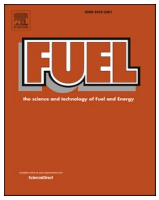
Fuel 316 (2022) 123330

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel



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

Victor Okoro [a], Ulugbek Azimov [a,*], Jose Munoz [b]

[a] Faculty of Engineering and Environment, Northumbria University, NE1 8ST Newcastle upon Tyne, United Kingdom
[b] Faculty of Health and Life Sciences, Northumbria University, NE1 8ST Newcastle upon Tyne, United Kingdom

ARTICLE INFO

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

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.

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 microbes to improve biofuels yields and production of energy carrying or new fuel molecules (next generation fuels) from microalgae and

* Corresponding author.
E-mail address: ulugbek.azimov@northumbria.ac.uk (U. Azimov).

https://doi.org/10.1016/j.fuel.2022.123330
Received 23 August 2021; Received in revised form 17 December 2021; Accepted 16 January 2022
Available online 25 January 2022

other microbes such as bacteria and yeast [1,4].

The use of microorganisms to produce renewable energy fuels can simultaneously resolve energy and ecological concerns [4]. Microbial fuels are renewable fuel molecules produced by microorganisms grown on different substrate and can supplement or/and in some instances replace conventional fuels [4–6]. Ogunkunle et al. [5] reviewed global current scenario of biodiesel adoption and combustion in vehicular diesel engines. Pater et al. [6] studied de novo and ex novo lipid fermentation by oleaginous yeast using glucose and sonicated waste cooking oil. To date, various feedstocks, metabolic engineering and production technologies have been utilized for the production of microbial liquid fuels through general metabolic routes as shown in Fig. 1. These pathways have failed to yield commercial scale volume of biofuels due to microbe strain complexity, complex biological processes and poor understanding of metabolic pathways. Increasing the production of biofuels in order to compete with fossil fuels is a major challenge, which synthetic biology is ameliorating.

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.

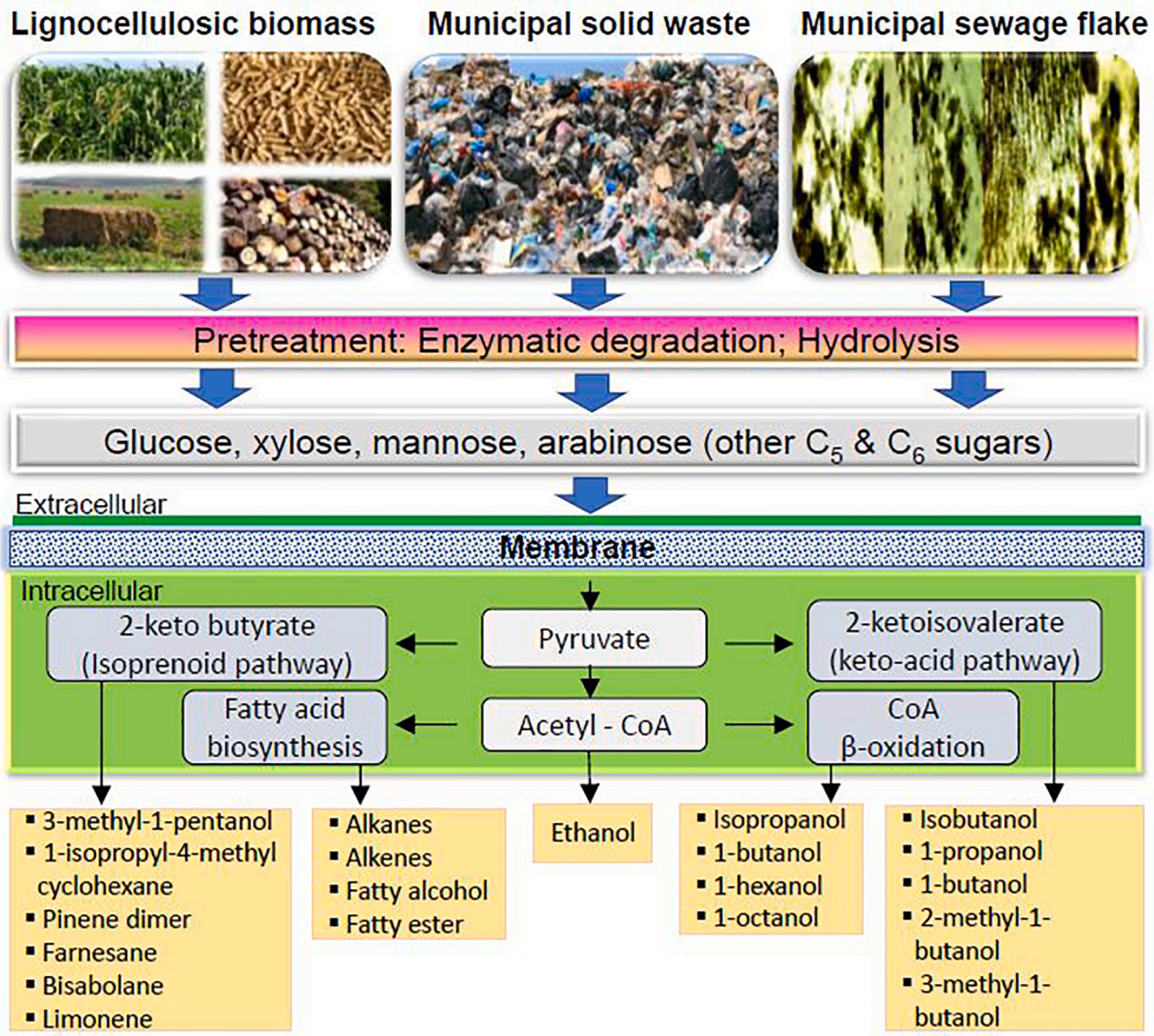


Fig. 1. Overview of pathways towards the production of microbial fuels. Extracellular: conventional lignocellulosic biomass, municipal solid waste and sewage flake substrates can be degraded enzymatically to produce C5 and C6 sugars that are utilized as carbon sources for microbial fuel production; Intracellular: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

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 engineered microbial platforms along with the engineering approaches employed. Das et al. [18] reviewed metabolic engineering for enhancing microbial biosynthesis of advanced biofuels. They provided a comprehensive outlook on the trends and developments in metabolic engineering strategies for advanced biofuel production using different hosts. Choi et al. [19] also reviewed metabolic engineering strategies toward production of biofuels. They discussed metabolic engineering strategies recently exploited to enhance biofuel production and facilitate utilization of non-edible low-value carbon sources. These strategies include engineering enzymes, exploiting new pathways, and systematically optimizing metabolism and fermentation processes, among others. They also discussed metabolic and bioprocess engineering strategies to achieve competitiveness of current biofuel production systems compared to those of fossil fuels. Carmona-Cabello et al. [20] investigated biodiesel production using microbial lipids derived from food waste discarded by catering services. Their study demonstrated that food industry waste from the hospitality sector can be used for biodiesel production via microbial oil production. Suitability of food waste to produce an alternative fermentation medium for microbial oil production in two steps (solid-state fermentation using potato peels and food waste hydrolysis) has been demonstrated. All these studies have focused mainly on biological production of microbial fuels and never considered challenges associated with application. 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.

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.

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

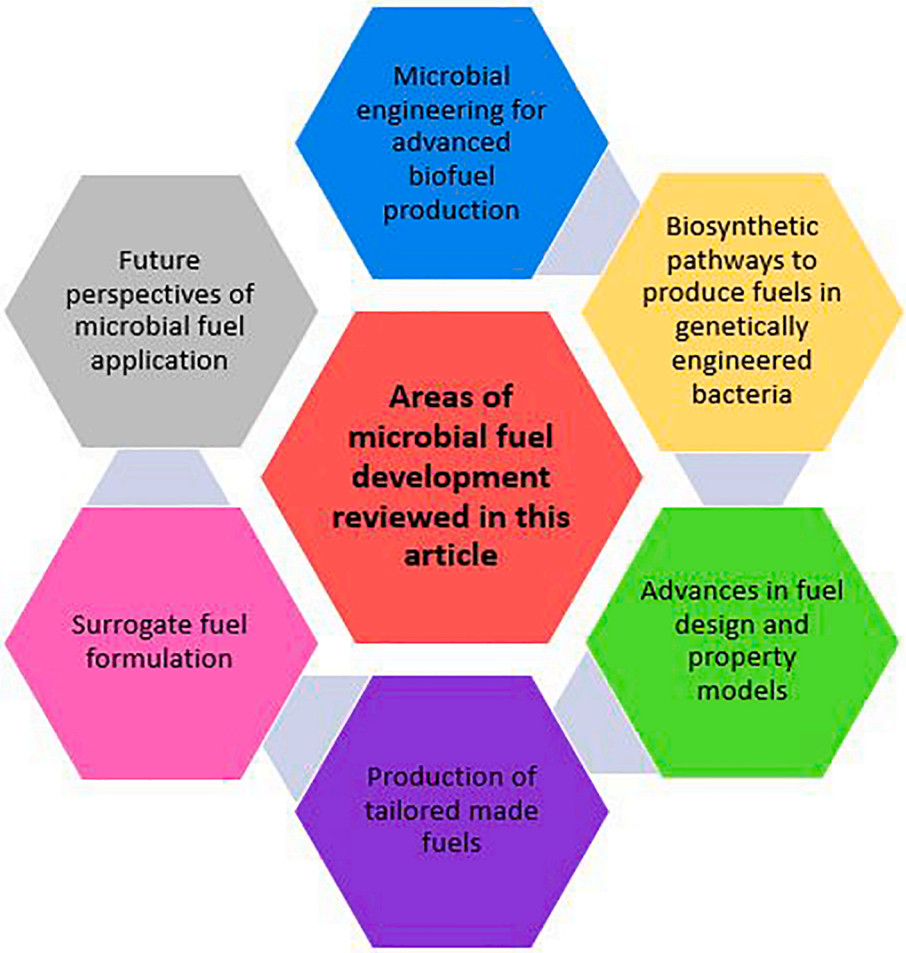


Fig. 2. Overview structure of this review article.

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 acceptance by consumers. To achieve this, it will be necessary to provide more effective community education and to ensure greater transparency regarding the legal provisions governing approval of GMOs for biotech applications. Cravens et al. [29] described recent developments in metabolic engineering at the level of host, pathway, and enzyme, and discussed how the field is approaching ever more complex biosynthetic opportunities.

Two reasons to employ synthetic biology for metabolic engineering in microorganisms are specificity and ease of genetic manipulations. In metabolic engineering, the host's metabolic pathways are modified to improving endogenous metabolic pathways or to initiate exogenous pathways to enhance the production of desired microbial fuel precursors. In addition, synthetic biology can also be used for microbe strain development for enhanced cell factories, metabolic pathway reconstruction and process regulation and optimization [24,30].

The challenges incurred in building cells into resourceful factories are due to incomplete annotated microbe metabolic networks, lack of understanding of genomic regulatory networks and complex interaction between molecular pathways that impede attempts to divert resources [21,31].

