. Furthermore, during surrogate formulation, the properties of chemical components could be chosen to improved engine functionality, emission reduction and costs optimization [241].

Although surrogate fuel formulation has been proposed, it is faced with challenges in implementation such as low functional group resolution and accommodation of hydrocarbons only, without oxygenated fuels. Consequently, group contribution method (GCM) was recently proposed as a method to overcome these challenges [242-246]. In formulating a surrogate fuel using this method, the molecular structure, functional groups and physicochemical properties of *target* and *surrogate* fuels must be well understood [247]. The basic ideology of GCM is that the physicochemical, combustion and emission characteristics of fuel are totally dependent on the fuel's functional groups and molecular structure [248]. Thus, if the functional groups of a surrogate fuel match those of target fuel, physiochemical, combustion and emission properties/features would also match. Going forward, it is important to identify and define key terms associated with surrogate fuel formulation, which include; target fuel, target property, surrogate fuel and surrogate palette. The necessary steps and criteria/decisive factor of each step of surrogate fuel formulation are illustrated in Fig 8.

- *Target fuel* is a chosen fuel whose selected properties are to correspond with those of the surrogate fuel. It is usually a real fuel with numerous individual compounds.

- *Target property* is a set of selected properties of the target fuel to correspond with surrogate fuel, e.g. cetane number or octane number for IC engines.
- *Surrogate fuel* is a fuel made up of a smaller number of compounds of known chemical composition formulated to have similar properties with target fuel.
- *Surrogate palette* is a set of compounds of known chemical compositions, which could be blended in specific proportions to form a surrogate fuel [249].

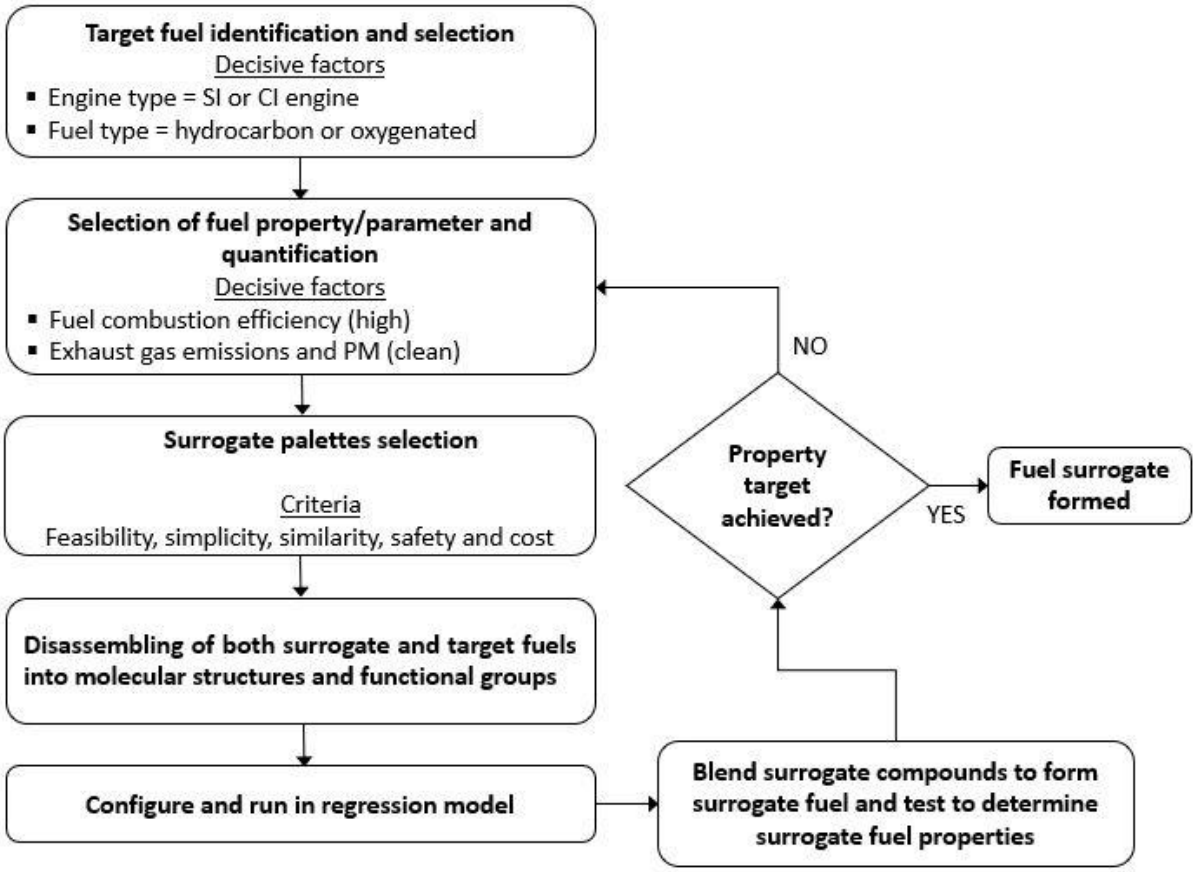


Fig. 8. General overview of surrogate fuel formulation, considering molecular structure and functional groups, with factors/criteria considered in each step. This figure was made by authors using information from [249].

The first step is target fuel identification and selection, which is mostly selected from AGO, PMS, jet fuel and biodiesel, with known characteristics determined from ASTM test methods or fuel characterization database. These properties are expected to match as close as possible

953 those of surrogate fuel. It is important to select a target fuel compatible with test engine in
954 order to avoid much error due to technical limitations to testing the surrogate fuel formed.
955 For instance, hydrocarbon fuels such as AGO and PMS run better in CI and SI engines
956 respectively due to the availability of necessary engine accessories and mode of combustion.
957 As a result, engine and fuel types are key factors considered in the process of target fuel
958 selection. Otherwise, the formed surrogate fuel would be tested in every available engine
959 type to demonstrate its suitability and efficiency across each engine type and mode of
960 combustion.
961 Secondly, the molecular structures and functional groups of the selected target fuel which
962 the surrogate fuel is expected to mimic should fall within an acceptable tolerance. Studies
963 have emphasized the importance of molecular structure and functional group of fuel in its
964 oxidative and pyrolytic combustion chemistries [250]. The combustion chemistry of a fuel of
965 single molecular structure has shown a robust relationship between the molecular structure,
966 physicochemical properties and combustion performances in diverse experimental and
967 numerical settings. Notably, ignition delay duration decreases as the alkyl straight chain
968 increases. The presence of unsaturated carbon bonds and structural isomerism (alkyl
969 branching) can increase ignition delay. The effect of the position of the double bond and the
970 presence of oxygen for the oxygenated fuels depends on the overall molecular structure of
971 the fuel [251, 252].
972 Thirdly, compounds of surrogate palettes are selected. No palette compound is perfect, but
973 some palettes are preferable to others. The selected surrogate palette compounds criteria
974 include feasibility (available kinetic mechanism), similarity (sooting tendency, volatility, and
975 combustion properties), simplicity (fewer number of carbons), safety, cost and more than
976 98% pure [253, 254]. It is advisable to choose fewer number of palette compounds to enhance

accuracy, minimize complexity and increase systems control. Every selected palette compound would stand for a class of compounds seen in the target fuels, with available chemical kinetic mechanism to enable computational simulation of the combustion process. Fourthly, the chosen palette compounds and target fuel molecules are broken down into functional groups. These functional groups are input into model and run to determine the surrogate recipe; that is, the amount of each palette compound needed in the surrogate fuel to accomplish the desired target. After ascertaining the desired properties and chemical composition of each pure palettes compound, the selected palettes are blended to form the surrogate fuel. The formulated surrogate fuel hereafter will be subjected to various tests to determine whether the property targets fall within the acceptable tolerances. If met, the surrogate fuel will be produced and if not, the processes of target fuel and surrogate palette selection and property will be revisited alongside the regression model assumptions. These steps will be adjusted and iterated until the surrogate fuel property targets are met [253, 255]. Experimental and computational studies using surrogates will inform researchers the upshots of fuel composition and features on IC engine performance and extent of exhaust gas emissions.

Summary of section 4: In this section we have reviewed key features associated with surrogate fuel formulation that sets requirements on the target fuel, target property, surrogate fuel, and surrogate palette. It was discussed that the physicochemical, combustion and emission characteristics of fuels are totally dependent on the fuel's functional groups and molecular structure. These functional groups are input into model and run to determine the surrogate recipe; that is, the amount of each palette compound needed in the surrogate fuel to accomplish the desired target.
A key challenge in surrogate-fuel formulation is determining the set of palette-compound mole fractions such that the resultant surrogate mixture best matches the desired properties of the target fuel (i.e., the property targets). Usually, a regression model can be used to provide an automated technique for surrogate formulation. Furthermore, a multi-property regression algorithm can be applied to determine the optimal surrogate formulation by matching the surrogate-design properties to the property targets as closely as possible through the use of an objective function.

