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# Towards sustainable production of clean energy carriers from biomass resources

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## HIGHLIGHTS

- ▶ Review of various biomass types, including first-generation, second-generation, and third-generation feedstock.
- ▶ Review of various transformation methods for conversion of biomass.
- ▶ Review of various clean energy carriers, including bioethanol, biodiesels, biogas, biohydrogen, biobutanol, and others.

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## ABSTRACT

A great fraction of the world's energy requirements are presently met through the unfettered use of fossil-derived fuels. However, due to the anticipated demise of these energy sources and the environmental and socioeconomic concerns associated with their use, a recent paradigm shift is to displace conventional fuels with renewable energy sources. Among various alternatives, biomasses have garnered tremendous interests as potential feedstock for clean energy production. While numerous biorefinery schemes and conversion technologies exist for the transformation of biomass into usable energy forms, they are not cost-efficient and economically viable to compete with the existing petroleum-refinery technologies. In particular, the recalcitrant nature of several feedstock presents a major technological obstacle for their processing and transformation. Providentially, the synergistic integration of various biochemical and bioprocessing technologies is aiding in the establishment of future biomass energy programs. This article reviews the state of the art and future challenges in the recent development of biomass and associated transformation technologies for clean production of biofuels.



## 1. Introduction

Conventional fuels, primarily including coal, oil, and gas, are indispensable resources whose availability has been integral to the rapid technological progresses over the past few centuries. Currently, it is estimated that more than 85% of the world's energy requirements are supplied based on the utilization of conventional fuels [1]. In addition to supplying energy, they are also an important feedstock for the majority of commodity products produced today (e.g. plastics and fabrics) [2]. However, conventional fuels are non-sustainable and currently having two major issues, i.e. (1) the prognosticated demise of natural reserves in the years to come and (2) the substantial environmental impacts associated with their use. In light of the uncertainties, the recent fluctuating prices, and the environmental disturbances associated with the use of conventional fuels, a recent paradigm shift is to displace conventional fuels with sustainable, renewable, and environmentally-friendly/clean energy sources, among which biomass-derived energy appears to be the most attractive [2,3]. Interconversion of various biomass and energy forms in the carbon cycle is schematically illustrated in Fig. 1. While biomass can be directly burned to obtain energy, it can also serve as a feedstock to be converted to various liquid or gas fuels for practical applications. Hence, a recently emerging strategy is to develop biorefinery and biotransformation technologies to covert renewable biomass feedstock into clean energy fuels and other commodities [4–6].

*Abbreviations:* ABE, acetone, butanol, and ethanol; ACP, acyl-carrier protein; AD, anaerobic digestion; CBP, consolidated bioprocessing; CoA, coenzyme A; CoM, coenzyme M; DMAPP, dimethylallyl pyrophosphate; DME, dimethyl ether; EJ, exajoules; FAEEs, fatty acid ethyl esters; FAMEs, fatty acid methyl esters; FTS, Fischer–Tropsch synthesis; IPP, isopentenyl pyrophosphate; MT, metric ton; MTBE, methyl tert-butyl ether; MW, megawatt; RME, diesel derived from rapeseed crops; SCP, single cell protein; SHF, separate hydrolysis and fermentation; SSF, simultaneous saccharification and fermentation; TAL, transaldolase; TKL, transketolase; XDH, xyitol dehydrogenase; XI, xylose isomerase; XK, xylulokinase; XR, xylose reductase.