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

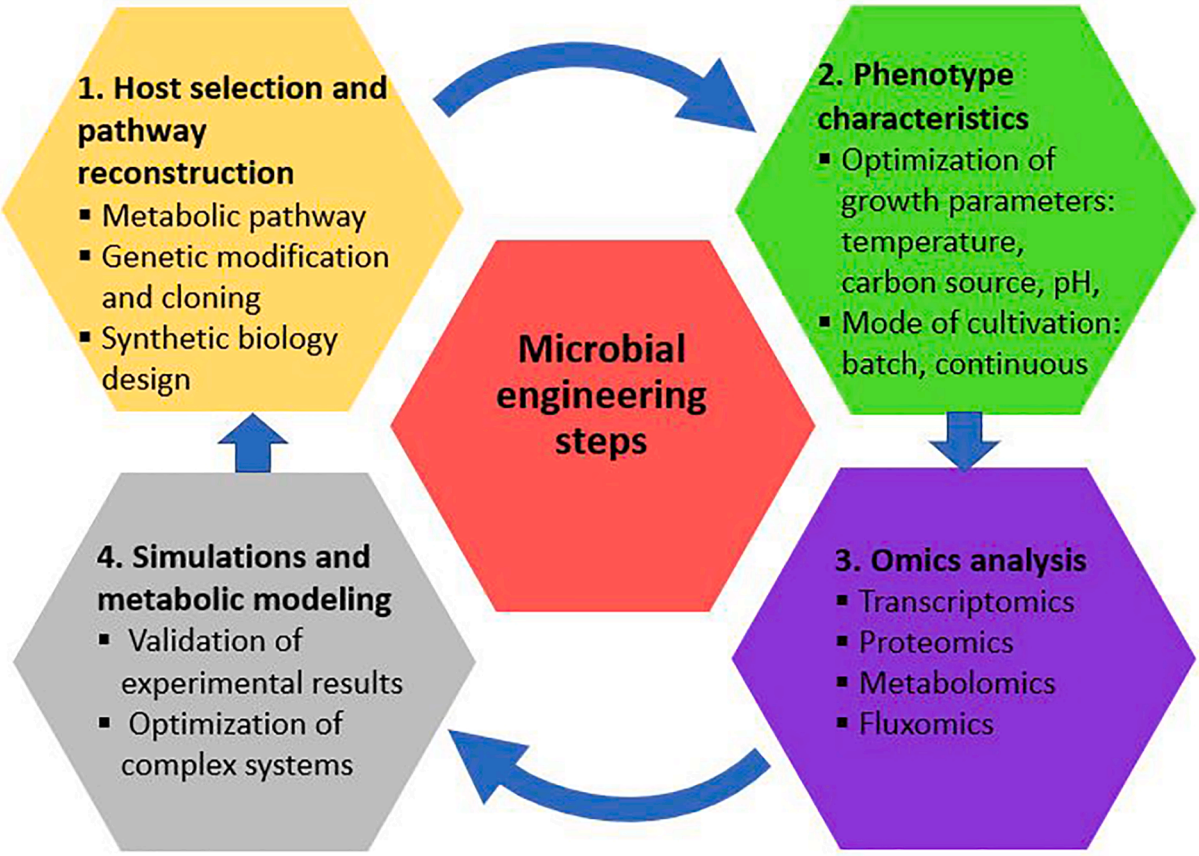


Fig. 3. Schematic display of microbial engineering steps: 1. Host selection and development of engineered or improved host towards yielding targeted biofuel; 2. Culturing the engineered host by simple optimization of growth parameters and conditions; 3. Analysis of complex and modified pathways generated 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.

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

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, depending of the product generated by the metabolic pathway, we could choose between several examples in both types [45]. When selecting a host, we need to consider the product(s) we would like to produce, the feedstocks available, and the inherent metabolic pathways of the host; we could use a native host, when the natural metabolic pathway is present but the molecular tolls have not

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.

been developed, and non-native host, when we have the genetic tools available to engineer the above metabolic pathway but sometimes they need to overlap with stress problems.

2.2.2. Genetic tools developed for biofuel production

Several methods have been described for the manipulation of the genomes in the selected host, but they could be further classified into DNA assembly and Genome-editing techniques [46]. A cornerstone of synthetic biology, DNA assembly uses multiple DNA fragments and physically linking them together. We could include into this section on-step PCR, Gibson assembly, BioBricks or golden gate [47-49]. On-step PCR would be the ligation of several genes into one genetic construct by overlap extension PCR.

Gibson Assembly was first used by Gibson et al. in 2009 to assemble the artificial genome of *Mycoplasma genitalium* to overcome the difficulties in assembling the 600,000 bp genome [48]. This method uses three different enzymes; exonuclease, DNA polymerase, and DNA ligase, to join multiple DNA fragments into a single DNA strand [49,50]. Gibson Assembly is a single reaction technique that facilitates the assembly or deletion of several parts into the genome. Gibson Assembly has several advantages over other DNA assembly methods in that there is no need for a restriction digest site in the sequence of interest, it uses fewer reagents and steps, and has no remaining unwanted DNA, or scars, between the ligated fragments. This strategy was successfully used by Gao et al. [51] to assemble the ß-carotene pathway 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 production of desired high value products.

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

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

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

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

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	Enhanced 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]

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].

The 2-ketoacid pathway to higher alcohols has been used due to the fact they are intermediates in amino acid biosynthesis pathways. The 2-ketoacids undergo decarboxylation, forming aldehydes or ketones, which are further metabolized to alcohols by alcohol dehydrogenases. Atsumi et al. [76] took advantage of this native pathway and introduced 2 non-native enzymes into *E. coli* to produce alcohols. The authors used six different 2-ketoacids, produced their respective alcohol derivatives. Another strategy to produce higher alcohols has been the elimination of competitive metabolic pathways [77]. This has been used to increase the titers of 1-butanol in microbes by preventing the key precursor, acetyl-CoA, from being shunted into competitive pathways. This approach was used to delete the enzyme acetate kinase in *Clostridium tyrobutylicum* to decrease the acetyl-CoA conversion into acetate, which led to a nine-fold increase in 1-butanol titer [77,78]. In a separate work, Saini et al. deleted the native *frdA* subunit of fumarate reductase, lactate dehydrogenase, aldehyde-alcohol dehydrogenase and phosphate acetyltransferase genes in *E. coli* and introduced the acetoacetyl-CoA transferase and butyraldehyde-butanol dehydrogenase genes from *Clostridium spp*. to produce 1-butanol using glucose and butyrate as carbon sources [78,79]. A strategy of upscaling the production of higher alcohols is the reducing cofactors imbalance. Matsuda et al. [80] increased the production of isobutanol in an isobutanol producing *S. cerevisiae* strain from 0.23 g/l to 1.62 g/l by introducing a shunt pathway to maintain the NADP + to NADPH ratio in the cell as well as deletion of contending pathways. Combining these techniques clearly improved product yield but titer is still low. Hence, optimizing the process could lead to commercial production of isobutanol, as compared to 1-butanol, isobutanol has a comparable energy content and a higher-octane number.

Other non-clostridial host, such as *E. coli, S. cerevisiae* and *S. elongatum* have recently been used to produce 1-butanol [73,80]. Shen et al. [81] constructed a modified clostridial 1-butanol pathway in *Escherichia coli* to provide an irreversible reaction catalyzed by *trans*-enoyl-coenzyme A (CoA) reductase (Ter) and created NADH and acetyl-CoA driving forces to direct the flux. They achieved high-titer (30 g/liter) and high-yield (70 to 88% of the theoretical) production of 1 butanol anaerobically, comparable to or exceeding the levels demonstrated by native producers. Steen et al. [74] engineered *Saccharomyces cerevisiae* with an n-butanol biosynthetic pathway, in which isozymes from a number of different organisms (*S. cerevisiae*, *Escherichia coli*, *Clostridium beijerinckii*, and *Ralstonia eutropha*) were substituted for the *Clostridial enzymes* and their effect on n-butanol production was compared. By choosing the appropriate isozymes, they were able to improve production of n-butanol ten-fold to 2.5 mg/L. Dekishima et al. [82] investigated metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. To produce 1-butanol from CO2, they transferred a modified CoA-dependent 1-butanol production pathway into a cyanobacterium, *Synechococcus elongatus* PCC 7942, and demonstrated the activity of each enzyme in the pathway by chromosomal integration and expression of the genes.

Production of 1-butanol was also accomplished by introducing the clostridial CoA-dependent reversed oxidation pathways into these microorganisms. Dekishima et al. [82] produced 1-hexanol from 1-butanol by heterologous expression of the β-ketothiolase (BktB) from *Ralstonia eutropha* and the 3-hydroxybutyryl-CoA dehydrogenase and crotonase *C. acetobutylicum* in *E. coli*. The isoprenoid pathway is the typical platform to produce diesel-like fuels. In this pathway, monoterpenes or sesquiterpenes are converted into limonene or farnesene through the mevalonate or the D-xylulose-5- phosphate pathways [83]. These biodiesel-like fuels are close to be commercialized into the jet fueling. Therefore, these isoprenoids-type biofuels are an excellent biosource for the diesel and jet fuel substitute because their chemical properties are very similar to the traditional fossil fuels.

2.2.4. Omics analysis

Recent advances in multiple omics tools, such as genomics, transcriptomics or proteomics, has facilitated the global analysis of the genotype to phenotype association in engineered and non-engineered microorganism [84]. The new “omics” era is driving our understanding of the regulation and interconnectedness of biological processes through the ability to simultaneously analyze DNA, RNA, proteins and metabolites extracted from the same experimental samples. In this sense, next generation sequencing of nucleic acids has facilitated the identification of cryptic metabolic pathways in new improved strains decreasing dramatically the cost of engineered microorganisms. RNA microarrays can detect the transcriptome expression and mass spectroscopy can obtain the metabolite profile of strains of interest. Finally, we can obtain glycome and lipidome through LC/MS, GC/MS, NMR and flux analysis [84].