5. Future perspectives

5.1 Application of bioenergy molecules and microbial fuels in transport, aviation and shipping sectors

When applying biofuels in transport, aviation and shipping sectors, the fuel sustainability, combustion efficiency, and emissions production are of primary concern [256]. Notably, microbial fuels may be derived from non-edible plant biomass, municipal and sewage wastes, and food wastes, implying that microbial fuels' sustainability and economic feasibility will improve as biofuel production technology evolve. Physicochemical characteristics have been found in studies to have a substantial impact on clean and efficient combustion. As a result, these qualities are required for the design of IC engines and accessories that govern fuel injection, spray/atomization characteristics, and combustion regime. The molecular structure and functioning of biofuels have been shown to have a considerable impact on their uses, resulting in technical incompatibility with current fuel infrastructure [257] . Microbial biofuels may be reconstructed, refunctionalized, and adjusted using fuel design ideas to mimic the composition of actual fuel, reducing the consequences of structural variances from traditional hydrocarbon fuels. Integrating fuel design and engine design, as well as further co-optimizing performance, might help biofuels gain traction in the transportation, aviation, and shipping industries.

5.2 Application of bioenergy molecules and microbial fuels in energy conversion and storage

As a sort of biological energy storage, the generation of liquid fuels and energy molecules is an appealing renewable energy source. In the recent decade, remarkable progress has been achieved in the design and manufacturing of fuels and energy molecules utilising bacteria. Even yet, the number of recognised metabolic processes that create hydrocarbon compounds

1018 important to fuel is restricted [258, 259]. These include derivations of the amino acid pathway
1019 to produce isobutanol [260], the mevalonate pathway to produce farnesene [261, 262], the
1020 polyketide pathway to produce a variety of fuel molecules [263] and the fatty acid pathway
1021 to produce fatty acid methyl esters [264, 265]. These native pathways are found in many
1022 different micro-organisms [266, 267] and can be genetically manipulated or placed into a
1023 simple host to increase an organism's biosynthesis capacity for a specific bioenergy product.
1024 Renewable energy technologies are becoming more widely available across the world as a
1025 result of their maturing maturity and lower cost structure. However, large-scale electrical
1026 energy storage and retrieval would almost likely be required to enhance the penetration of
1027 renewable sources into the grid. The perfect combination of high power and energy density,
1028 low economic and environmental costs, lack of site limitations, long period and calendar
1029 lifetime, convenient supply of materials, and quick reaction time is required to ensure rapid
1030 and effective penetration of renewable energy technologies into the grid. Engineered
1031 microorganisms might solve many of the problems of present energy storage systems by
1032 permitting rewired carbon fixation, a process that spatially splits reactions generally done
1033 jointly in a photosynthetic cell and replaces the least effective with biological counterparts.
1034 Microbial or enzymatic carbon dioxide fixation and subsequent delivery of materials as
1035 carbon-based energy storage molecules, if effective, will allow high-density storage of
1036 renewable energy, including hydrocarbons and non-volatile polymers.

1037 *5.3 Application of energy molecules and microbial fuels in built environment*

1038 Bioenergy application in built environments, like energy conversion and storage, is a rapidly
1039 growing field. However, there is little research on the application of microbial populations in
1040 constructed settings. Lal et al. [268] revived the idea of using microorganisms in buildings and
1041 homes to generate bioenergy by processing waste materials. They did, however, identify a

few issues that must be addressed in order to efficiently employ this microbial technology in households. The initial investment is significant, the anaerobic digestion efficiency is low, and further research is needed. Finally, while there have been few bacteria identified as potential electron producers, next-generation sequencing technologies may aid in the discovery of novel and efficient microbes.

6. Conclusions

In this paper we have reviewed the recent advancements in microbial fuel development and fuel design with the focus of their impact on sustainability, exhaust gas emissions, and compatibility with conventional and future transport and energy applications. Several studies have explored the potential to utilize microorganisms to convert biogenic residue and waste into energy carrying molecules and fuels. Advanced microbial biofuels are renewable energy sources produced by microorganisms grown on different available organic substrates. These substrates are sourced from cost effective and sustainable feedstock and organic wastes. The key difficulty in converting feedstocks into advanced biofuels using native hosts is the ability to manipulate or modify the native biofuel producing metabolic pathways to achieve high product yields or produce new fuels. It is crucial to utilize genetically tractable microorganisms and biocatalysts that can be induced to produce desired fuels from a variety of feedstocks. Microbial metabolic engineering and fuel design have enhanced the production and utilization of advanced biofuels. New metabolic pathways were identified and then utilized for metabolite production, but these developments are in very early stages. For most of the metabolic pathways, the yields of produced fuels and energy molecules are still low. However, with the introduction of CRISPR genome editing tools and advancements in synthetic and systems biology, production of microbial fuel molecules can be enhanced with potential to outperform fossil fuels in terms of clean and efficient combustion. In addition, the application

of predictive models using fuel properties of energy molecules should be developed which can help predicting processes to produce tailor-made fuels with desired properties required to run in combustion systems. For example, technical incompatibility of oxygenated fuels, seen as a major hurdle against the utilization of biofuels in IC engines, can be addressed through advancements in fuel molecular design and fuel property modeling.

7. References

[1] Kumar R, Ghosh AK, Pal P. Synergy of biofuel production with waste remediation along with value-added co-products recovery through microalgae cultivation: A review of membrane-integrated green approach. Science of The Total Environment. 2020;698:134169.

[2] Patel A, Mikes F, Matsakas L. An Overview of Current Pretreatment Methods Used to Improve Lipid Extraction from Oleaginous Micro-Organisms. Molecules (Basel, Switzerland). 2018;23.

[3] Dutta K, Daverey A, Lin J-G. Evolution retrospective for alternative fuels: First to fourth generation. Renewable Energy. 2014;69:114-22.

[4] Matuszewska A. Microorganisms as Direct and Indirect Sources of Alternative Fuels. Alternative Fuels, Technical and Environmental Conditions: IntechOpen; 2016.

[5] Ogunkunle O, Ahmed NA. A review of global current scenario of biodiesel adoption and combustion in vehicular diesel engines. Energy Reports. 2019;5:1560-79.

[6] Patel A, Matsakas L. A comparative study on de novo and ex novo lipid fermentation by oleaginous yeast using glucose and sonicated waste cooking oil. Ultrasonics sonochemistry. 2019;52:364-74.

[7] Rafay R, Uratani JM, Hernandez HH, Rodríguez J. Growth and Nitrate Uptake in Nannochloropsis gaditana and Tetraselmis chuii Cultures Grown in Sequential Batch Reactors. Frontiers in Marine Science. 2020;7.

[8] Hollinshead W, He L, Tang YJ. Biofuel production: an odyssey from metabolic engineering to fermentation scale-up. Frontiers in Microbiology. 2014;5.

[9] Peralta-Yahya PP, Keasling JD. Advanced biofuel production in microbes. Biotechnology journal. 2010;5:147-62.

[10] Festel G, Würmseher M, Rammer C, Boles E, Bellof M. Modelling production cost scenarios for biofuels and fossil fuels in Europe. Journal of Cleaner Production. 2014;66:242-53.

[11] Abdullah B, Muhammad SAFaS, Shokravi Z, Ismail S, Kassim KA, Mahmood AN, et al. Fourth generation biofuel: A review on risks and mitigation strategies. Renewable and sustainable energy reviews. 2019;107:37-50.

[12] Costa M, Piazzullo D. Biofuel Powering of Internal Combustion Engines: Production Routes, Effect on Performance and CFD Modeling of Combustion. Frontiers in Mechanical Engineering. 2018;4.

[13] Setiyo M, Saifudin S, Jamin A, Nugroho R, Karmiadji D. The effect of ethanol on fuel tank corrosion rate. Jurnal Teknologi. 2018;80.

[14] Hellier P, Talibi M, Eveleigh A, Ladommatos N. An overview of the effects of fuel molecular structure on the combustion and emissions characteristics of compression ignition engines. Proceedings of the Institution of Mechanical Engineers, Part D: Journal of Automobile Engineering. 2018;232:90-105.

[15] Keasling J, Garcia Martin H, Lee TS, Mukhopadhyay A, Singer SW, Sundstrom E. Microbial production of advanced biofuels. Nature Reviews Microbiology. 2021;19:701-15.

[16] Shanmugam S, Ngo H-H, Wu Y-R. Advanced CRISPR/Cas-based genome editing tools for microbial biofuels production: A review. Renewable Energy. 2020;149:1107-19.

[17] Mehmood MA, Shahid A, Malik S, Wang N, Rizwan Javed M, Nabeel Haider M, et al. Advances in developing metabolically engineered microbial platforms to produce fourth-generation biofuels and high-value biochemicals. Bioresource technology. 2021;337:125510.

[18] Das M, Patra P, Ghosh A. Metabolic engineering for enhancing microbial biosynthesis of advanced biofuels. Renewable and Sustainable Energy Reviews. 2020;119:109562.