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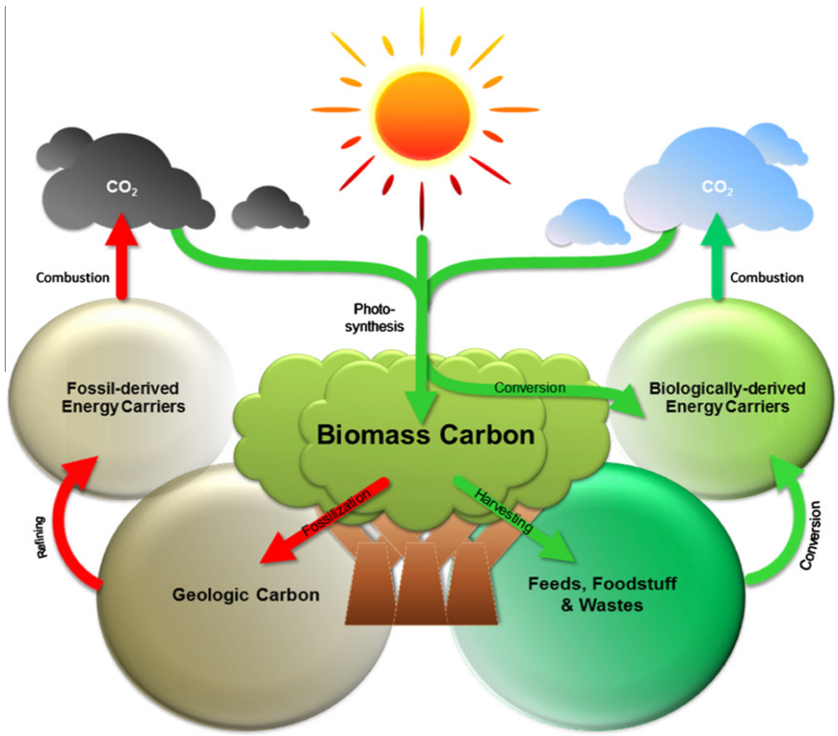


**Fig. 1.** Model of carbon cycle illustrating how energy carriers are derived from biomass. Biomass carbon is generated via photosynthesis upon fixing atmospheric $CO_2$ with a simultaneous conversion of solar energy into chemical energy stored in biomass. Biomass carbon could be transformed into several energy carriers through either an environmentally amicable route (shown in green) or environmentally unfriendly route (shown in red). If biomass carbon, harvested crops, or wastes are converted into fuel, the process is renewable with no atmospheric $CO_2$ build-up. Conversely, biomass decomposed over several epochs (geologic carbon) can also be partially recovered and utilized. However, the later process is lethargic, non-sustainable, and potentially deleterious to the natural environment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Biomass feedstock are energy sources derived from plants, microbial cells, and the wastes and residues associated with their processing (e.g. agricultural residues, forestry and municipal wastes). They are generally formed through photosynthesis, whereby plants (and some microbial cells) garner atmospheric $CO_2$ and sunlight to produce high energy carbonaceous compounds (i.e. biomass) and oxygen [3,7]. The dry biomass is a carbohydrate polymer containing carbon, hydrogen and oxygen in a ratio of approximately 1:1.4:0.6 [8]. When the energy constrained within biomass is released, the carbon is oxidized to $CO_2$, which can be recycled to produce new biomass. Theoretically, no additional greenhouse gas is produced since the emitted $CO_2$ is part of the current carbon cycle. Therefore, if efficiently utilized, biomass is regarded as an alternative clean and renewable source for energy and other commodities due to its abundance (~100 and 50 billion tons of land and aquatic biomass, respectively, is produced on the Earth), high energy content, sustainability, biodegradability, and generation of recyclable exhaust gases. Moreover, the utilization of biomass-derived fuels will also greatly mitigate current energy security and trade balance issues, and foster socioeconomic developments for many rural communities in developing nations (see Table 1) [2,3,8]. Nevertheless, given the recalcitrant nature of certain biomass feedstock and the current technological bottlenecks associated with various transformation processes, the economical feasibility of biomass-derived fuels are far too low to compete with the existing fossil fuel technologies. Fortunately, recent advances in biotechnology and bioengineering are synergistically aiding in realizing the applicative potential of biomass-derived feedstock and improving its conversion technologies to create economically feasible platforms for more sustainable production of biofuels in the future. Herein, we survey various biomass feedstock and the clean biofuels derived from their transformation. Moreover, the advances and challenges associated with the refinery and conversion technologies of biomass feedstock are also delineated, with special attention given to biologically derived processes and energy carriers.

## 2. Biomass feedstock

Currently, biomass-derived energy sources supply ~50 EJ (exajoules) of the world's energy, which represents 10% of global annual primary energy consumption and ~75% of the energy derived from alternative renewable energy sources [9]. Moreover, it is expected that biomass-derived energy may have to contribute ~1500 EJ by 2050. At this time, only 2% of the biomass-derived energy sources are utilized in the transportation sector whilst the rest is generally for household uses [10,11]. Transportation fuels derived from biomass (i.e. biofuels) can be produced using the feedstock of conventional agricultural crops (first-generation), lignocellulosic crops and unused agricultural wastes (second-generation) or microscopic organisms (third-generation) [12]. Feedstock are categorized on the basis of the type of raw materials and transformation processes, and their features are compared in Table 2 and Fig. 2.