These techniques are being used more frequently due to the lower cost for analysis and more robust analysis models. For example, *Synechocystis* sp. PCC6803 has been analyzed using high throughput transcriptomics to detect small RNA molecules in response to light or substrate depletion stress [85,86]. In addition, the same strain was analyzed with proteomics to understand the proteins involved in the redirection of carbon flow *Tricoderma reseei*, a well-characterized fungi used in the cellulose conversion into biofuel, has been fully explored and

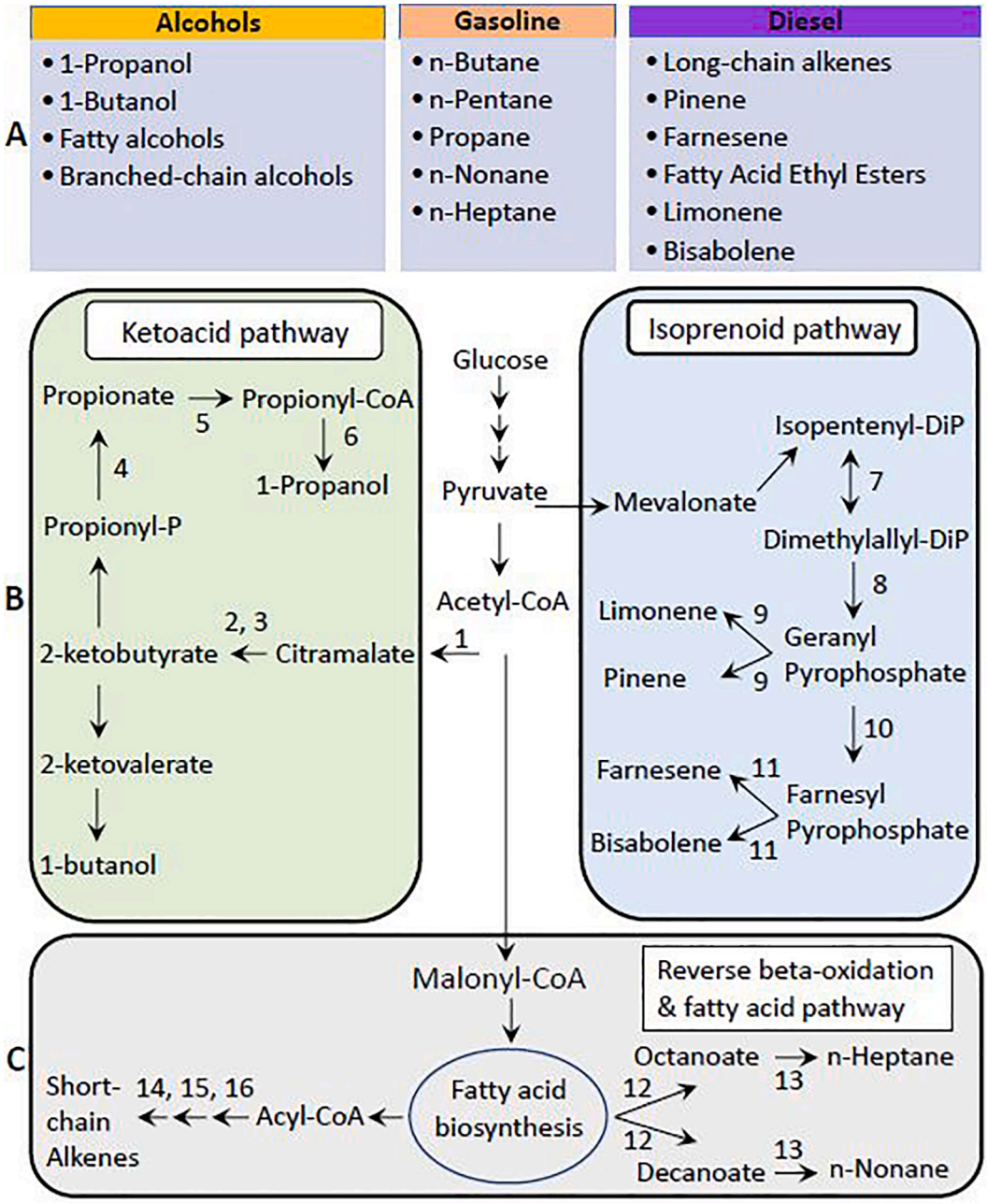


Fig. 4. Main bioenergy carrying molecules and metabolic pathways generated in biosynthetic fuels. This figure was made by authors using information from [9,74,75]. **A.** Molecules according to the fuel category; orange-alcohols; pink-gasoline, and purple-diesel. **B.** Metabolic pathways involved in 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**, acyl-ACP thioesterase I; **15**, acyl-CoA synthetase and **16**, aldehyde deformylating oxygenase (or aldehyde decarbonylase.

characterized with the help of advanced proteomics to correlate the regulation of gene transcripts (transcriptomics) with protein expression (proteomics) and generate novel strategies to maximize this conversion with a most cost-effective way [87,88].

2.2.5. *Simulation and metabolic modeling*

Omics techniques have produced a large set of metadata on the microorganism genomes and their potential engineering for industrial use. This has led to the development of biological databases, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) [89], Biochemical Genetic and Genomic (BiGG) [90] and ENZYME [91], which act as repositories for use with bioinformatics tools. The mining of these databases by bioinformatics tools has proven indispensable to predicting potential metabolic routes toward production of novel biofuels. For example, The *Y. lipolytica*'s genome has been mined in order to understand potential metabolic pathways involved in this lipid synthesis [92].

The construction of genome-scale models (GEM) is vital to simulating *in vitro* the cellular behavior under physiological and stress environments. Pan and Hua [93] reconstructed a metabolic network in this yeast using genome annotation and the biochemical databases previously listed to predict the growth in minimal media with some carbon sources. This helped in the knowledge of new carbon uses degradation

by *Y. lipolytica*.

All this data provides GEMs with the necessary data for the evolution of metabolic prediction algorithms, with the recent introduction of enzymatic constraints. These new model algorithms increase the accuracy of current models by taking into consideration the kinetic parameters of enzymes involved in the metabolic pathways along with the relative concentration of secondary metabolites observed in the cellular environment [94].

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 [95]. 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 [96-98].

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 [99]. Lignocellulose is composed of cellulose and hemicellulose as carbohydrate component, tightly packed by phenol aldehyde lignin polymer [100]. The predigestion 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. [101] used laccase from *Picnoporus cinnabarinus* to pretreat wheat straw obtaining a 60% increase in glucose yield. In addition, Asgher et al. [102] 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 [103]. 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) [104]. 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 [105]. 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 [106].

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	[115]
Cellulases	Cellulose	Cellobiose inhibition decrease	[116,117]
β-glucosidases	Cellulose	Glucose inhibition decrease	[116,117]
Xylosidases	hemicellulose	Xylose inhibition decrease	[116,117]
cellobiohydrolases	Cellulose	Cellobiose inhibition decrease	[118]
endoglucanases	Cellulose	Cellobiose inhibition decrease	[118]
CBM-endoglucanase	Cellulose	Higher cellulose affinity	[107]
Enzymatic cocktails			
Laccase, Lignin and Manganese Peroxidase mix	Lignin	34% delignification obtaining 16 g/l ethanol	[102]
Pectinases, Laccases LPMOs mix	Cellulose and chitin	122 g/l and 80% glucose release from cellulose	[107]
Multicomplex cellulosome	Cellulose	Higher cellulose affinity and catalytic efficiency	[113,119,120]

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 [107]. 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. [108] 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* [109] or the more complex in *Ruminococcus flavefaciens* [110] or *Pseudo)Bacteroides cellulosolvens* [111]. Recent efforts have been made to synthetize recombinant cellulosome composed of modules from different organism, including thermostable or salt tolerant bacteria or fungi [112,113]. This proximity strategy naturally employed by bacteria has been employed as a cellulosome design approach using a combinatorial set of enzymes, including auxiliary LPMOs, as

biotechnological nanomachines [114].

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 as 'surrogate fuel formulation' [121,122]. Villeda et al. [121] developed a model-based fuel design methodology which is based on an integrated product and process design approach, considering aspects of both fuel combustion and fuel production. They aimed at identifying possible fossil fuel surrogates from a database of rigorously generated molecular structures. Li et al. [122] developed surrogate formulation methodology for biodiesel based on chemical deconstruction in consideration of molecular structure and engine combustion factors. Tailored-made biofuels are fuels made from biomasses that are upgraded to exhibit certain desired properties for high efficiency and clean combustion [123]. It has been reported that the production and utilization of biofuels/oxygenates have several advantages such as carbon neutrality, renewability, clean and high combustion efficiency, low sulfur and aromatic content [124,125]. The presence of 10–45% oxygen distinguishes biofuels from fossil fuels [126]. However, some drawbacks like emission of toxic nitrogen oxides and PM, limited infrastructures, high fuel consumption and less energy content have been observed from various literature [127,128]. Attempts have been made to enhance the efficiency and emission related drawbacks associated with the use of biofuels through model-based design of TMFB [129]. Reports of advances in model-based design of some novel fuels are scanty.

In this section, we will identify various pathways for the production of different oxygenated fuel species, advances in model-based fuel design and demonstrate the model-base formulation of single fuel species, multi-species and various blends (multiple fuel species) of four new fuel species such as 2-pentanol, cyclopentanol, ethyl furfuryl ether and 5-(ethoxymethly)furfuryl amongst others. The effects of the properties of these fuels on engine performance will be 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 [123]. 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 [130,131]. vom Stein et al [130] 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. [131] 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 [132]. Den et al. [133] 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. [134] 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 [135].

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 [136]. 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. [137] 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. [138] 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 pre-treatment 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 various biomasses can either be used as carbon source for microbial cell factories, as mentioned earlier, or catalytically reconfigured to yield biofuels [139].

Many enhanced clean combustible tailor-made fuels and fuel intermediates could be produced from C5 and C6 sugars sourced from diverse biomasses/feedstock by simple bond-breaking and bond-formation processes through selective hydrogenation, dehydration, hydrolysis, esterification and other new processes like ethanolysis and use of novel catalysts for catalytic transformations [140,141]. For the sake of simplicity, the routes of catalytic production of various platform chemicals, leading to the formation of novel oxygenated fuels utilizing C6 and C5, and each chemical process/reaction and corresponding chemical structure have been outlined in Fig. 5 and Table 4 respectively. The processes leading to the production of hydrocarbon fuels, especially from ϒ-valerolactone were excluded since our focus lies strictly on chemical processes to produce oxygenated fuels, and identifying the physical, chemical and combustion characteristics of these new fuels. The overview of various biomasses that can be converted into various oxygenated fuels and fuel platforms is shown in Fig. 5.

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 [144]. 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 [145,146]. Furfural which exists in large quantity in hemicelluloses, and mainly produced by hydrogenation and dehydration of xylose can be converted into novel biofuels such as cyclopentanol, 2-pentanol and ethyl furfuryl ethers, and other biofuel producing chemical platforms. Li et al. [144] reviewed the conversion of furfural to C4 and C5 chemicals by various catalytic processes. They divided the chemical products from furfural into several groups according to their carbon numbers and synthesis routes, with emphasis on the catalysts and reaction mechanisms. Ma et al. [147] prepared series of cobalt catalysts with different supports for the selective conversion of biomass-derived furfural to cyclopentanol in one step. Zhao et al. [148] studied the conversion of furfuryl alcohol into ethyl levulinate over glucose-derived carbon-based solid acid in ethanol.

Most of these fuels are yet to be tested in IC engines. Apart from chemo-catalyst process, pathways for direct production of these biofuels, bypassing the intermediate products can engender faster and easier production process. Fig. 6 shows various steps/pathways required to produce tailored-made biofuels from various feedstocks. Notably, intermediate fuel molecules are hardly chosen from bulky database of physiochemical properties and are therefore prone to be neglected. These intermediates can also be upgraded into incredible variety of molecular structures which could be tested as fuel species. Produced tailored biofuels and their blends with other fuels are investigated and validated through experimentations in IC engines [149].