[19] Choi KR, Jiao S, Lee SY. Metabolic engineering strategies toward production of biofuels. Curr Opin Chem Biol. 2020;59:1-14.

[20] Carmona Cabello M, García IL, Papadaki A, Tsouko E, Koutinas A, Dorado MP. Biodiesel production using microbial lipids derived from food waste discarded by catering services. Bioresource technology. 2020;323:124597.

[21] Kumar R, Kumar P. Future microbial applications for bioenergy production: a perspective. Frontiers in microbiology. 2017;8:450.

[22] Peralta-Yahya PP, Zhang F, Del Cardayre SB, Keasling JD. Microbial engineering for the production of advanced biofuels. Nature. 2012;488:320-8.

[23] Chubukov V, Mukhopadhyay A, Petzold CJ, Keasling JD, Martín HG. Synthetic and systems biology for microbial production of commodity chemicals. npj Systems Biology and Applications. 2016;2:1-11.

[24] Yuan S-F, Alper HS. Metabolic engineering of microbial cell factories for production of nutraceuticals. Microbial cell factories. 2019;18:46.

[25] Liao JC, Mi L, Pontrelli S, Luo S. Fuelling the future: microbial engineering for the production of sustainable biofuels. Nature Reviews Microbiology. 2016;14:288-304.

[26] Mukhopadhyay A. Tolerance engineering in bacteria for the production of advanced biofuels and chemicals. Trends in microbiology. 2015;23:498-508.

[27] Ostergaard S, Olsson L, Nielsen J. Metabolic engineering of Saccharomyces cerevisiae. Microbiology and molecular biology reviews. 2000;64:34-50.

[28] Nevoigt E. Progress in metabolic engineering of Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews. 2008;72:379-412.

[29] Cravens A, Payne J, Smolke CD. Synthetic biology strategies for microbial biosynthesis of plant natural products. Nature communications. 2019;10:1-12.

[30] Gustavsson M, Lee SY. Prospects of microbial cell factories developed through systems metabolic engineering. Microbial Biotechnology. 2016;9:610-7.

[31] Balan V. Current challenges in commercially producing biofuels from lignocellulosic biomass. International Scholarly Research Notices. 2014;2014.

[32] Kopf M, Hess WR. Regulatory RNAs in photosynthetic cyanobacteria. FEMS Microbiology Reviews. 2015;39:301-15.

[33] Kopf M, Klähn S, Scholz I, Matthiessen JK, Hess WR, Voß B. Comparative analysis of the primary transcriptome of Synechocystis sp. PCC 6803. DNA research : an international journal for rapid publication of reports on genes and genomes. 2014;21:527-39.

[34] García-Granados R, Lerma-Escalera JA, Morones-Ramírez JR. Metabolic Engineering and Synthetic Biology: Synergies, Future, and Challenges. Frontiers in Bioengineering and Biotechnology. 2019;7.

[35] Wang F, Zhang W. Synthetic biology: recent progress, biosafety and biosecurity concerns, and possible solutions. Journal of Biosafety and Biosecurity. 2019;1:22-30.

[36] Young E, Alper H. Synthetic biology: tools to design, build, and optimize cellular processes. BioMed Research International. 2010;2010.

[37] Nielsen J, Keasling Jay D. Engineering Cellular Metabolism. Cell. 2016;164:1185-97.

[38] Yu T, Dabirian Y, Liu Q, Siewers V, Nielsen J. Strategies and challenges for metabolic rewiring. Current Opinion in Systems Biology. 2019;15:30-8.

[39] Wu G, Yan Q, Jones JA, Tang YJ, Fong SS, Koffas MAG. Metabolic Burden: Cornerstones in Synthetic Biology and Metabolic Engineering Applications. Trends in Biotechnology. 2016;34:652-64.

[40] Bosi E, Bacci G, Mengoni A, Fondi M. Perspectives and Challenges in Microbial Communities Metabolic Modeling. Frontiers in Genetics. 2017;8.

[41] Fondi M, Liò P. Multi -omics and metabolic modelling pipelines: Challenges and tools for systems microbiology. Microbiological Research. 2015;171:52-64.

[42] Pourmir A, Johannes TW. Directed evolution: selection of the host organism. Comput Struct Biotechnol J. 2012;2:e201209012-e.

[43] Jin H, Chen L, Wang J, Zhang W. Engineering biofuel tolerance in non-native producing microorganisms. Biotechnology Advances. 2014;32:541-8.

[44] Nawab S, Wang N, Ma X, Huo Y-X. Genetic engineering of non-native hosts for 1-butanol production and its challenges: a review. Microbial Cell Factories. 2020;19:1-16.

[45] Keller MW, Lipscomb GL, Nguyen DM, Crowley AT, Schut GJ, Scott I, et al. Ethanol production by the hyperthermophilic archaeon Pyrococcus furiosus by expression of bacterial bifunctional alcohol dehydrogenases. Microbial biotechnology. 2017;10:1535-45.

[46] Liu S, Xiao H, Zhang F, Lu Z, Zhang Y, Deng A, et al. A seamless and iterative DNA assembly method named PS-Brick and its assisted metabolic engineering for threonine and 1-propanol production. Biotechnology for biofuels. 2019;12:1-20.

[47] Gaj T, Sirk SJ, Shui S-l, Liu J. Genome-editing technologies: principles and applications. Cold Spring Harbor perspectives in biology. 2016;8:a023754.

[48] Celińska E, Ledesma-Amaro R, Larroude M, Rossignol T, Pauthenier C, Nicaud JM. Golden Gate Assembly system dedicated to complex pathway manipulation in Yarrowia lipolytica. Microbial biotechnology. 2017;10:450-5.

[49] Gibson DG, Young L, Chuang R-Y, Venter JC, Hutchison CA, Smith HO. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nature methods. 2009;6:343-5.

[50] Gibson DG. Enzymatic assembly of overlapping DNA fragments. Methods in enzymology: Elsevier; 2011. p. 349-61.

[51] Gao S, Han L, Zhu L, Ge M, Yang S, Jiang Y, et al. One-step integration of multiple genes into the oleaginous yeast Yarrowia lipolytica. Biotechnology letters. 2014;36:2523-8.

[52] Xu X, Liu Y, Du G, Ledesma-Amaro R, Liu L. Microbial chassis development for natural product biosynthesis. Trends in Biotechnology. 2020;38:779-96.

[53] Engler C, Kandzia R, Marillonnet S. A One Pot, One Step, Precision Cloning Method with High Throughput Capability. PLOS ONE. 2008;3:e3647.

[54] Knight T. Idempotent vector design for standard assembly of biobricks. 2003.

[55] Shetty RP, Endy D, Knight TF. Engineering BioBrick vectors from BioBrick parts. Journal of biological engineering. 2008;2:5.

[56] Yamazaki K-i, de Mora K, Saitoh K. BioBrick-based 'Quick Gene Assembly' in vitro. Synthetic Biology. 2017;2.

[57] Keller MW, Lipscomb GL, Loder AJ, Schut GJ, Kelly RM, Adams MW. A hybrid synthetic pathway for butanol production by a hyperthermophilic microbe. Metabolic engineering. 2015;27:101-6.

[58] Park Y-k, Ledesma-Amaro R, Nicaud J-M. De novo biosynthesis of odd-chain fatty acids in Yarrowia lipolytica enabled by modular pathway engineering. Frontiers in Bioengineering and Biotechnology. 2020;7:484.

[59] Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315:1709-12.

[60] Li H, Shen CR, Huang C-H, Sung L-Y, Wu M-Y, Hu Y-C. CRISPR-Cas9 for the genome engineering of cyanobacteria and succinate production. Metabolic engineering. 2016;38:293-302.

[61] Lee Y-G, Seo J-H. Production of 2, 3-butanediol from glucose and cassava hydrolysates by metabolically engineered industrial polyploid Saccharomyces cerevisiae. Biotechnology for biofuels. 2019;12:204.

[62] Qiao K, Wasylenko TM, Zhou K, Xu P, Stephanopoulos G. Lipid production in Yarrowia lipolytica is maximized by engineering cytosolic redox metabolism. Nature Biotechnology. 2017;35:173-7.

[63] Kim HM, Chae TU, Choi SY, Kim WJ, Lee SY. Engineering of an oleaginous bacterium for the production of fatty acids and fuels. Nature Chemical Biology. 2019;15:721-9.

[64] Liu D, Yang Z, Wang P, Niu H, Zhuang W, Chen Y, et al. Towards acetone-uncoupled biofuels production in solventogenic Clostridium through reducing power conservation. Metabolic engineering. 2018;47:102-12.

[65] Cho C, Hong S, Moon HG, Jang Y-S, Kim D, Lee SY. Engineering clostridial aldehyde/alcohol dehydrogenase for selective butanol production. MBio. 2019;10.

[66] Jang Y-S, Lee JY, Lee J, Park JH, Im JA, Eom M-H, et al. Enhanced butanol production obtained by reinforcing the direct butanol-forming route in Clostridium acetobutylicum. MBio. 2012;3.