### 2.1. First-generation feedstock

First-generation feedstock are edible feedstock from the agricultural sector such as corn, wheat, sugarcane, and oilseeds. These basic feedstock are generally harvested with a high carbohydrate or oil content, and transformed into fuels such as biodiesel (bio-esters), alcohols and biogas (mixture of $CH_4$ and $CO_2$). The biofuels based on the first-generation feedstock are normally derived through conventional technologies (delineated further in Section

**Table 1**
Potential benefits and technical limitations of biofuels.

| Potential benefits | Technical limitations |
|---|---|
| *Environmental gains*<br>• Reduced dependency on environmentally damaging fossil fuels and petroleum products<br>• Lowered levels of greenhouse gas (GHG) emissions<br>• Reduced smog and toxic chemical emissions<br>• Use of waste materials reducing the need for landfill sites | *Environmental threats*<br>• Use of protected land for biomass production<br>• Depleting local water supplies<br>• High demand for fertilizers, herbicides and pesticides leading to an increase in air and soil pollution<br>• Possibility of global climate change with increased atmospheric $CO_2$ production<br>• Use of genetically engineered crops and microorganisms can possibly affect ecosystems<br>• Reduced biodiversity due to soil pollution and/or industrial cultivation of favoured crop species<br>• Increased particulate carbon emissions from wood burning |
| *Economic benefits*<br>• Relatively inexpensive resources<br>• Locally distributed energy sources provide constancy and reliability<br>• More widely distributed access to energy<br>• Price stability<br>• Generation of employment opportunities in rural communities<br>• Biomass and bio-energy technology export opportunities<br>• Use of underutilized biomass resources as a renewable and inexhaustible fuel source | *Associated technologies*<br>• Collection storage of feed stock<br>• Pre-treatment of biomass<br>• Enzyme production<br>• Cost of technology manufacturing and maintenance |

3). Conventional crops are already available in high quantities as these crops are produced in a large scale for human consumption and animal feed. Currently, the three most popular edible feedstock that are exploited for biofuel production are sugar canes (in Brazil for bioethanol), corn (in the United States for bioethanol) and lastly rapeseed (in various European nations for biodiesel). While the use of edible feedstock content may potentially enhance the conversion and yield of biofuels from biomass, it tends to impact food prices [13].

### 2.1.1. Starch crops

Domesticated cereal grains and cultivated crops such as corn (maize), wheat, sorghum, cassava, and potatoes possess a high starch content and can be obtained in high yields if cultivated properly. Corn is the largest fuel crop for producing bioethanol and one of the most important agricultural crops globally principally because it utilizes a unique and highly efficient 'C4' photosynthesis system for carbon fixation. This photosynthesis system, in contrast to the conventional 'C3' one for most plants, yields a higher starch content [3,12]. The global production of corn grain is ~822 million MT (metric tons) with major producers being the United States, China, and some nations in southern Africa. Through genetic modifications, numerous desirable traits have been obtained to enhance of the crop production, such as resistance to various pathogens (e.g. *Bacillus thuringiensis* endotoxins) and stresses (e.g. drought and high salinity) [3,12,14]. While wheat and rice are also important grains with a high starch content, their use to produce biofuels is uncommon as these crops are harvested primarily for human food consumption [12].

Two other important cultivated crops that may potentially be used for biofuel production are cassava and sorghum. Cassava is a perennial plant cultivated as an annual crop in the tropical and subtropical countries. The largest producers of cassava are currently various African and south Asian nations. It also possesses a high starch content, and is recognized as an alternative to corn and sugarcane for the production of bioethanol [12]. Moreover, the cassava ethanol production schemes are compatible with current corn ethanol technologies and infrastructures. However, cassava cultivation is rather labor-intensive and the ethanol yield obtained from cassava is substantially lower than those from sugarcane and corn [12]. Sorghum is cultivated in temperate-to-hot and dry regions and is the one of the most widely grown cereal crop in the world. It contains ~30 species providing human food, animal feed and forage, and sugar. As a 'C4 plant', it also has a high grain, starch, and biomass content, and thus is now being developed as a potential bioenergy crop [15]. Its conversion process for biofuel production depends on the type and part of sorghum to be used. Multiple systems are available for biofuel production using starch from grain sorghum, stalk sugar from sweet sorghum, and cellulose from the crop residue The properties of sorghum are also improved by conventional breeding and genetic approaches [15,16].