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

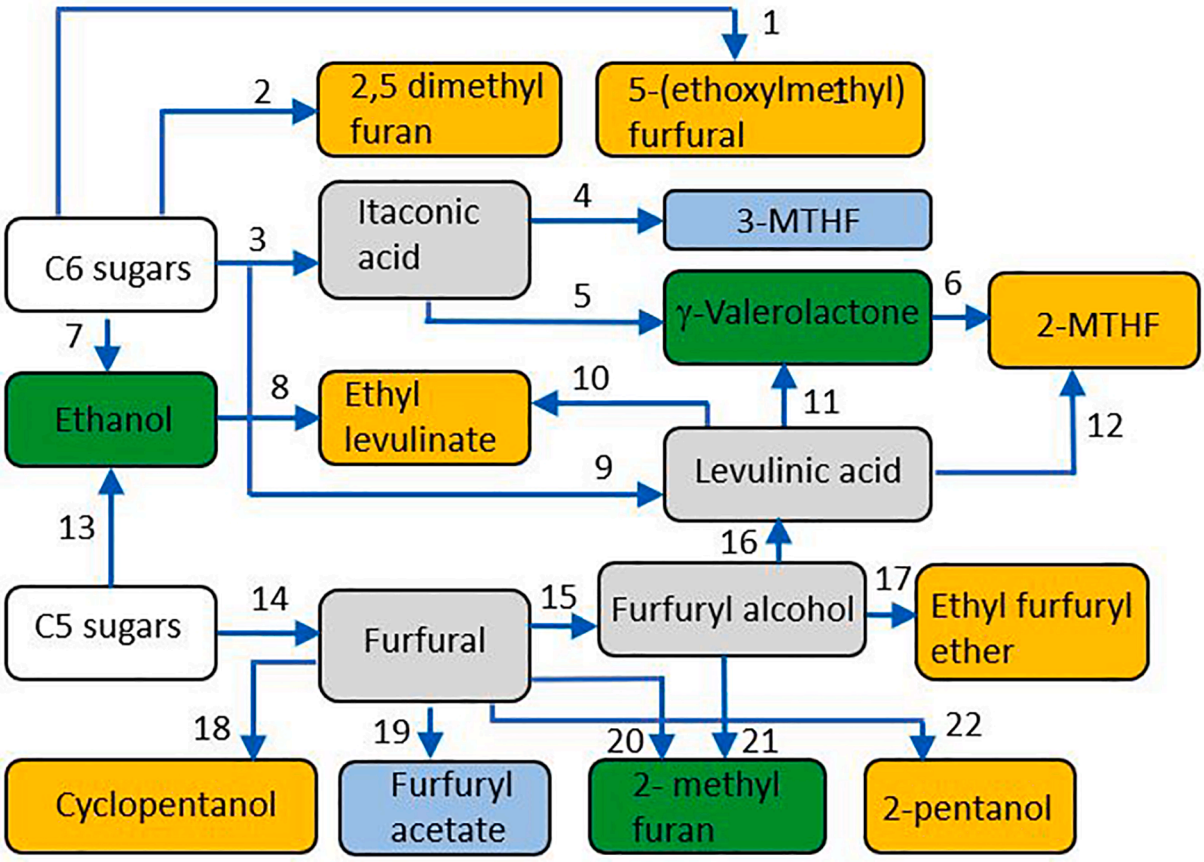
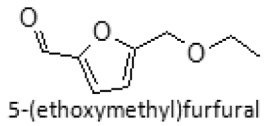
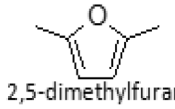
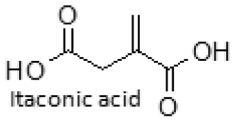
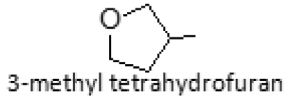
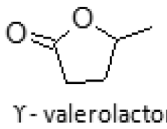
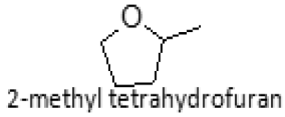
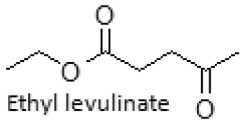
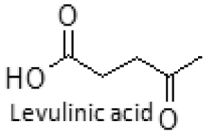
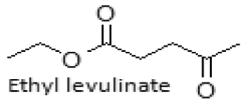
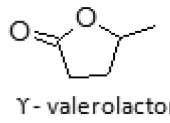
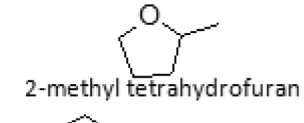


Fig. 5. Pathways leading to the production of new fuel molecules using C6 and C5 sugar sources. This figure was made by authors using information from [136,142-144]. For convenience, only pathways/routes are outlined in Fig. 5 and chemical processes with references are outlined in Table 4. Each color 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.

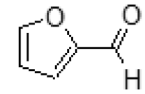
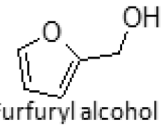
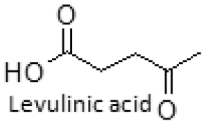
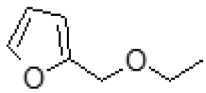
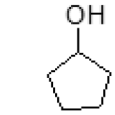
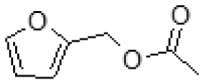
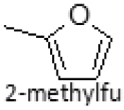
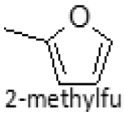
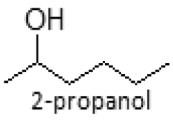
Table 4
Chemical processes to produce intermediate and fuel molecules.

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	[154]
2	Dehydration and Hydrogenolysis	Ru/15CuZr catalysts, 200^{O}C, 15 bar of hydrogen, 5.5 hrs (biofuel)	 2,5-dimethylfuran	45.6	[155]
3	Fermentation	Produced from *Aspergillus terreus* fungal strain from glucose (intermediate)	 Itaconic acid	26.3[a]	[156]
4	Hydrogenation	Pd − Re/C catalysts, 200^{O}C, 1000 psig H_2, 24 hrs (potential biofuel)	 3-methyl tetrahydrofuran	80	[157]
5	Hydrogenation	(Nanoparticle catalysis): $Ru_3(CO)_{12}$, 190 °C, syngas ($2H_2$/CO)100 bar, 20hrs (intermediate and biofuel)	 Υ- valerolactone	70	[158]
6	Hydrogenation and dehydration	Ru/C + zeolite HY, 200^{O}C, 3 MPa H_2, 46 hrs (biofuel)	 2-methyl tetrahydrofuran	88.8	[159]
7	Fermentation	Electrostatic fermentation of *S. cerevisiae* from glucose (intermediate and biofuel)	 Ethanol	12.3	[160]
8	Esterification	Amberlyst-15 and sulfated SnO_2 catalysts, 70^{O}C, 5 hrs (biofuel)	 Ethyl levulinate	56	[161]
9	Dehydration and Hydrolysis	Fe/HY zeolite catalysts, agitation speed 200 rpm, 180^{O}C, 240 mins (intermediate)	 Levulinic acid	66	[162]
10	Esterification	Amberlyst-15 and sulfated SnO_2 catalysts, 70^{O}C, 5 hrs (biofuel)	 Ethyl levulinate	56	[163]
11	Hydrogenation	Ni/NiO catalyst, 110^{O}C, 40 bar pressure (intermediate and biofuel)	 Υ- valerolactone	94	[164]

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	[165]
13	Fermentation	*S. passalidarum* CMUWF1–2, 30^{O}C (intermediate and biofuel)	 Ethanol	43	[166]

(*continued on next page*)

Table 4 (*continued*)

Process number	Process type	Description	Structure of product	Max. Yield	Ref.
14	Dehydration	Arenesulfonic SBA-15 catalysts, 160^{O}C, 15 bar, 200 rpm, 20 hrs (intermediate)	 Furfuryl	80	[167]
15	Hydrogenation	N-doped carbon encapsulated Co catalysts (Co-N-C), 150 °C, 6 hrs (intermediate)	 Furfuryl alcohol	99	[168]
16	Hydrolysis	$ArSO_3H$-Et-HNS catalyst, 120^{O}C, 120 min. (intermediate)	 Levulinic acid	81.3	[169]
17	Etherification	$H_1Cs_2PW_{12}O_{40}$ catalyst, 130^{O}C, stirring speed 800 rpm, 2.5 hrs (biofuel)	 Ethyl furfuryl ether	65	[170]
18	Hydrogenation and Hydrolysis	Cobalt, supported on ZrO_2-La_2O_3, 160^{O}C, 2 MPa H_2, (biofuel)	 Cyclopentanol	82	[171]
19	Hydrogenation and Esterification	$RHSiO_2$-Cu-Al-Mg catalyst, 150^{O}C, at 40 bar (potential biofuel)	 Furfuryl acetate	24.5	[172]
20	Hydrogenation	Ni-Cu/Al_2O_3 catalyst, 210^{O}C, 4 hrs (intermediate and biofuel)	 2-methylfuran	75.6	[173]
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	[174]
22	Hydrogenation	Co–Cu/Al_2O_3 catalyst, 240 °C, 45 bar H_2 pressure, 12 hrs (biofuel)	 2-propanol	71.1	[175]

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

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. [149] 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. [150] 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. [151] 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 [152,153], which are also considered for the identification of fuel molecules from a database of millions of fuel chemicals through computational and experimental approaches.

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

The chemical structures of fuel were shown to have direct influence

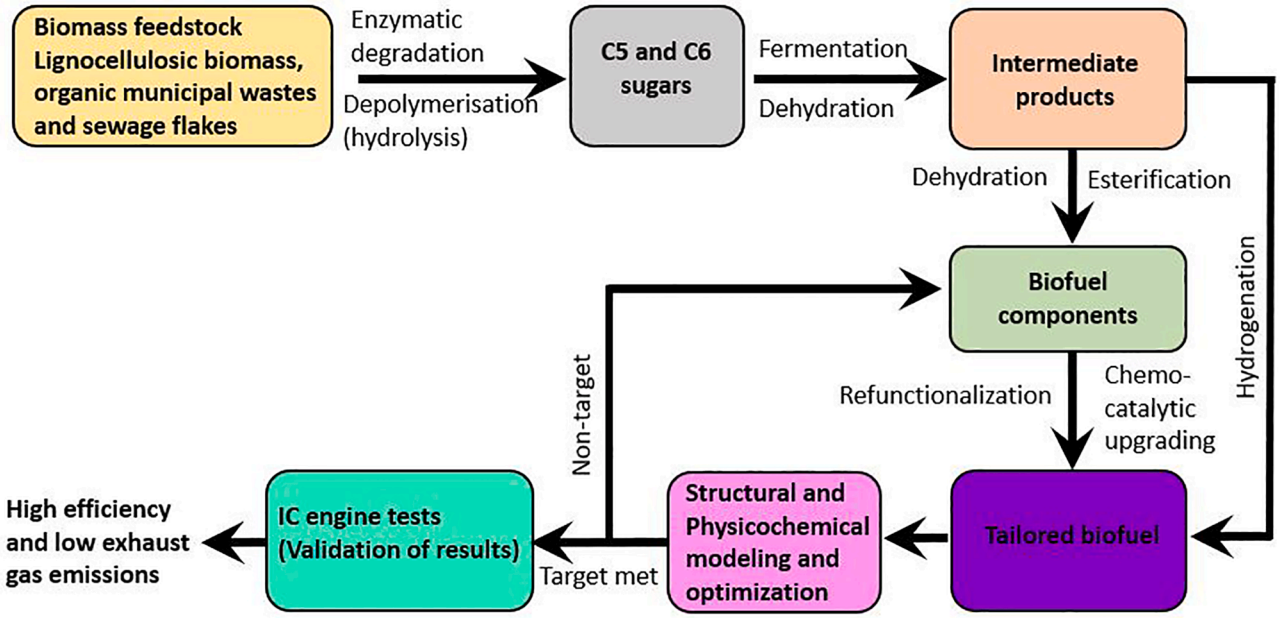


Fig. 6. Overview of biofuel production pathway from biomass and municipal wastes tailored for clean combustion. This figure was made by authors using information from [123].