[67] Wess J, Brinek M, Boles E. Improving isobutanol production with the yeast Saccharomyces cerevisiae by successively blocking competing metabolic pathways as well as ethanol and glycerol formation. Biotechnology for biofuels. 2019;12:173.

[68] Zhang Y, Zhang X, Xiao S, Qi W, Xu J, Yuan Z, et al. Engineering Corynebacterium glutamicum Mutants for 3-Methyl-1-butanol Production. Biochemical Genetics. 2019;57:443-54.

[69] Gérando HMd, Fayolle-Guichard F, Rudant L, Millah SK, Monot F, Ferreira NL, et al. Improving isopropanol tolerance and production of Clostridium beijerinckii DSM 6423 by random mutagenesis and genome shuffling. Applied Microbiology and Biotechnology. 2016;100:5427-36.

[70] George KW, Thompson MG, Kang A, Baidoo E, Wang G, Chan LJG, et al. Metabolic engineering for the high-yield production of isoprenoid-based C 5 alcohols in E. coli. Scientific reports. 2015;5:11128.

[71] Angermayr SA, Van der Woude AD, Correddu D, Vreugdenhil A, Verrone V, Hellingwerf KJ. Exploring metabolic engineering design principles for the photosynthetic production of lactic acid by Synechocystis sp. PCC6803. Biotechnology for biofuels. 2014;7:99.

[72] Liu H, Zhang J, Yuan J, Jiang X, Jiang L, Zhao G, et al. Omics-based analyses revealed metabolic responses of Clostridium acetobutylicum to lignocellulose-derived inhibitors furfural, formic acid and phenol stress for butanol fermentation. Biotechnology for biofuels. 2019;12:1-20.

[73] Cheon S, Kim HM, Gustavsson M, Lee SY. Recent trends in metabolic engineering of microorganisms for the production of advanced biofuels. Current Opinion in Chemical Biology. 2016;35:10-21.

[74] Steen EJ, Chan R, Prasad N, Myers S, Petzold CJ, Redding A, et al. Metabolic engineering of Saccharomyces cerevisiae for the production of n-butanol. Microbial Cell Factories. 2008;7:36.

[75] Zhang F, Rodriguez S, Keasling JD. Metabolic engineering of microbial pathways for advanced biofuels production. Curr Opin Biotechnol. 2011;22:775-83.

[76] Peralta-Yahya PP, Keasling JD. Advanced biofuel production in microbes. Biotechnol J. 2010;5:147-62.

[77] Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature. 2008;451:86-9.

[78] Toogood HS, Scrutton NS. Retooling microorganisms for the fermentative production of alcohols. Current Opinion in Biotechnology. 2018;50:1-10.

[79] Yu M, Zhang Y, Tang I-C, Yang S-T. Metabolic engineering of Clostridium tyrobutyricum for n-butanol production. Metabolic engineering. 2011;13:373-82.

[80] Saini M, Chen MH, Chiang C-J, Chao Y-P. Potential production platform of n-butanol in Escherichia coli. Metabolic engineering. 2015;27:76-82.

[81] Matsuda F, Ishii J, Kondo T, Ida K, Tezuka H, Kondo A. Increased isobutanol production in Saccharomyces cerevisiae by eliminating competing pathways and resolving cofactor imbalance. Microb Cell Fact. 2013;12:119.

[82] Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC. Driving forces enable high-titer anaerobic 1-butanol synthesis in Escherichia coli. Applied and environmental microbiology. 2011;77:2905-15.

[83] Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC. Driving forces enable high-titer anaerobic 1-butanol synthesis in Escherichia coli. Applied and environmental microbiology. 2011;77:2905-15.

[84] Dekishima Y, Lan EI, Shen CR, Cho KM, Liao JC. Extending Carbon Chain Length of 1-Butanol Pathway for 1-Hexanol Synthesis from Glucose by Engineered Escherichia coli. Journal of the American Chemical Society. 2011;133:11399-401.

[85] Gupta P, Phulara S. Metabolic engineering for isoprenoid-based biofuel production. Journal of applied microbiology. 2015;119:605-19.

[86] Misra N, Panda PK, Parida BK. Agrigenomics for microalgal biofuel production: an overview of various bioinformatics resources and recent studies to link OMICS to bioenergy and bioeconomy. Omics: a journal of integrative biology. 2013;17:537-49.

[87] Palazzotto E, Weber T. Omics and multi-omics approaches to study the biosynthesis of secondary metabolites in microorganisms. Current Opinion in Microbiology. 2018;45:109-16.

[88] Kopf M, Klähn S, Scholz I, Matthiessen JKF, Hess WR, Voß B. Comparative analysis of the primary transcriptome of Synechocystis sp. PCC 6803. DNA Res. 2014;21:527-39.

[89] Borirak O, De Koning LJ, Van Der Woude AD, Hoefsloot HC, Dekker HL, Roseboom W, et al. Quantitative proteomics analysis of an ethanol-and a lactate-producing mutant strain of Synechocystis sp. PCC6803. Biotechnology for biofuels. 2015;8:111.

[90] Gupta VK, Steindorff AS, de Paula RG, Silva-Rocha R, Mach-Aigner AR, Mach RL, et al. The post-genomic era of Trichoderma reesei: what's next? Trends in Biotechnology. 2016;34:970-82.

[91] Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic acids research. 2017;45:D353-D61.

[92] Norsigian CJ, Pusarla N, McConn JL, Yurkovich JT, Dräger A, Palsson BO, et al. BiGG Models 2020: multi-strain genome-scale models and expansion across the phylogenetic tree. Nucleic acids research. 2020;48:D402-D6.

[93] Bairoch A. The ENZYME database in 2000. Nucleic acids research. 2000;28:304-5.

[94] Larroude M, Rossignol T, Nicaud JM, Ledesma-Amaro R. Synthetic biology tools for engineering Yarrowia lipolytica. Biotechnology Advances. 2018;36:2150-64.

[95] Pan P, Hua Q. Reconstruction and in silico analysis of metabolic network for an oleaginous yeast, Yarrowia lipolytica. PloS one. 2012;7:e51535.

[96] Sánchez BJ, Zhang C, Nilsson A, Lahtvee PJ, Kerkhoven EJ, Nielsen J. Improving the phenotype predictions of a yeast genome-scale metabolic model by incorporating enzymatic constraints. Molecular systems biology. 2017;13:935.

[97] Cho S, Shin J, Cho B-K. Applications of CRISPR/Cas System to Bacterial Metabolic Engineering. Int J Mol Sci. 2018;19:1089.

[98] Blin K, Pedersen LE, Weber T, Lee SY. CRISPy-web: An online resource to design sgRNAs for CRISPR applications. Synthetic and Systems Biotechnology. 2016;1:118-21.

[99] Liu H, Wei Z, Dominguez A, Li Y, Wang X, Qi LS. CRISPR-ERA: a comprehensive design tool for CRISPR-mediated gene editing, repression and activation. Bioinformatics. 2015;31:3676-8.

[100] Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nature protocols. 2013;8:2180-96.

[101] Zoghlami A, Paës G. Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. Frontiers in Chemistry. 2019;7:874.

[102] Vu HP, Nguyen LN, Vu MT, Johir MAH, McLaughlan R, Nghiem LD. A comprehensive review on the framework to valorise lignocellulosic biomass as biorefinery feedstocks. Science of The Total Environment. 2020;743:140630.

[103] Rencoret J, Pereira A, José C, Martínez AT, Gutiérrez A. Laccase-mediator pretreatment of wheat straw degrades lignin and improves saccharification. BioEnergy Research. 2016;9:917-30.

[104] Asgher M, Ahmad Z, Iqbal HMN. Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production. Industrial Crops and Products. 2013;44:488-95.

[105] Hsieh C-wC, Cannella D, Jørgensen H, Felby C, Thygesen LG. Cellulase Inhibition by High Concentrations of Monosaccharides. Journal of Agricultural and Food Chemistry. 2014;62:3800-5.

[106] da Silva ASA, de Souza MF, Ballesteros I, Manzanares P, Ballesteros M, Bon EP. High-solids content enzymatic hydrolysis of hydrothermally pretreated sugarcane bagasse using a laboratory-made enzyme blend and commercial preparations. Process Biochemistry. 2016;51:1561-7.

[107] Gruno M, Väljamäe P, Pettersson G, Johansson G. Inhibition of the Trichoderma reesei cellulases by cellobiose is strongly dependent on the nature of the substrate. Biotechnology and bioengineering. 2004;86:503-11.

[108] Andrić P, Meyer AS, Jensen PA, Dam-Johansen K. Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: II. Quantification of inhibition and suitability of membrane reactors. Biotechnology advances. 2010;28:407-25.

[109] Xu C, Zhang J, Zhang Y, Guo Y, Xu H, Xu J, et al. Enhancement of high-solids enzymatic hydrolysis efficiency of alkali pretreated sugarcane bagasse at low cellulase dosage by fed-batch strategy based on optimized accessory enzymes and additives. Bioresource Technology. 2019;292:121993.