### 2.1.2. Sugar crops

Sugarcane is a perennial grass commonly cultivated in the tropics and subtropics, with a worldwide production of ~1.74 billion MT. The largest producer of sugarcane is Brazil, followed by Australia, India, South Africa, and Thailand [3,12]. As a 'C4 plant' with a fast growth rate, fecundity, and high sucrose content (~20%), it is the preeminent choice for biofuel production by supplying more than 40% of all fuel ethanol. With the advantages from its vast arable land, cheap feedstock price, and advanced agricultural technologies, Brazil has developed a green and sustainable sugarcane ethanol industry. Stem cutting has been the reproduction method for prop-

**Table 2**
Major characteristics of globally available biomass feedstock.

| Feedstock | Advantage | Development of associated technology | Limitation | Share of total renewable energy in the world (%) | Share of total energy in the world (%) |
|---|---|---|---|---|---|
| First-generation (e.g.: food crops) | Excellent energy content | Relatively mature (e.g.: bioethanol refineries) | Requires tropical arable land | ~9 | ~1 |
| Second-generation (e.g.: energy crops) | Devoid of competition with food industries | Relatively immature | Laborious and costly treatment technologies | ~87 | ~10 |
| Third-generation (e.g.: microalgae) | Devoid of farming and land inputs | Immature | Low yield of energy carriers | ~0 | ~0 |
| Other (e.g.: municipal solid wastes) | No cost associated with feedstock | Mature (e.g.: anaerobic digestion) | Size of feedstock inconsistent | ~4 | ~0.5 |

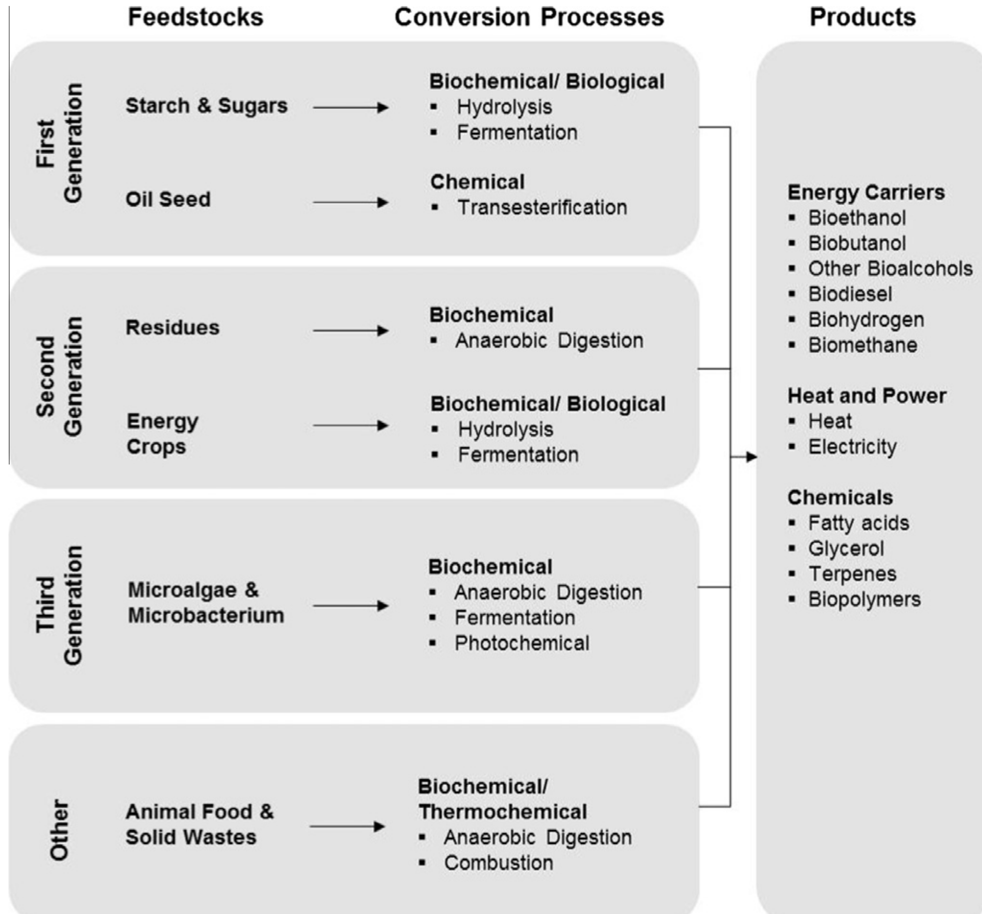


**Fig. 2.** Summary of various major biomass feedstock, conversion processes, and final products associated with biorefinery.

agation with subsequent milling and biorefinery process to produce ethanol [3,17]. The byproduct and residue (bagasse and molasses) from sugarcane milling process are also useful for ethanol fermentation and power generation, making the net energy ratio of sugarcane ethanol relatively higher than corn ethanol. Other alternatives to sugarcanes are sugar beets and sweet sorghums. However these crops are generally not utilized for biofuel production owing to their low harvest yields and labor-intensive cultivation schemes [3,17].