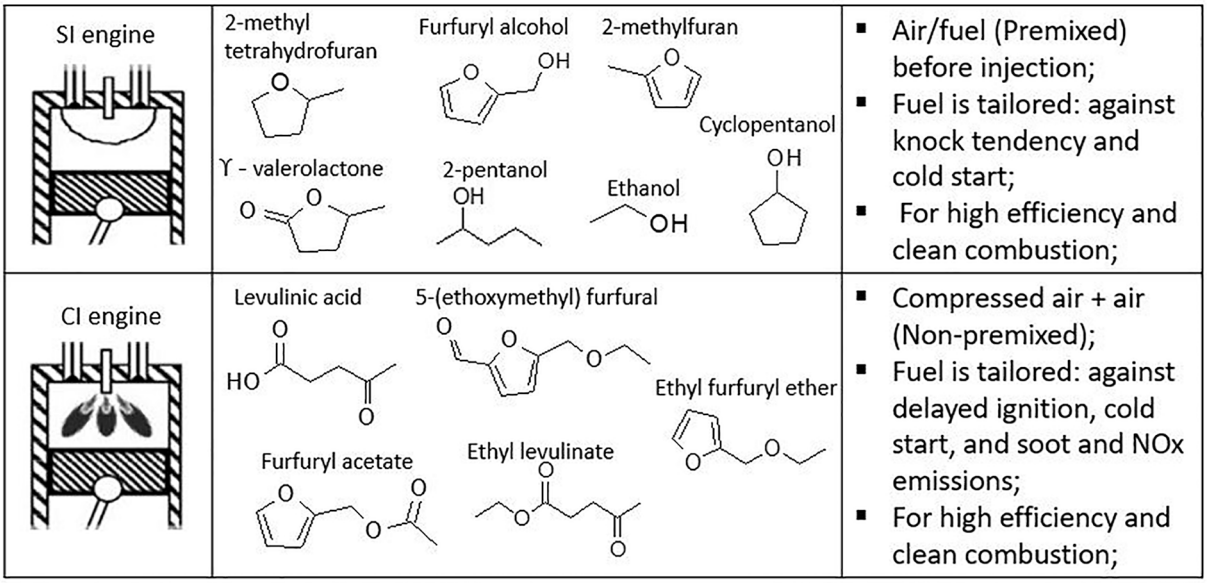


Fig. 7. Promising pure/single fuel species combustible in spark ignition (SI) and compression ignition (CI) engines, depending on molecular structure, functional group, and physicochemical properties such as cetane/octane numbers and volatility. Fuels of lengthy alkyl seemed to perform better in CI engines. This figure was made by authors using information from [14,123].

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 [176-178]. 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 [179] 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 [180]. 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

Table 5
Physicochemical properties of various oxygenates compared to AGO and PMS. Note: **AGO**: diesel fuel ($C_{14}H_{24}$) [180-182]; **PMS**: gasoline (C_8H_{18}) [180,183-185]; **ETH**: ethanol (C_2H_6O) [180,182,184,186]; **VAL**: ϒ-valerolactone ($C_5H_8O_2$) [187,188]; **2MF**: 2-methylfuran (C_5H_6O) [184,187,189,190]; **ELE**: ethyl levulinate ($C_7H_{12}O_3$) [191-193]; **2,5 DMF**: 2,5-dimethylfuran (C_6H_8O) [184,194,195]; **2 MTHF**: 2-methyl tetrahydrofuran ($C_5H_{10}O$) [189,196]; **EFE**: ethyl furfuryl ether ($C_7H_{10}O_2$) [190,193]; **EMF**: ethoxy methyl furfural ($C_8H_{10}O_3$) [197,198]; **CPNTL**: cyclopentanol ($C_5H_{10}O$) [190,199,200]; **PNTL**: 2-pentanol ($C_5H_{12}O$) [200-203]; 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

biofuels in transportation (internal combustion engines), power generation systems and heating sectors, as it devalues the efficiency of biofuels and promotes the dominance of the existing diesel and gasoline transport infrastructures [181,182].

Ideally, the properties of future fuels may differ from those of fossil fuels, thus the established fossil-based fuels standards should not be a measure for defining the performance of future fuels. Therefore, to maximize the full potential of existing and emerging future fuels, the effects of fuel properties such as density, viscosity, oxygen content, boiling point, cetane/octane numbers and enthalpy of vaporization of these fuels and their blends in premix and non-premix engine modes of combustion need to be exhaustive studied.

As can be seen in Table 5, oxygenated fuels are denser (higher specific gravity) than conventional hydrocarbon fuels. Density is the mass per unit volume of fuel injected into the cylinder for combustion. It varies inversely to volume; hence the density of injected fuel into the cylinder could be regulated by the injector control system through fuel volume regulation and not by mass [204]. Density, viscosity and surface tension are related in physical nature, and can be affected by temperature. At the same temperature, fuel of higher density tends to have higher viscosity and surface tension, and these properties greatly influence fuel injection and tribology characteristics, atomisation and efficiency [205]. Some authors have reported that lowering the values of these fuel parameters could lead to the production of fuel of better spray characteristics and atomization, while others attributed better fuel atomization, evaporation and spray characteristics to the integration of high-pressure injection nozzle as part of engine accessory. However, the later attribute is key to the investigation of spray characteristics of oxygenates [206-209]. In general, increases in fuel density increases viscosity, leading to the injection of larger fuel droplets that can result in incomplete combustion. Consequently, efficiency decreases as NOx and PM increase [210].

Fuel cetane/octane number and ignition temperature plays key roles in the smooth and efficient running of IC engines. Cetane number is an index used in measuring the combustion quality of fuel in a diesel engine. Higher cetane number can cause quick ignition tendency of injected fuel [180]. Injection of high cetane number fuel into the cylinder of a CI engine (non-premixed) requires instant auto-ignition before the end of compression stroke to maximize power output. Thus, high cetane number and lower ignition temperature are key conditions for enhanced fuel combustion efficiency in CI engines. It is worth noting that fuels of higher cetane number can cause quick ignition tendency leading to high power output, but fuel of lower cetane number could increase ignition delay, leading to a more homogenous air/fuel mixture, which will in turn reduce soot formation (by reducing fuel rich mixture zone) and particulate matter PM [211,212]. This trade-off needs to be balanced through fuel design process and optimization. There exists an ignition delay database of pure single fuel molecules generated from automated ignition quality tester (IQT) and structural group contribution model used in predicting ignition delay of new fuel species [213].

Conversely, in SI engines, premixed air/fuel mixtures do not need to auto-ignite, even at the end of the compression stroke, as the spark plug ignites the compressed mixture [214]. Setting the correct ignition timing is necessary to prevent engine knock, backfiring, wasted spark and excessive vibration [215]. Also, the availability of end gas (air/fuel mixture region not ignited) which can later ignite due to high temperature and pressure of the cylinder environs can lead to the occurrence of knock. Octane number measures the ability of fuel to resist knocking. Fuel of higher-octane number has a better anti-knock quality [216,217]. Octane number and cetane number vary inversely even though the interplay between octane number and auto-ignition is still unclear [218]. Fuel of high-octane number and low cetane will have a high knock resistance characteristic when run in SI engine and hardly auto-ignite as in the case of gasoline [219]. Fuel with high cetane number and low octane number is prone to quick auto-ignition. Looking at Table 5, most oxygenated fuels therein have higher-octane numbers when compared with gasoline and appreciable values of cetane number. As a result, oxygenated fuels can be run in SI, CI and other engines of various combustion mode, as well as serve as better additives for fuel blending.

Other properties such as oxygen content and boiling point are also critical to engine performance and exhaust gas emissions [220]. The presence of oxygen content differentiates 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 [221]. 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 [222,223]. 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 [224]. 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 [14,220], leading to more efficient combustion, thereby cutting-down toxic exhaust gas emissions in IC engines. Donahue et al. [225] 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 [225]. 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 energy content observed in oxygenated biofuels ought not to be a major concern because oxygenated biofuels have lower air–fuel ratio than fossil fuels. Consequently, with increased air volume, the resulting in-cylinder energy could be higher using ethanol vs. PMS in SI engines [226]. More so, the effects of the number of carbons to oxygen bond (whether single or double) has been studied. They show that biofuels of single carbon to oxygen ratio such as alkanols are more ignitable and effective in reducing soot emission than those of double bonds like alkanones or ketones [220,222,227]. The effects of having the presence of both single and double carbon to oxygen bonds in biofuels such as biodiesel and ethyl levulinate have not been studied.

The boiling point of fuel is one of the key properties that determines fuel storage feasibility, IC engine operation (vaporization and combustion) and the extent of particulate deposits [228]. In SI engines, fuel of low boiling point tends to have shorter liquid penetration length, diminishing problems associated with wall wetting and fuel-in-oil dilution. It has been reported that fuel of low boiling point can reduce particulate matter emissions with increased NOx [229]. High boiling point and enthalpy of vaporization of oxygenated fuels can lead to engine operation difficulty in low ambient temperature, but after the engine warm-up phase, high enthalpy of vaporization, filling efficiency and knock resistance are enhanced [223]. Comparatively, the CI engines burn less volatile fuel when compared with SI. Apparently, wall wetting, and fuel-in-oil dilution abound if fuel particles are large. To alleviate these concerns, modern fuel injection methods are employed to achieve fuel combustion at low temperature and reduced soot and NO_X emissions [230,231]. Fisher et al. [230] emphasized that a quantitative understanding of liquid-phase penetration for biodiesel fuels is needed to help mitigate the accelerated dilution of engine lubrication oil with unburned fuel. They reported liquid penetration lengths measured in an optical engine under time-varying in-cylinder conditions for soy- and cuphea-derived biodiesel fuels. Dec [231] emphasized the importance of achieving highly diluted and well-premixed charge to achieve low emissions and reviewed the principles of HCCI and diesel LTC engines along with the effects of in-cylinder fuel injection processes. The physicochemical properties of single pure oxygenated fuels may not resolve all energy and ecological concerns linked with the use of conventional fossil fuels. At times, single species fuels fail to exhibit all the necessary properties, thus, the model could also be used to predict the properties of a mixture of multiple fuel blend species [232]. Also, public demand may not be met due to insufficient fuel production volume. Going forward, these pressing issues could be alleviated through target-oriented fuel design, which involves blending different categories of liquid fuels and fuel molecules to produce blended fuels of unique and desired properties through the application of fuel design concepts. By this, biofuels and blends could be modified to desired physicochemical properties that could 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.