[110] Reyes-Ortiz V, Heins RA, Cheng G, Kim EY, Vernon BC, Elandt RB, et al. Addition of a carbohydrate-binding module enhances cellulase penetration into cellulose substrates. Biotechnol Biofuels. 2013;6:93.

[111] Lamed R, Setter E, Bayer EA. Characterization of a cellulose-binding, cellulase-containing complex in Clostridium thermocellum. Journal of bacteriology. 1983;156:828-36.

[112] Dassa B, Borovok I, Ruimy-Israeli V, Lamed R, Flint HJ, Duncan SH, et al. Rumen cellulosomics: divergent fiber-degrading strategies revealed by comparative genome-wide analysis of six ruminococcal strains. PLoS One. 2014;9:e99221.

[113] Zhivin-Nissan O, Dassa B, Morag E, Kupervaser M, Levin Y, Bayer EA. Unraveling essential cellulosomal components of the (Pseudo) Bacteroides cellulosolvens reveals an extensive reservoir of novel catalytic enzymes. Biotechnology for biofuels. 2019;12:115.

[114] Kahn A, Moraïs S, Galanopoulou AP, Chung D, Sarai NS, Hengge N, et al. Creation of a functional hyperthermostable designer cellulosome. Biotechnology for biofuels. 2019;12:1-15.

[115] Gilmore SP, Lillington SP, Haitjema CH, de Groot R, O'Malley MA. Designing chimeric enzymes inspired by fungal cellulosomes. Synthetic and Systems Biotechnology. 2020;5:23-32.

[116] Arfi Y, Shamshoum M, Rogachev I, Peleg Y, Bayer EA. Integration of bacterial lytic polysaccharide monooxygenases into designer cellulosomes promotes enhanced cellulose degradation. Proceedings of the National Academy of Sciences. 2014;111:9109-14.

[117] Deng Z, Xia A, Liao Q, Zhu X, Huang Y, Fu Q. Laccase pretreatment of wheat straw: effects of the physicochemical characteristics and the kinetics of enzymatic hydrolysis. Biotechnology for Biofuels. 2019;12:159.

[118] Hsieh CW, Cannella D, Jørgensen H, Felby C, Thygesen LG. Cellulase inhibition by high concentrations of monosaccharides. Journal of agricultural and food chemistry. 2014;62:3800-5.

[119] Silva A, Souza M, Ballesteros I, Manzanares P, Ballesteros M, Bon E. High-solids content enzymatic hydrolysis of hydrothermally pretreated sugarcane bagasse using a laboratory-made enzyme blend and commercial preparations. Process Biochemistry. 2016;51.

[120] Puurand M, Väljamäe P, Pettersson G, Johansson G. Inhibition of the Trichoderma reesei cellulases by cellobiose is strongly dependent on the nature of the substrate. Biotechnology and bioengineering. 2004;86:503-11.

[121] Asgher M, Ahmad Z, Iqbal H. Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production. Industrial Crops and Products. 2013;44:488–95.

[122] Xu C, Zhang J, Zhang Y, Guo Y, Xu H, Xu J-L, et al. Enhancement of high-solids enzymatic hydrolysis efficiency of alkali pretreated sugarcane bagasse at low cellulase dosage by fed-batch strategy based on optimized accessory enzymes and additives. Bioresource technology. 2019;292:121993.

[123] Eibinger M, Ganner T, Plank H, Nidetzky B. A Biological Nanomachine at Work: Watching the Cellulosome Degrade Crystalline Cellulose. ACS Central Science. 2020;6:739-46.

[124] Kahn A, Moraïs S, Galanopoulou AP, Chung D, Sarai NS, Hengge N, et al. Creation of a functional hyperthermostable designer cellulosome. Biotechnology for Biofuels. 2019;12:44.

[125] Victoria Villeda JJ, Dahmen M, Hechinger M, Voll A, Marquardt W. Towards Model-Based Design of Tailor-Made Fuels from Biomass. In: Klaas M, Pischinger S, Schröder W, editors. Fuels From Biomass: An Interdisciplinary Approach. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 193-211.

[126] Li A, Zhu L, Mao Y, Zhai J, Han D, Lu X, et al. Surrogate formulation methodology for biodiesel based on chemical deconstruction in consideration of molecular structure and engine combustion factors. Combustion and Flame. 2019;199:152-67.

[127] Dahmen M, Marquardt W. Model-Based Design of Tailor-Made Biofuels. Energy & Fuels. 2016;30:1109-34.

[128] Liew F, Martin ME, Tappel RC, Heijstra BD, Mihalcea C, Köpke M. Gas fermentation—a flexible platform for commercial scale production of low-carbon-fuels and chemicals from waste and renewable feedstocks. Frontiers in microbiology. 2016;7:694.

[129] Datta A, Hossain A, Roy S. An Overview on Biofuels and Their Advantages and Disadvantages. Asian Journal of Chemistry. 2019;31.

[130] Demirbas A. Combustion efficiency impacts of biofuels. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects. 2009;31:602-9.

[131] Guarieiro LLN, de Almeida Guerreiro ET, dos Santos Amparo KK, Manera VB, Regis ACD, Santos AG, et al. Assessment of the use of oxygenated fuels on emissions and performance of a diesel engine. Microchemical Journal. 2014;117:94-9.

[132] Baskar P, Senthilkumar A. Effects of oxygen enriched combustion on pollution and performance characteristics of a diesel engine. Engineering Science and Technology, an International Journal. 2016;19:438-43.

[133] McLinden M, Bruno T, Frenkel M, Huber M. Standard reference data for the thermophysical properties of biofuels. Biofuels: ASTM International; 2011.

[134] vom Stein T, Klankermayer J, Leitner W. Tailor-Made Fuels and Chemicals from Biomass. 2013.

[135] Palkovits R, Delidovich I. Efficient utilization of renewable feedstocks: the role of catalysis and process design. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences. 2018;376:20170064.

[136] Janssen AJ, Kremer FW, Baron JH, Muether M, Pischinger S, Klankermayer J. Tailor-Made Fuels from Biomass for Homogeneous Low-Temperature Diesel Combustion. Energy & Fuels. 2011;25:4734-44.

[137] Den W, Sharma VK, Lee M, Nadadur G, Varma RS. Lignocellulosic biomass transformations via greener oxidative pretreatment processes: access to energy and value-added chemicals. Frontiers in chemistry. 2018;6:141.

[138] Guczi L, Erdôhelyi A. Catalysis for alternative energy generation: Springer Science & Business Media; 2012.

[139] Wilson K, Lee A, Dacquin J-P. Catalysis for Alternative Energy Generation. 2012. p. 263-304.

[140] Hoppe F, Burke U, Thewes M, Heufer A, Kremer F, Pischinger S. Tailor-Made Fuels from Biomass: Potentials of 2-butanone and 2-methylfuran in direct injection spark ignition engines. Fuel. 2016;167:106-17.

[141] Dornau A, Robson JF, Thomas GH, McQueen-Mason SJ. Robust microorganisms for biofuel and chemical production from municipal solid waste. Microbial Cell Factories. 2020;19:1-18.

[142] Bharathiraja DB, Yogendran D, Kumar.R R, Muninathan C, Subramani P. Biofuels from sewage sludge- A review. International journal of chem tech research. 2014;6:4417-27.

[143] Alonso DM, Bond JQ, Dumesic JA. Catalytic conversion of biomass to biofuels. Green chemistry. 2010;12:1493-513.

[144] Liu B, Zhang Z. Catalytic conversion of biomass into chemicals and fuels over magnetic catalysts. Acs Catalysis. 2016;6:326-38.

[145] Hommes A, Heeres HJ, Yue J. Catalytic Transformation of Biomass Derivatives to Value-Added Chemicals and Fuels in Continuous Flow Microreactors. ChemCatChem. 2019;11:4671-708.

[146] Isikgor F, Becer R. Lignocellulosic Biomass: A Sustainable Platform for Production of Bio-Based Chemicals and Polymers. Polym Chem. 2015;6.

[147] Mariscal R, Maireles-Torres P, Ojeda M, Sádaba I, López Granados M. Furfural: a renewable and versatile platform molecule for the synthesis of chemicals and fuels. Energy & Environmental Science. 2016;9:1144-89.

[148] Li X, Jia P, Wang T. Furfural: A Promising Platform Compound for Sustainable Production of C4 and C5 Chemicals. ACS Catalysis. 2016;6:7621-40.

[149] Mulik N, Niphadkar P, Bokade V. Synthesis of ethyl furfuryl ether (potential biofuel) by etherification of furfuryl alcohol with ethanol over heterogenized reusable H1Cs2PW12O40 catalyst. Research on Chemical Intermediates. 2020.