*2.1.3. Oilseed crops*

Oilseed crops such as rapeseed, soybean, sunflower, peanut, palm, coconut, safflower, linseed and hemp are valuable feedstock for the production of liquid biofuels [18]. Aside from fuels, these oils may also be used for culinary purposes, as well as for deriving other commodities such as soaps, skin products, and perfumes. The unsaturated oils from these crops can be transformed by hydrogenation into fat with high melting points. More importantly, the vegetable oils yielded by these crops can be directly used in conventional or modified diesel engines, or can be refined via transesterification with a short-chain alcohol to produce alkyl (methyl, ethyl or propyl) esters, namely, biodiesels [19,20].

## 2.2. Second-generation feedstock

Although the first-generation feedstock are attractive options for biofuel production in terms of their high sugar and starch composition, abundance in nature and combined ease of cultivation and processing, this production scheme is considered unsustainable. As the demand for renewable energy increases exponentially, the practicability of the production first-generation feedstock becomes tentative and limited since large arable croplands in tropical and temperate regions are required for their cultivation. Moreover, the direct competition of biofuels with human food and animal feed results in significant price increases of these crops. Second-generation feedstock are non-edible and comprise of raw materials derived from lignocellulosic biomass and crop waste residues from various agricultural and forestry processes [21,22]. These raw materials are far more ideal for fuel production since their utilization will not impact the food industry. Accordingly, second-generation feedstock can be cultivated in a large scale solely for the purpose of energy production. Cellulosic biomasses are also far more versatile than conventional energy crops and can be cultivated in a much wider range of soils and environments with comparable yields. Finally, if accrued crude agricultural and forestry residues are processed efficiently for biofuel production, it will greatly reduce the current disposal problems associated with these materials. However, the conversion processes (i.e. thermo-chemical and biochemical conversions, see Section 3) are far more complex and sophisticated because of the recalcitrant nature of cellulosic biomass, which is associated with the composition of tenaciously complex polysaccharides such as cellulose, hemicelluloses, and lignins. Moreover, due to the present bottlenecks in the production scheme, second-generation feedstock are not cost-competitive with existing petroleum-derived fuels. In general, the second-generation feedstock can be categorized into two major groups, i.e. organic waste residues and dedicated energy crops [21,23,24].

*2.2.1. Organic waste residues*

Every year, approximately 40 dry tons per hectare of lignocellulosic residues are produced and most of which are underutilized.

These lignocelluloses derived from an assortment of agricultural processes include corn cobs, corn stover, wheat straw, rice hulls, and cane bagasse. In many developing nations, these wastes are currently combusted for the generation of heat and electricity or for forage, or are ploughed back into croplands [12,25]. Considering their distributive variety, large quantity available, and high carbohydrate content, the energy potential of these residues is enormous. Nevertheless, it should be noted that the energy content of waste residues greatly varies from one crop to another. Among organic waste residues, woody wastes, i.e. the byproducts from logging operations, sawmill processes, pulp- and plywood factories, and the lumber industry, are also excellent feedstock for fuel production. Although biofuel production from woody biomass is still in its infancy, the importance of these feedstock has been perceived because of their high cellulose and low hemicelluloses composition [12,25,26].

#### 2.2.2. Dedicated energy crops

With the substantially increasing demand for producing biofuels from the lignocellulosic feedstock in recent years, it becomes important to identify and cultivate crops exclusively for generating energy. Desired merits of energy crops include: fast growth rate, fecundity, high tolerance to various environmental stresses, high energy content, and relative ease of cultivation in comparison to grain crops. To date, the following energy crops are of great interest: perennial grasses (such as switch grass and Miscanthus) and woody energy crops (such as polars, willows, and eucalyptus) [3,17]. Compared to conventional grain crops, these 'short-rotation' and fast-growing crops are excellent feedstock largely due to their superior growth on cold, wet or temperate soils with high annual biomass yield and their ability to be co-produced with grain crops in the same soil, a cultivation strategy known as "double-cropping" [3,17].