4. Surrogate fuel formulation

Real fuels such as gasoline, diesel and jet aviation are composed of variable, complex mixtures of chemical components. Thus, numerical simulations of their combustion remain challenging. The use of surrogate fuel as an attractive option to circumvent this problem has been reviewed in various literature [233-235]. A surrogate fuel ought to be a blend of a limited number of chemical components that exhibits some physicochemical properties of interest [236]. 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 [237].

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 [238-242]. 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 [243]. 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 [244]. 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 [245].

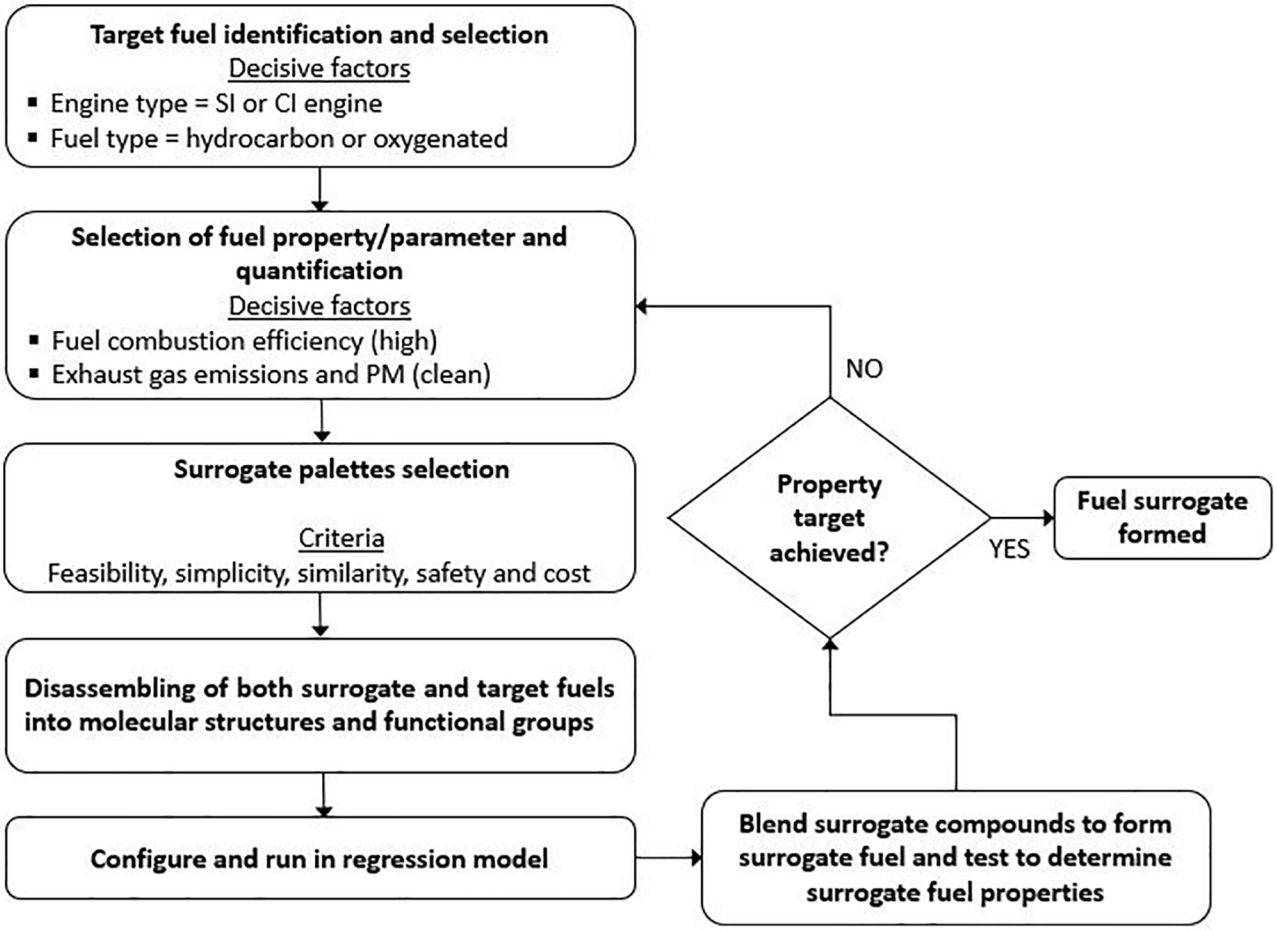


Fig. 8. General overview of surrogate fuel formulation, considering molecular structure and functional groups, with factors/criteria considered in each step. This figure was made by authors using information from [245].

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 those of surrogate fuel. It is important to select a target fuel compatible with test engine in order to avoid much error due to technical limitations to testing the surrogate fuel formed. For instance, hydrocarbon fuels such as AGO and PMS run better in CI and SI engines respectively due to the availability of necessary engine accessories and mode of combustion. As a result, engine and fuel types are key factors considered in the process of target fuel selection. Otherwise, the formed surrogate fuel would be tested in every available engine type to demonstrate its suitability and efficiency across each engine type and mode of combustion.

Secondly, the molecular structures and functional groups of the selected target fuel which the surrogate fuel is expected to mimic should fall within an acceptable tolerance. Studies have emphasized the importance of molecular structure and functional group of fuel in its oxidative and pyrolytic combustion chemistries [246]. The combustion chemistry of a fuel of single molecular structure has shown a robust relationship between the molecular structure, physicochemical properties and combustion performances in diverse experimental and numerical settings. Notably, ignition delay duration decreases as the alkyl straight chain increases. The presence of unsaturated carbon bonds and structural isomerism (alkyl branching) can increase ignition delay. The effect of the position of the double bond and the presence of oxygen for the oxygenated fuels depends on the overall molecular structure of the fuel [247,248].

Thirdly, compounds of surrogate palettes are selected. No palette compound is perfect, but some palettes are preferable to others. The selected surrogate palette compounds criteria include feasibility (available kinetic mechanism), similarity (sooting tendency, volatility, and combustion properties), simplicity (fewer number of carbons), safety, cost and more than 98% pure [245,249]. 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 [245,250]. 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 [251]. 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 [252]. 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 important to fuel is restricted [253,254]. These include derivations of the amino acid pathway to produce isobutanol [255], the mevalonate pathway to produce farnesene [22,256], the polyketide pathway to produce a variety of fuel molecules [257] and the fatty acid pathway to produce fatty acid methyl esters [258,259]. These native pathways are found in many different micro-organisms [260,261] and can be genetically manipulated or placed into a simple host to increase an organism's biosynthesis capacity for a specific bioenergy product. Renewable energy technologies are becoming more widely available across the world as a result of their maturing maturity and lower cost structure. However, large-scale electrical energy storage and retrieval would almost likely be required to enhance the penetration of renewable sources into the grid. The perfect combination of high power and energy density, low economic and environmental costs, lack of site limitations, long period and calendar lifetime, convenient supply of materials, and quick reaction time is required to ensure rapid and effective penetration of renewable energy technologies into the grid. Engineered microorganisms might solve many of the problems of present energy storage systems by permitting rewired carbon fixation, a process that spatially splits reactions generally done jointly in a photosynthetic cell and replaces the least effective with biological counterparts. Microbial or enzymatic carbon dioxide fixation and subsequent delivery of materials as carbon-based energy storage molecules, if effective, will allow high-density storage of renewable energy, including hydrocarbons and non-volatile polymers.

5.3. Application of energy molecules and microbial fuels in built environment

Bioenergy application in built environments, like energy conversion and storage, is a rapidly growing field. However, there is little research on the application of microbial populations in constructed settings. Lal et al. [262] revived the idea of using microorganisms in buildings and 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This work was funded by the Royal Society grant for the International Exchanges Scheme (Project number IES\R2\192108).