[150] Insyani R, Verma D, Kim SM, Kim J. Direct one-pot conversion of monosaccharides into high-yield 2, 5-dimethylfuran over a multifunctional Pd/Zr-based metal–organic framework@ sulfonated graphene oxide catalyst. Green Chemistry. 2017;19:2482-90.

[151] Ma Y-F, Wang H, Xu G-Y, Liu X-H, Zhang Y, Fu Y. Selective conversion of furfural to cyclopentanol over cobalt catalysts in one step. Chinese Chemical Letters. 2017;28:1153-8.

[152] Zhao G, Liu M, Xia X, Li L, Xu B. Conversion of Furfuryl alcohol into ethyl levulinate over glucose-derived carbon-based solid acid in ethanol. Molecules. 2019;24:1881.

[153] Hellier P, Ladommatos N, Allan R, Payne M, Rogerson J. The impact of saturated and unsaturated fuel molecules on diesel combustion and exhaust emissions. SAE International Journal of Fuels and Lubricants. 2011;5:106-22.

[154] Hellier P, Ladommatos N. The influence of biodiesel composition on compression ignition combustion and emissions. Proceedings of the Institution of Mechanical Engineers, Part A: Journal of Power and Energy. 2015;229:714-26.

[155] Heuser B, Jakob M, Kremer F, Pischinger S, Kerschgens B, Pitsch H. Tailor-Made Fuels from Biomass: Influence of Molecular Structures on the Exhaust Gas Emissions of Compression Ignition Engines. SAE International; 2013.

[156] Jakob M, Heuser B, Kremer F, Pischinger S, Kerschgens B, Pitsch H. Tailor-Made Fuels from Biomass: Influence of Molecular Structures on the Exhaust Gas Emissions of Compression Ignition Engines. SAE TEchnical Paper. 2013;SAE 2013-36-0571.

[157] Kalghatgi G, Stone R. Fuel requirements of spark ignition engines. Proceedings of the Institution of Mechanical Engineers, Part D: Journal of Automobile Engineering. 2018;232:22-35.

[158] Li H, Saravanamurugan S, Yang S, Riisager A. Direct transformation of carbohydrates to the biofuel 5-ethoxymethylfurfural by solid acid catalysts. Green Chemistry. 2016;18:726-34.

[159] Requies JM, Frias M, Cuezva M, Iriondo A, Agirre I, Viar N. Hydrogenolysis of 5-Hydroxymethylfurfural To Produce 2, 5-Dimethylfuran over ZrO2 Supported Cu and RuCu Catalysts. Industrial & Engineering Chemistry Research. 2018;57:11535-46.

[160] Nemestóthy N, Komáromy P, Bakonyi P, Tóth AL, Tóth G, Gubicza L, et al. Carbohydrate to itaconic acid conversion by Aspergillus terreus and the evaluation of process monitoring based on the measurement of CO 2. Waste and Biomass Valorization. 2020;11:1069-75.

[161] Abdelrahman OA, Park DS, Vinter KP, Spanjers CS, Ren L, Cho HJ, et al. Renewable isoprene by sequential hydrogenation of itaconic acid and dehydra-decyclization of 3-methyl-tetrahydrofuran. ACS Catalysis. 2017;7:1428-31.

[162] Gowda RR, Chen EYX. Regioselective Hydrogenation of Itaconic Acid to γ-Isovalerolactone by Transition-Metal Nanoparticle Catalysts. ChemSusChem. 2019;12:973-7.

[163] Licursi D, Antonetti C, Fulignati S, Giannoni M, Raspolli Galletti AM. Cascade strategy for the tunable catalytic valorization of levulinic acid and γ-valerolactone to 2-methyltetrahydrofuran and alcohols. Catalysts. 2018;8:277.

[164] Joshi J, Dhungana P, Prajapati B, Maharjan R, Poudyal P, Yadav M, et al. Enhancement of Ethanol Production in Electrochemical Cell by Saccharomyces cerevisiae (CDBT2) and Wickerhamomyces anomalus (CDBT7). 2019.

[165] Fernandes D, Rocha A, Mai E, Mota CJ, Da Silva VT. Levulinic acid esterification with ethanol to ethyl levulinate production over solid acid catalysts. Applied Catalysis A: General. 2012;425:199-204.

[166] Ramli NAS, Amin NAS. Kinetic study of glucose conversion to levulinic acid over Fe/HY zeolite catalyst. Chemical Engineering Journal. 2016;283:150-9.

[167] Singh H, Iyengar N, Yadav R, Rai A, Sinha AK. Facile conversion of levulinic acid to γ-valerolactone using a high surface area magnetically separable Ni/NiO catalyst. Sustainable Energy & Fuels. 2018;2:1699-706.

[168] Xie Z, Chen B, Wu H, Liu M, Liu H, Zhang J, et al. Highly efficient hydrogenation of levulinic acid into 2-methyltetrahydrofuran over Ni–Cu/Al 2 O 3–ZrO 2 bifunctional catalysts. Green Chemistry. 2019;21:606-13.

[169] Rodrussamee N, Sattayawat P, Yamada M. Highly efficient conversion of xylose to ethanol without glucose repression by newly isolated thermotolerant Spathaspora passalidarum CMUWF1–2. BMC microbiology. 2018;18:73.

[170] Agirrezabal-Telleria I, Requies J, Güemez M, Arias P. Dehydration of d-xylose to furfural using selective and hydrothermally stable arenesulfonic SBA-15 catalysts. Applied catalysis B: environmental. 2014;145:34-42.

[171] Xu L, Nie R, Lyu X, Wang J, Lu X. Selective hydrogenation of furfural to furfuryl alcohol without external hydrogen over N-doped carbon confined Co catalysts. Fuel Processing Technology. 2020;197:106205.

[172] Arjunan S. Microalgae Harvesting via Flocculation: Impact of PH, Algae Species and Biomass Concentration. 2018.

[173] Hashim LH, Halilu A, Sudarsanam P, Umar YB, Johan MRB, Bhargava SK. Bifunctional rice husk-derived SiO2-Cu-Al-Mg nanohybrid catalyst for one-pot conversion of biomass-derived furfural to furfuryl acetate. Fuel. 2020;275:117953.

[174] Fu Z, Wang Z, Lin W, Song W, Li S. High efficient conversion of furfural to 2-methylfuran over Ni-Cu/Al2O3 catalyst with formic acid as a hydrogen donor. Applied Catalysis A: General. 2017;547:248-55.

[175] Aldosari OF. Selective conversion of furfuryl alcohol to 2-methylfuran over nanosilica supported Au: Pd bimetallic catalysts at room temperature. Journal of Saudi Chemical Society. 2019;23:938-46.

[176] Seemala B, Kumar R, Cai CM, Wyman CE, Christopher P. Single-step catalytic conversion of furfural to 2-pentanol over bimetallic Co–Cu catalysts. Reaction Chemistry & Engineering. 2019;4:261-7.

[177] Dobovišek Ž, Vajda B, Pehan S, Kegl B. Influence of fuel properties on engine characteristics and tribology parameters. Goriva i maziva: časopis za tribologiju, tehniku podmazivanja i primjenu tekućih i plinovitih goriva i inžinjerstvo izgaranja. 2009;48:145-58.

[178] TAN P-q, ZHAO J-y, HU Z-y, LOU D-m, DU A-m, DU D-m. Effects of fuel properties on exhaust emissions from diesel engines. Journal of Fuel Chemistry and Technology. 2013;41:347-55.

[179] Jeihouni Y, Pischinger S, Ruhkamp L, Koerfer T. Relationship between fuel properties and sensitivity analysis of non-aromatic and aromatic fuels used in a single cylinder heavy duty diesel engine. SAE Technical Paper; 2011.

[180] Ritchie H, Roser M. Fossil fuels. Our World in Data. 2017.

[181] Tesfa B, Mishra R, Gu F, Powles N. Prediction models for density and viscosity of biodiesel and their effects on fuel supply system in CI engines. Renewable energy. 2010;35:2752-60.

[182] Hassan MH, Kalam MA. An Overview of Biofuel as a Renewable Energy Source: Development and Challenges. Procedia Engineering. 2013;56:39-53.

[183] Hassan MH, Kalam MA. An overview of biofuel as a renewable energy source: development and challenges. Procedia Engineering. 2013;56:53.

[184] Kumar N, Sonthalia A, Pali H. Alternative Fuels for Diesel Engines: New Frontiers. 2018.

[185] Rajamohan S, Kasimani R, Ramakrishnan P, Shameer PM. A review on the properties, performance and emission aspects of the third generation biodiesels. Renewable and Sustainable Energy Reviews. 2017.

[186] No S-Y. Utilization of Pentanol as Biofuels in Compression Ignition Engines. Frontiers in Mechanical Engineering. 2020;6.

[187] Paloboran M, Sutantra I, Sudarmanta B. Performances and Emissions Characteristics of Three Main Types Composition of Gasoline-Ethanol Blended in Spark Ignition Engines. International Review of Mechanical Engineering (IREME). 2016;10:552.

[188] Wei H, Feng D, Shu G, Pan M, Guo Y, Gao D, et al. Experimental investigation on the combustion and emissions characteristics of 2-methylfuran gasoline blend fuel in spark-ignition engine. Applied energy. 2014;132:317-24.

[189] Anitescu G, Bruno TJ. Liquid Biofuels: Fluid Properties to Optimize Feedstock Selection, Processing, Refining/Blending, Storage/Transportation, and Combustion. Energy & Fuels. 2012;26:324-48.

[190] El-Faroug MO, Yan F, Luo M, Fiifi Turkson R. Spark ignition engine combustion, performance and emission products from hydrous ethanol and its blends with gasoline. Energies. 2016;9:984.

[191] Yan K, Wu G, Lafleur T, Jarvis C. Production, properties and catalytic hydrogenation of furfural to fuel additives and value-added chemicals. Renewable and sustainable energy reviews. 2014;38:663-76.

[192] Bereczky Á, Lukács K, Farkas M, Dóbé S. Effect of γ-valerolactone blending on engine performance, combustion characteristics and exhaust emissions in a diesel engine. Natural Resources. 2014;5:177-91.

[193] Thewes M, Muether M, Pischinger S, Budde M, Brunn A, Sehr A, et al. Analysis of the impact of 2-methylfuran on mixture formation and combustion in a direct-injection spark-ignition engine. Energy & Fuels. 2011;25:5549-61.

[194] Natsir TA, Shimazu S. Fuels and fuel additives from furfural derivatives via etherification and formation of methylfurans. Fuel Processing Technology. 2020;200:106308.

[195] Unlu D, Boz N, Ilgen O, Hilmioglu N. Improvement of fuel properties of biodiesel with bioadditive ethyl levulinate. Open Chemistry. 2018;16:647-52.

[196] Ahmad E, Alam MI, Pant K, Haider MA. Catalytic and mechanistic insights into the production of ethyl levulinate from biorenewable feedstocks. Green Chemistry. 2016;18:4804-23.

[197] Tian M, McCormick RL, Luecke J, de Jong E, van der Waal JC, van Klink GP, et al. Anti-knock quality of sugar derived levulinic esters and cyclic ethers. Fuel. 2017;202:414-25.

[198] Xiao H, Zeng P, Li Z, Zhao L, Fu X. Combustion performance and emissions of 2-methylfuran diesel blends in a diesel engine. Fuel. 2016;175:157-63.

[199] da Silva Jr JL, Aznar M. Thermophysical properties of 2, 5-dimethylfuran and liquid–liquid equilibria of ternary systems water+ 2, 5-dimethylfuran+ alcohols (1-butanol or 2-butanol or 1-hexanol). Fuel. 2014;136:316-25.

[200] Kessler T, Sacia ER, Bell AT, Mack JH. Predicting the cetane number of furanic biofuel candidates using an improved artificial neural network based on molecular structure. Internal Combustion Engine Division Fall Technical Conference: American Society of Mechanical Engineers; 2016. p. V001T02A10.

[201] de Jong E, Gruter G-J. Furanics: A Novel Diesel Fuel with Superior Characteristics. SAE Technical Papers. 2009.

[202] Malinowski A, Wardzińska D. Catalytic conversion of furfural towards fuel biocomponents. Chemik. 2012;66:982-90.

[203] Zhao L, Ye D, Guo Y, Fang W. Density and Viscosity of Ternary Mixture of Cyclopentanol+ exo-Tetrahydrodicyclopentadiene+ 1, 3-Dimethyladamantane. Journal of Chemical & Engineering Data. 2019;64:2558-67.

[204] Gaspar DJ, West BH, Ruddy D, Wilke TJ, Polikarpov E, Alleman TL, et al. Top Ten Blendstocks Derived From Biomass For Turbocharged Spark Ignition Engines: Bio-blendstocks With Potential for Highest Engine Efficiency. Pacific Northwest National Lab.(PNNL), Richland, WA (United States); 2019.

[205] Hechinger M, Voll A, Marquardt W. Towards an integrated design of biofuels and their production pathways. Computers & Chemical Engineering. 2010;34:1909-18.

[206] Vargas-Ibáñez LT, Iglesias-Silva GA, Cano-Gómez JJ, Escamilla-Alvarado C, Berrones-Eguiluz MA. Densities and Viscosities for Binary Liquid Mixtures of Biodiesel+ 1-Pentanol, 2-Pentanol, or 2-Methyl-1-Butanol from (288.15 to 338.15) K at 0.1 MPa. Journal of Chemical & Engineering Data. 2018;63:2438-50.

[207] McCormick RL, Fioroni G, Fouts L, Christensen E, Yanowitz J, Polikarpov E, et al. Selection criteria and screening of potential biomass-derived streams as fuel blendstocks for advanced spark-ignition engines. SAE International Journal of Fuels and Lubricants. 2017;10:442-60.

[208] Tüccar G, Tosun E, Uludamar E. Investigations of effects of density and viscosity of diesel and biodiesel fuels on NOx and other emission formations. Akademik Platform Mühendislik ve Fen Bilimleri Dergisi. 2018;6:81-5.

[209] Lee S-w, Tanaka D, Kusaka J, Daisho Y. Effects of diesel fuel characteristics on spray and combustion in a diesel engine. Jsae Review - JSAE REV. 2002;23:407-14.

[210] Groendyk MA, Rothamer D. Effects of fuel physical properties on auto-ignition characteristics in a heavy duty compression ignition engine. SAE International Journal of Fuels and Lubricants. 2015;8:200-13.

[211] Zhang X, Li T, Ma P, Wang B. Spray combustion characteristics and soot emission reduction of hydrous ethanol diesel emulsion fuel using color-ratio pyrometry. Energies. 2017;10:2062.

[212] Shrivastava P, Verma TN. Effect of fuel injection pressure on the characteristics of CI engine fuelled with biodiesel from Roselle oil. Fuel. 2020;265:117005.

[213] Agarwal AK, Dhar A, Gupta JG, Kim WI, Choi K, Lee CS, Park S. Effect of Fuel Injection Pressure and Injection. Applied Energy. 2014;130:212-21.

[214] Woo DG, Kim TH. Effect of kinematic viscosity variation with blended-oil biodiesel on engine performance and exhaust emission in a power tiller engine. Environmental Engineering Research. 2019;25:946-59.

[215] Cataluna R, Da Silva R. Effect of cetane number on specific fuel consumption and particulate matter and unburned hydrocarbon emissions from diesel engines. Journal of Combustion. 2012;2012.

[216] McGann B, Kim K, Lee T, Temme J, Kweon C-B. Effect of the Cetane Number on Jet Fuel Spray Ignition at High-Temperature and-Pressure Conditions. Energy & Fuels. 2020;34:1337-46.

[217] Dahmen M, Marquardt W. A novel group contribution method for the prediction of the derived cetane number of oxygenated hydrocarbons. Energy & Fuels. 2015;29:5781-801.

[218] Kalghatgi G, Johansson B. Gasoline compression ignition approach to efficient, clean and affordable future engines. Proceedings of the Institution of Mechanical Engineers, Part D: Journal of Automobile Engineering. 2018;232:118-38.

[219] Yip HL, Srna A, Yuen ACY, Kook S, Taylor RA, Yeoh GH, et al. A Review of Hydrogen Direct Injection for Internal Combustion Engines: Towards Carbon-Free Combustion. Applied Sciences. 2019;9:4842.

[220] Stradling R, Williams J, Hamje H, Rickeard D. Effect of octane on performance, energy consumption and emissions of two Euro 4 passenger cars. Transportation Research Procedia. 2016;14:3159-68.

[221] Kalghatgi GT. The outlook for fuels for internal combustion engines. International Journal of Engine Research. 2014;15:383-98.

[222] Yanowitz J, Ratcliff MA, McCormick RL, Taylor JD, Murphy MJ. Compendium of experimental cetane numbers. National Renewable Energy Lab.(NREL), Golden, CO (United States); 2017.

[223] Cung KD, Ciatti SA, Tanov S, Andersson Ö. Low-Temperature Combustion of High Octane Fuels in a Gasoline Compression Ignition Engine. Frontiers in Mechanical Engineering. 2017;3.

[224] Song H, Quinton KS, Peng Z, Zhao H, Ladommatos N. Effects of oxygen content of fuels on combustion and emissions of diesel engines. Energies. 2016;9:28.

[225] Bergthorson JM, Thomson MJ. A review of the combustion and emissions properties of advanced transportation biofuels and their impact on existing and future engines. Renewable and sustainable energy reviews. 2015;42:1393-417.

[226] Westbrook CK, Pitz WJ, Curran HJ. Chemical kinetic modeling study of the effects of oxygenated hydrocarbons on soot emissions from diesel engines. The journal of physical chemistry A. 2006;110:6912-22.