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. Sci Total Environ 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 Rep 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. Ultrason Sonochem 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. Front Mar Sci 2020;7.
[8] Hollinshead W, He L, Tang YJ. Biofuel production: an odyssey from metabolic engineering to fermentation scale-up. Front Microbiol 2014;5.
[9] Peralta-Yahya PP, Keasling JD. Advanced biofuel production in microbes. Biotechnol J 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. J Cleaner Prod 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. Front Mech Eng 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. Proc Inst Mech Eng Part D: J Automob Eng 2018;232:90–105.
[15] Keasling J, Garcia Martin H, Lee TS, Mukhopadhyay A, Singer SW, Sundstrom E. Microbial production of advanced biofuels. Nat Rev Microbiol 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. Bioresour Technol 2021;337:125510.
[18] Das M, Patra P, Ghosh A. Metabolic engineering for enhancing microbial biosynthesis of advanced biofuels. Renew Sustain Energy Rev 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. Bioresour Technol 2020;323:124597.
[21] Kumar R, Kumar P. Future microbial applications for bioenergy production: a perspective. Front Microbiol 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 Syst Biol Appl 2016;2:1–11.
[24] Yuan S-F, Alper HS. Metabolic engineering of microbial cell factories for production of nutraceuticals. Microb Cell Fact 2019;18:46.
[25] Liao JC, Mi L, Pontrelli S, Luo S. Fuelling the future: microbial engineering for the production of sustainable biofuels. Nat Rev Microbiol 2016;14:288–304.
[26] Mukhopadhyay A. Tolerance engineering in bacteria for the production of advanced biofuels and chemicals. Trends Microbiol 2015;23:498–508.
[27] Ostergaard S, Olsson L, Nielsen J. Metabolic engineering of Saccharomyces cerevisiae. Microbiol Mol Biol Rev 2000;64:34–50.
[28] Nevoigt E. Progress in metabolic engineering of Saccharomyces cerevisiae. Microbiol Mol Biol Rev 2008;72:379–412.
[29] Cravens A, Payne J, Smolke CD. Synthetic biology strategies for microbial biosynthesis of plant natural products. Nat Commun 2019;10:1–12.
[30] Gustavsson M, Lee SY. Prospects of microbial cell factories developed through systems metabolic engineering. Microb Biotechnol 2016;9:610–7.
[31] Balan V. Current challenges in commercially producing biofuels from lignocellulosic biomass. Int Scholar Res Notices 2014;2014.
[32] Kopf M, Hess WR. Regulatory RNAs in photosynthetic cyanobacteria. FEMS Microbiol Rev 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. Front Bioeng Biotechnol 2019;7.
[35] Wang F, Zhang W. Synthetic biology: recent progress, biosafety and biosecurity concerns, and possible solutions. J Biosafety Biosecurity 2019;1:22–30.
[36] Young E, Alper H. Synthetic biology: tools to design, build, and optimize cellular processes. Biomed Res Int 2010;2010.
[37] Nielsen J, Keasling JD. 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. Curr Opin Syst Biol 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 Biotechnol 2016;34:652–64.
[40] Bosi E, Bacci G, Mengoni A, Fondi M. Perspectives and Challenges in Microbial Communities Metabolic Modeling. Front Genet 2017;8.
[41] Fondi M, Liò P. Multi -omics and metabolic modelling pipelines: Challenges and tools for systems microbiology. Microbiol Res 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. Biotechnol Adv 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. Microb Cell Fact 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. Microb Biotechnol 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. Biotechnol Biofuels 2019;12:1–20.
[47] Gaj T, Sirk SJ, Shui S-l, Liu J. Genome-editing technologies: principles and applications. Cold Spring Harbor Perspect Biol. 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. Microb Biotechnol 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. Nat Methods 2009;6:343–5.
[50] Gibson DG. Enzymatic assembly of overlapping DNA fragments. Methods Enzymol Elsevier 2011:349–61.
[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. Biotechnol Lett 2014;36:2523–8.
[52] Xu X, Liu Y, Du G, Ledesma-Amaro R, Liu L. Microbial chassis development for natural product biosynthesis. Trends Biotechnol 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. J Biol Eng 2008;2:5.
[56] Yamazaki K-i, de Mora K, Saitoh K. BioBrick-based 'Quick Gene Assembly' in vitro. Synth Biol 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. Metab Eng 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. Front Bioeng Biotechnol 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. Metab Eng 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. Biotechnol 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. Nat Biotechnol 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. Nat Chem Biol 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. Metab Eng 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. Biotechnol 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. Biochem Genet 2019;57:443–54.
[69] Gérando HM, 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. Appl Microbiol Biotechnol 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. Sci Rep. 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. Biotechnol 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. Curr Opin Chem Biol 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. Microb Cell Fact 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] Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 2008;451:86–9.
[77] Toogood HS, Scrutton NS. Retooling microorganisms for the fermentative production of alcohols. Curr Opin Biotechnol 2018;50:1–10.
[78] Yu M, Zhang Y, Tang I-C, Yang S-T. Metabolic engineering of Clostridium tyrobutyricum for n-butanol production. Metab Eng 2011;13:373–82.
[79] Saini M, Chen MH, Chiang C-J, Chao Y-P. Potential production platform of n-butanol in Escherichia coli. Metab Eng 2015;27:76–82.
[80] 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.
[81] Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC. Driving forces enable high-titer anaerobic 1-butanol synthesis in Escherichia coli. Appl Environ Microbiol 2011;77:2905–15.
[82] 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. J Am Chem Soc 2011;133:11399–401.
[83] Gupta P, Phulara S. Metabolic engineering for isoprenoid-based biofuel production. J Appl Microbiol 2015;119:605–19.
[84] 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: J Integr Biol 2013;17:537–49.
[85] Palazzotto E, Weber T. Omics and multi-omics approaches to study the biosynthesis of secondary metabolites in microorganisms. Curr Opin Microbiol 2018;45:109–16.
[86] 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.
[87] 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. Biotechnol Biofuels 2015;8:111.
[88] 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 Biotechnol 2016;34:970–82.
[89] Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017;45:D353–61.
[90] 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 Res 2020;48:D402–6.
[91] Bairoch A. The ENZYME database in 2000. Nucleic Acids Res 2000;28:304–5.
[92] Larroude M, Rossignol T, Nicaud JM, Ledesma-Amaro R. Synthetic biology tools for engineering Yarrowia lipolytica. Biotechnol Adv 2018;36:2150–64.
[93] Pan P, Hua Q. Reconstruction and in silico analysis of metabolic network for an oleaginous yeast, Yarrowia lipolytica. PloS one 2012;7:e51535.
[94] 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. Mol Syst Biol 2017;13:935.
[95] Cho S, Shin J, Cho B-K. Applications of CRISPR/Cas System to Bacterial Metabolic Engineering. Int J Mol Sci 2018;19:1089.
[96] Blin K, Pedersen LE, Weber T, Lee SY. CRISPy-web: An online resource to design sgRNAs for CRISPR applications. Synth Syst Biotechnol 2016;1:118–21.
[97] 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.
[98] Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nat Protoc 2013;8:2180–96.
[99] Zoghlami A, Paës G. Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. Front Chem 2019;7:874.
[100] 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. Sci Total Environ 2020;743:140630.
[101] Rencoret J, Pereira A, José C, Martínez AT, Gutiérrez A. Laccase-mediator pretreatment of wheat straw degrades lignin and improves saccharification. Bioenergy Res 2016;9:917–30.
[102] Asgher M, Ahmad Z, Iqbal HMN. Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production. Ind Crops Prod 2013;44:488–95.
[103] C-wC H, Cannella D, Jørgensen H, Felby C, Thygesen LG. Cellulase Inhibition by High Concentrations of Monosaccharides. J Agric Food Chem 2014;62:3800–5.
[104] 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 Biochem 2016;51:1561–7.
[105] 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. Biotechnol Bioeng 2004;86:503–11.
[106] 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. Biotechnol Adv 2010;28:407–25.
[107] 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. Bioresour Technol 2019;292:121993.
[108] 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.
[109] Lamed R, Setter E, Bayer EA. Characterization of a cellulose-binding, cellulase-containing complex in Clostridium thermocellum. J Bacteriol 1983;156:828–36.
[110] 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.
[111] 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. Biotechnol Biofuels 2019;12:115.
[112] Kahn A, Moraïs S, Galanopoulou AP, Chung D, Sarai NS, Hengge N, et al. Creation of a functional hyperthermostable designer cellulosome. Biotechnol Biofuels 2019;12:1–15.
[113] Gilmore SP, Lillington SP, Haitjema CH, de Groot R, O'Malley MA. Designing chimeric enzymes inspired by fungal cellulosomes. Synth Syst Biotechnol 2020;5:23–32.
[114] Arfi Y, Shamshoum M, Rogachev I, Peleg Y, Bayer EA. Integration of bacterial lytic polysaccharide monooxygenases into designer cellulosomes promotes enhanced cellulose degradation. Proc Natl Acad Sci 2014;111:9109–14.
[115] 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. Biotechnol Biofuels 2019;12:159.
[116] Hsieh CW, Cannella D, Jørgensen H, Felby C, Thygesen LG. Cellulase inhibition by high concentrations of monosaccharides. J Agric Food Chem 2014;62:3800–5.
[117] 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 Biochem 2016;51.
[118] 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. Biotechnol Bioeng 2004;86:503–11.
[119] Eibinger M, Ganner T, Plank H, Nidetzky B. A Biological Nanomachine at Work: Watching the Cellulosome Degrade Crystalline Cellulose. ACS Cent Sci 2020;6:739–46.
[120] Kahn A, Moraïs S, Galanopoulou AP, Chung D, Sarai NS, Hengge N, et al. Creation of a functional hyperthermostable designer cellulosome. Biotechnol Biofuels 2019;12:44.
[121] 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. Springer, Berlin Heidelberg: Berlin, Heidelberg; 2015. p. 193–211.
[122] 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. Combust Flame 2019;199:152–67.
[123] Dahmen M, Marquardt W. Model-Based Design of Tailor-Made Biofuels. Energy Fuels 2016;30:1109–34.
[124] 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. Front Microbiol 2016;7:694.
[125] Datta A, Hossain A, Roy S. An Overview on Biofuels and Their Advantages and Disadvantages. Asian J Chem 2019;31.
[126] Demirbas A. Combustion efficiency impacts of biofuels. Energy Sources Part A 2009;31:602–9.

[127] 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. Microchem J 2014;117:94–9.
[128] Baskar P, Senthilkumar A. Effects of oxygen enriched combustion on pollution and performance characteristics of a diesel engine. Eng Sci Technol Int J 2016;19:438–43.
[129] McLinden M, Bruno T, Frenkel M, Huber M. Standard reference data for the thermophysical properties of biofuels. Biofuels: ASTM International; 2011.
[130] vom Stein T, Klankermayer J, Leitner W. Tailor-Made Fuels and Chemicals from Biomass. 2013.
[131] Palkovits R, Delidovich I. Efficient utilization of renewable feedstocks: the role of catalysis and process design. Philos Trans Royal Soc A: Math Phys Eng Sci 2018;376:20170064.
[132] 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.
[133] 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. Front Chem 2018;6:141.
[134] Guczi L, Erdôhelyi A. Catalysis for alternative energy generation. Springer Science & Business Media; 2012.
[135] Wilson K, Lee A, Dacquin J-P. Catalysis for Alternative Energy Generation 2012:263–304.
[136] 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.
[137] Dornau A, Robson JF, Thomas GH, McQueen-Mason SJ. Robust microorganisms for biofuel and chemical production from municipal solid waste. Microb Cell Fact 2020;19:1–18.
[138] Bharathiraja DB, Yogendran D, Kumar RR, Muninathan C, Subramani P. Biofuels from sewage sludge- A review. Int J Chem Tech Res 2014;6:4417–27.
[139] Alonso DM, Bond JQ, Dumesic JA. Catalytic conversion of biomass to biofuels. Green Chem 2010;12:1493–513.
[140] Liu B, Zhang Z. Catalytic conversion of biomass into chemicals and fuels over magnetic catalysts. ACS Catal 2016;6:326–38.
[141] 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.
[142] Isikgor F, Becer R. Lignocellulosic Biomass: A Sustainable Platform for Production of Bio-Based Chemicals and Polymers. Polym Chem 2015;6.
[143] Mariscal R, Maireles-Torres P, Ojeda M, Sádaba I, López GM. Furfural: a renewable and versatile platform molecule for the synthesis of chemicals and fuels. Energy Environ Sci 2016;9:1144–89.
[144] Li X, Jia P, Wang T. Furfural: A Promising Platform Compound for Sustainable Production of C4 and C5 Chemicals. ACS Catal 2016;6:7621–40.
[145] 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. Res Chem Intermed 2020.
[146] 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 Chem 2017;19:2482–90.
[147] 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. Chin Chem Lett 2017;28:1153–8.
[148] 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.
[149] 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 Int J Fuels Lubr 2011;5:106–22.
[150] Hellier P, Ladommatos N. The influence of biodiesel composition on compression ignition combustion and emissions. Proc Inst Mech Eng Part A: J Power Energy 2015;229:714–26.
[151] 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.
[152] 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..
[153] Kalghatgi G, Stone R. Fuel requirements of spark ignition engines. Proc Inst Mech Eng Part D: J Automob Eng 2018;232:22–35.
[154] Li H, Saravanamurugan S, Yang S, Riisager A. Direct transformation of carbohydrates to the biofuel 5-ethoxymethylfurfural by solid acid catalysts. Green Chem 2016;18:726–34.
[155] 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. Ind Eng Chem Res 2018;57:11535–46.
[156] 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 Biomass Valoriz 2020;11:1069–75.
[157] Abdelrahman OA, Park DS, Vinter KP, Spanjers CS, Ren L, Cho HJ, et al. Renewable isoprene by sequential hydrogenation of itaconic acid and dehydradecyclization of 3-methyl-tetrahydrofuran. ACS Catal 2017;7:1428–31.
[158] Gowda RR, Chen EYX. Regioselective Hydrogenation of Itaconic Acid to γ-Isovalerolactone by Transition-Metal Nanoparticle Catalysts. ChemSusChem 2019;12:973–7.
[159] 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.
[160] 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.
[161] Fernandes D, Rocha A, Mai E, Mota CJ, Da Silva VT. Levulinic acid esterification with ethanol to ethyl levulinate production over solid acid catalysts. Appl Catal A 2012;425:199–204.
[162] Ramli NAS, Amin NAS. Kinetic study of glucose conversion to levulinic acid over Fe/HY zeolite catalyst. Chem Eng J 2016;283:150–9.
[163] 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.
[164] 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 Chem 2019;21:606–13.
[165] 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 Microbiol 2018;18:73.
[166] Agirrezabal-Telleria I, Requies J, Güemez M, Arias P. Dehydration of d-xylose to furfural using selective and hydrothermally stable arenesulfonic SBA-15 catalysts. Appl Catal B 2014;145:34–42.
[167] 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 Process Technol 2020;197:106205.
[168] Arjunan S. Microalgae Harvesting via Flocculation: Impact of PH, Algae Species and Biomass Concentration. 2018.
[169] 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.
[170] 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. Appl Catal A 2017;547:248–55.
[171] Aldosari OF. Selective conversion of furfuryl alcohol to 2-methylfuran over nanosilica supported Au: Pd bimetallic catalysts at room temperature. J Saudi Chem Soc 2019;23:938–46.
[172] Seemala B, Kumar R, Cai CM, Wyman CE, Christopher P. Single-step catalytic conversion of furfural to 2-pentanol over bimetallic Co–Cu catalysts. React Chem Eng 2019;4:261–7.
[173] 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.
[174] P-q TAN, J-y ZHAO, Z-y HU, D-m LOU, A-m DU, D-m DU. Effects of fuel properties on exhaust emissions from diesel engines. J Fuel Chem Technol 2013;41:347–55.
[175] 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.
[176] Ritchie H, Roser M. Fossil fuels. Our World in Data 2017.
[177] 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.
[178] Hassan MH, Kalam MA. An Overview of Biofuel as a Renewable Energy Source: Development and Challenges. Procedia Eng 2013;56:39–53.
[179] Hassan MH, Kalam MA. An overview of biofuel as a renewable energy source: development and challenges. Procedia Eng 2013;56:53.
[180] Kumar N, Sonthalia A, Pali H. Alternative Fuels for Diesel Engines: New Frontiers. 2018.
[181] Rajamohan S, Kasimani R, Ramakrishnan P, Shameer PM. A review on the properties, performance and emission aspects of the third generation biodiesels. Renew Sustain Energy Rev 2017.
[182] No S-Y. Utilization of Pentanol as Biofuels in Compression Ignition Engines. Front Mech Eng 2020;6.
[183] Paloboran M, Sutantra I, Sudarmanta B. Performances and Emissions Characteristics of Three Main Types Composition of Gasoline-Ethanol Blended in Spark Ignition Engines. Int Rev Mech Eng (IREME) 2016;10:552.
[184] 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. Appl Energy 2014;132:317–24.
[185] 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.
[186] El-Faroug MO, Yan F, Luo M, Fiifi TR. Spark ignition engine combustion, performance and emission products from hydrous ethanol and its blends with gasoline. Energies 2016;9:984.
[187] Yan K, Wu G, Lafleur T, Jarvis C. Production, properties and catalytic hydrogenation of furfural to fuel additives and value-added chemicals. Renew Sustain Energy Rev 2014;38:663–76.
[188] 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. Nat Resour 2014;5:177–91.

[189] 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.
[190] Natsir TA, Shimazu S. Fuels and fuel additives from furfural derivatives via etherification and formation of methylfurans. Fuel Process Technol 2020;200:106308.
[191] Unlu D, Boz N, Ilgen O, Hilmioglu N. Improvement of fuel properties of biodiesel with bioadditive ethyl levulinate. Open Chem 2018;16:647–52.
[192] Ahmad E, Alam MI, Pant K, Haider MA. Catalytic and mechanistic insights into the production of ethyl levulinate from biorenewable feedstocks. Green Chem 2016;18:4804–23.
[193] 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.
[194] 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.
[195] 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.
[196] 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. In: Internal Combustion Engine Division Fall Technical Conference: American Society of Mechanical Engineers; 2016. p. V001T02A10.
[197] de Jong E, Furanics G-J. A Novel Diesel Fuel with Superior Characteristics. SAE Technical Papers 2009.
[198] Malinowski A, Wardzińska D. Catalytic conversion of furfural towards fuel biocomponents. Chemik 2012;66:982–90.
[199] Zhao L, Ye D, Guo Y, Fang W. Density and Viscosity of Ternary Mixture of Cyclopentanol+ exo-Tetrahydrodicyclopentadiene+ 1, 3-Dimethyladamantane. J Chem Eng Data 2019;64:2558–67.
[200] 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.
[201] Hechinger M, Voll A, Marquardt W. Towards an integrated design of biofuels and their production pathways. Comput Chem Eng 2010;34:1909–18.
[202] 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. J Chem Eng Data 2018;63:2438–50.
[203] 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 Int J Fuels Lubr 2017;10:442–60.
[204] 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.
[205] 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.
[206] Groendyk MA, Rothamer D. Effects of fuel physical properties on auto-ignition characteristics in a heavy duty compression ignition engine. SAE Int J Fuels Lubr 2015;8:200–13.
[207] 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.
[208] 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.
[209] Agarwal AK, Dhar A, Gupta JG, Kim WI, Choi K, Lee CS, et al. Effect of Fuel Injection Pressure and Injection. Appl Energy 2014;130:212–21.
[210] Woo DG, Kim TH. Effect of kinematic viscosity variation with blended-oil biodiesel on engine performance and exhaust emission in a power tiller engine. Environ Eng Res 2019;25:946–59.
[211] Cataluna R, Da Silva R. Effect of cetane number on specific fuel consumption and particulate matter and unburned hydrocarbon emissions from diesel engines. J Combust 2012;2012.
[212] 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.
[213] 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.
[214] Kalghatgi G, Johansson B. Gasoline compression ignition approach to efficient, clean and affordable future engines. Proc Inst Mech Eng Part D: J Automob Eng 2018;232:118–38.
[215] 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. Appl Sci 2019;9:4842.
[216] Stradling R, Williams J, Hamje H, Rickeard D. Effect of octane on performance, energy consumption and emissions of two Euro 4 passenger cars. Transp Res Procedia 2016;14:3159–68.
[217] Kalghatgi GT. The outlook for fuels for internal combustion engines. Int J Engine Res 2014;15:383–98.
[218] 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.
[219] Cung KD, Ciatti SA, Tanov S, Andersson Ö. Low-Temperature Combustion of High Octane Fuels in a Gasoline Compression Ignition Engine. Front Mech Eng 2017;3.
[220] 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.
[221] Bergthorson JM, Thomson MJ. A review of the combustion and emissions properties of advanced transportation biofuels and their impact on existing and future engines. Renew Sustain Energy Rev 2015;42:1393–417.
[222] Westbrook CK, Pitz WJ, Curran HJ. Chemical kinetic modeling study of the effects of oxygenated hydrocarbons on soot emissions from diesel engines. J Phys Chem A 2006;110:6912–22.
[223] 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 Convers Manage 2016;108:243–54.
[224] Cheng AS, Dibble R, Buchholz B. The effect of oxygenates on diesel engine particulate matter. SAE Technical Papers 2002.
[225] 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 Trans 2000;334–50.
[226] 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 Int J Fuels Lubr 2012;5:274–88.
[227] 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.
[228] Nagano S, Yokoo N, Kitano K, Nakata K. Effects of high boiling point fuel additives on deposits in a direct injection gasoline engine. SAE Int J Fuels Lubr 2017;10:789–802.
[229] 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.
[230] 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.
[231] Dec JE. Advanced compression-ignition engines—understanding the in-cylinder processes. Proc Combust Inst 2009;32:2727–42.
[232] König A, Marquardt W, Mitsos A, Viell J, Dahmen M. Integrated design of renewable fuels and their production processes: recent advances and challenges. Curr Opin Chem Eng 2020;27:45–50.
[233] Piehl JA, Zyada A, Bravo L, Samimi-Abianeh O. Review of Oxidation of Gasoline Surrogates and Its Components. J Combust 2018;2018:8406754.
[234] 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.
[235] Pitz WJ, Mueller CJ. Recent progress in the development of diesel surrogate fuels. Prog Energy Combust Sci 2011;37:330–50.
[236] 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. Combust Flame 2012;159:1444–66.
[237] 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.
[238] 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.
[239] 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.
[240] Krishnasamy A, Reitz RD, Willems W, Kurtz E. Surrogate Diesel Fuel Models for Low Temperature Combustion. SAE International 2013.
[241] 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.
[242] Su X, Ra Y, Reitz R. A Surrogate Fuel Formulation Approach for Real Transportation Fuels with Application to Multi-Dimensional Engine Simulations. SAE Int J Fuels Lubr 2014;7:236–49.
[243] Ranzi E. A wide-range kinetic modeling study of oxidation and combustion of transportation fuels and surrogate mixtures. Energy Fuels 2006;20:1024–32.
[244] Marrero J, Gani R. Group-contribution based estimation of pure component properties. Fluid Phase Equilib 2001;183–184:183–208.
[245] 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.
[246] Sarathy SM, Farooq A, Kalghatgi GT. Recent progress in gasoline surrogate fuels. Prog Energy Combust Sci 2018;65:67–108.
[247] 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.
[248] 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.
[249] 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.
[250] 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.

[251] Jeswani HK, Chilvers A, Azapagic A. Environmental sustainability of biofuels: a review. Proc Math Phys Eng Sci. 2020;476:20200351-.
[252] 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.
[253] Keasling JD. Manufacturing molecules through metabolic engineering. Sci (New York, NY). 2010;330:1355–8.
[254] 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.
[255] 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.
[256] Harvey BG, Meylemans HA, Gough RV, Quintana RL, Garrison MD, Bruno TJ. High-density biosynthetic fuels: the intersection of heterogeneous catalysis and metabolic engineering. PCCP 2014;16:9448–57.
[257] Zargar A, Bailey CB, Haushalter RW, Eiben CB, Katz L, Keasling JD. Leveraging microbial biosynthetic pathways for the generation of 'drop-in'biofuels. Curr Opin Biotechnol 2017;45:156–63.
[258] Ledesma-Amaro R, Nicaud JM. Yarrowia lipolytica as a biotechnological chassis to produce usual and unusual fatty acids. Prog Lipid Res 2016;61:40–50.
[259] 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. Biotechnol Biofuels 2018;11:239.
[260] Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB. Microbial biosynthesis of alkanes. Sci (New York, NY) 2010;329:559–62.
[261] 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. J Ind Microbiol Biotechnol 2017;44:237–46.
[262] Lal D. Microbes to generate electricity. Indian J Microbiol 2013;53:120–2.