[227] García A, Monsalve-Serrano J, Heuser B, Jakob M, Kremer F, Pischinger S. Influence of fuel properties on fundamental spray characteristics and soot emissions using different tailor-made fuels from biomass. Energy Conversion and Management. 2016;108:243-54.

[228] Cheng AS, Dibble R, Buchholz B. The effect of oxygenates on diesel engine particulate matter. SAE Technical Papers. 2002.

[229] Donahue RJ, Foster DE. Effects of oxygen enhancement on the emissions from a DI diesel via manipulation of fuels and combustion chamber gas composition. SAE Transactions. 2000:334-50.

[230] Thewes M, Müther M, Brassat A, Pischinger S, Sehr A. Analysis of the Effect of Bio-Fuels on the Combustion in a Downsized DI SI Engine. SAE International Journal of Fuels and Lubricants. 2012;5:274-88.

[231] Labeckas G, Slavinskas S, Kanapkienė I. Study of the effects of Biofuel-Oxygen of various origins on a CRDI diesel engine combustion and emissions. Energies. 2019;12:1241.

[232] Nagano S, Yokoo N, Kitano K, Nakata K. Effects of high boiling point fuel additives on deposits in a direct injection gasoline engine. SAE International Journal of Fuels and Lubricants. 2017;10:789-802.

[233] Pickett LM, Kook S. Effect of Fuel Volatility and Ignition Quality on Combustion and Soot Formation at Fixed Premixing Conditions. Sandia National Lab.(SNL-CA), Livermore, CA (United States); 2009.

[234] Fisher BT, Knothe G, Mueller CJ. Liquid-phase penetration under unsteady in-cylinder conditions: soy-and cuphea-derived biodiesel fuels versus conventional diesel. Energy & fuels. 2010;24:5163-80.

[235] Dec JE. Advanced compression-ignition engines—understanding the in-cylinder processes. Proceedings of the combustion institute. 2009;32:2727-42.

[236] König A, Marquardt W, Mitsos A, Viell J, Dahmen M. Integrated design of renewable fuels and their production processes: recent advances and challenges. Current Opinion in Chemical Engineering. 2020;27:45-50.

[237] Piehl JA, Zyada A, Bravo L, Samimi-Abianeh O. Review of Oxidation of Gasoline Surrogates and Its Components. Journal of Combustion. 2018;2018:8406754.

[238] Luning Prak DJ, Luning Prak PJ, Trulove PC, Cowart JS. Formulation of surrogate fuel mixtures based on physical and chemical analysis of Hydrodepolymerized Cellulosic Diesel Fuel. Energy & Fuels. 2016;30:7331-41.

[239] Pitz WJ, Mueller CJ. Recent progress in the development of diesel surrogate fuels. Progress in Energy and Combustion Science. 2011;37:330-50.

[240] Dooley S, Won SH, Heyne J, Farouk TI, Ju Y, Dryer FL, et al. The experimental evaluation of a methodology for surrogate fuel formulation to emulate gas phase combustion kinetic phenomena. Combustion and Flame. 2012;159:1444-66.

[241] Li R, Herreros JM, Tsolakis A, Yang W. Novel Functional Group Contribution Method for Surrogate Formulation with Accurate Fuel Compositions. Energy & Fuels. 2020;34:2989-3012.

[242] Mueller CJ, Cannella WJ, Bays JT, Bruno TJ, DeFabio K, Dettman HD, et al. Diesel Surrogate Fuels for Engine Testing and Chemical-Kinetic Modeling: Compositions and Properties. Energy & Fuels. 2016;30:1445-61.

[243] Yu J, Wang Z, Zhuo X, Wang W, Gou X. Surrogate Definition and Chemical Kinetic Modeling for Two Different Jet Aviation Fuels. Energy & Fuels. 2016;30:1375-82.

[244] Krishnasamy A, Reitz RD, Willems W, Kurtz E. Surrogate Diesel Fuel Models for Low Temperature Combustion. SAE International; 2013.

[245] Naik CV, Puduppakkam K, Wang C, Kottalam J, Liang L, Hodgson D, et al. Applying Detailed Kinetics to Realistic Engine Simulation: the Surrogate Blend Optimizer and Mechanism Reduction Strategies. SAE International; 2010.

[246] Su X, Ra Y, Reitz R. A Surrogate Fuel Formulation Approach for Real Transportation Fuels with Application to Multi-Dimensional Engine Simulations. SAE International Journal of Fuels and Lubricants. 2014;7:236-49.

[247] Ranzi E. A wide-range kinetic modeling study of oxidation and combustion of transportation fuels and surrogate mixtures. Energy & fuels. 2006;20:1024-32.

[248] Marrero J, Gani R. Group-contribution based estimation of pure component properties. Fluid Phase Equilibria. 2001;183-184:183-208.

[249] Mueller CJ, Cannella WJ, Bruno TJ, Bunting B, Dettman HD, Franz JA, et al. Methodology for formulating diesel surrogate fuels with accurate compositional, ignition-quality, and volatility characteristics. Energy & Fuels. 2012;26:3284-303.

[250] Sarathy SM, Farooq A, Kalghatgi GT. Recent progress in gasoline surrogate fuels. Progress in Energy and Combustion Science. 2018;65:67-108.

[251] Perez PL, Boehman AL. Effects of the Chemical Structure and Composition of Surrogate Gasoline Fuels on Homogeneous Charge Compression Ignition Combustion in a Single-Cylinder Engine. Energy & Fuels. 2014;28:3377-90.

[252] Mehl M, Chen JY, Pitz WJ, Sarathy SM, Westbrook CK. An Approach for Formulating Surrogates for Gasoline with Application toward a Reduced Surrogate Mechanism for CFD Engine Modeling. Energy & Fuels. 2011;25:5215-23.

[253] Mueller CJ, Cannella WJ, Bruno TJ, Bunting B, Dettman HD, Franz JA, et al. Methodology for Formulating Diesel Surrogate Fuels with Accurate Compositional, Ignition-Quality, and Volatility Characteristics. Energy & Fuels. 2012;26:3284-303.

[254] Yuan H, Chen Z, Zhou Z, Yang Y, Brear MJ, Anderson JE. Formulating gasoline surrogate for emulating octane blending properties with ethanol. Fuel. 2020;261:116243.

[255] Yu J, Ju Y, Gou X. Surrogate fuel formulation for oxygenated and hydrocarbon fuels by using the molecular structures and functional groups. Fuel. 2016;166:211-8.

[256] Jeswani HK, Chilvers A, Azapagic A. Environmental sustainability of biofuels: a review. Proc Math Phys Eng Sci. 2020;476:20200351-.

[257] Anitescu G, Bruno T. Liquid Biofuels: Fluid Properties to Optimize Feedstock Selection, Processing, Refining/Blending, Storage/Transportation, and Combustion. Energy & Fuels. 2011;26:324–48.

[258] Keasling JD. Manufacturing molecules through metabolic engineering. Science (New York, NY). 2010;330:1355-8.

[259] Peralta-Yahya PP, Ouellet M, Chan R, Mukhopadhyay A, Keasling JD, Lee TS. Identification and microbial production of a terpene-based advanced biofuel. Nat Commun. 2011;2:483-.

[260] Akita H, Watanabe M, Suzuki T, Nakashima N, Hoshino T. Characterization of the Kluyveromyces marxianus strain DMB1 YGL157w gene product as a broad specificity NADPH-dependent aldehyde reductase. AMB Express. 2015;5:17.

[261] Peralta-Yahya PP, Zhang F, del Cardayre SB, Keasling JD. Microbial engineering for the production of advanced biofuels. Nature. 2012;488:320-8.

[262] Harvey BG, Meylemans HA, Gough RV, Quintana RL, Garrison MD, Bruno TJ. High-density biosynthetic fuels: the intersection of heterogeneous catalysis and metabolic engineering. Physical Chemistry Chemical Physics. 2014;16:9448-57.

[263] Zargar A, Bailey CB, Haushalter RW, Eiben CB, Katz L, Keasling JD. Leveraging microbial biosynthetic pathways for the generation of 'drop-in'biofuels. Current opinion in biotechnology. 2017;45:156-63.

[264] Ledesma-Amaro R, Nicaud JM. Yarrowia lipolytica as a biotechnological chassis to produce usual and unusual fatty acids. Progress in lipid research. 2016;61:40-50.

[265] Santos-Merino M, Garcillán-Barcia MP, de la Cruz F. Engineering the fatty acid synthesis pathway in Synechococcus elongatus PCC 7942 improves omega-3 fatty acid production. Biotechnology for Biofuels. 2018;11:239.

[266] Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB. Microbial biosynthesis of alkanes. Science (New York, NY). 2010;329:559-62.

[267] Chen L, Li Y, Zhang Q, Wang D, Akhberdi O, Wei D, et al. Improved pestalotiollide B production by deleting competing polyketide synthase genes in Pestalotiopsis microspora. Journal of industrial microbiology & biotechnology. 2017;44:237-46.

[268] Lal D. Microbes to generate electricity. Indian J Microbiol. 2013;53:120-2.