### 2.3. Third-generation feedstock

While a wide collection of fermentative and photosynthetic bacteria and algae are currently being explored as biocatalysts, they are also recognized as excellent feedstock, so-called "third-generation feedstock", primarily due to their high oil/lipid, carbohydrate, or protein contents. In comparison to the first- and second- generation feedstock, microbial cells can be obtained in high yields via bioreactors with no requirement of arable crop lands and other farming inputs (i.e. fertilizers, water, and pesticides) [22,27]. The impetus for exploring microalgae as an alternative energy source stems from its highly efficient photosynthetic systems for carbon fixation and carbohydrate production, and high lipid content (20–40% dry weight). Algal strains are capable of accruing oils through three types of production schemes, i.e. phototrophic (via photosynthesis), heterotrophic (via dissimilation of carbonaceous substrates such as glucose), or mixtropic (a mixture of phototrophic and heterotrophic). While the current algal-based oil production platform is technologically immature, a few genetically modified algal strains can produce oil with an extremely high yield (up to 75% dry weight). It is estimated that microalgae may produce $\sim$10–300 times more oil (used for biodiesel production) than convention and dedicated energy crops in near future [22].

## 3. Biomass conversion routes for clean energy production

### 3.1. Biorefineries

Akin to petroleum-based refineries, bio-based refineries are facilities that integrate conversion processes based on the use of biomass feedstock to produce transportation fuels, direct power, high-value chemicals, and other useful commodities with minimal wastes and emissions. It is expected that in the future the product palette of a biorefinery will be significantly broadened. Three major types of conversion are often included in a typical biorefinery process, i.e. (1) thermo-chemical and mechanical conversions, (2) biochemical and biological conversions, and (3) physicochemical conversions. All these conversion routes are aiming to concomitantly deoxygenize and depolymerize the biomass feedstock to release monomeric sugar for subsequent conversions [13]. Many of these conversion routes demand extensive pretreatment or upgrading of the feedstock (e.g. heat generated via combustion) due to the complex and recalcitrant nature of biomass, particularly lignocelluloses. Biorefineries are categorized into three groups, i.e. phase I, II, and III. Phase I biorefineries are of limited value as they utilize a single feedstock for the production of a single product. Phase II biorefineries also handle a single feedstock, but transform it through several conversion processes to produce multiple products. Phase III biorefineries are the most advanced ones aiming at employing numerous conversion processes to produce multiple products with the use of a selection of feedstock (e.g. whole-crop biorefineries). Nevertheless, current biorefinery operations are not cost-competitive with traditional petroleum-based refineries since the costs of biomass feedstock and their transportation and processing are extremely high in comparison to crude oil. Strenuous research and development in biorefinery is also needed to improve the performance of transformation processes [5].

### 3.2. Thermo-chemical conversion routes

Thermo-chemical conversion involves treating the biomass with high temperatures in either an oxygenic or anoxygenic condition to promote structural degradation. There are four main thermo-chemical routes for the production of fuels, i.e. direct combustion, gasification, pyrolysis, and liquefaction; each differing in the temperature, heating rate, and oxygen level present during the treatment.

#### 3.2.1. Direct combustion

The burning of biomass in an oxygen-rich environment has been one of the traditional methods for the generation of heat (and/or electricity) from biomass with the aid of a steam cycle (e.g. combustion boilers, steam turbines, power plants). Through combustion, the chemical energy from the biomass feedstock, such as fuelwood, agricultural (bagasse) and wood residues from the pulp and paper industry, and municipal solid wastes, can be harnessed. These feedstock are cheap, exist in large quantities, and generally contain a low water content for combustion [28,29]. Presently, different combustion systems, such as grate boilers and underfeed strokers, are available for the production of heat for large-scale industrial use (100–3000 MW) or for district heating (<100 MW). In regions that may demand both heat and electricity, cogeneration systems are also available through the use of steam turbines. With the advent of more advanced technologies such as fluidized bed combustion systems, the efficacy for power generation can be greatly enhanced with reduced emissions and increased tolerance to different types of biomass [26,29]. Although these advanced combustion systems may offer power outputs comparable to traditional carbonaceous fuels, the technology is currently not economically feasible due to the costs involved in the distribution networks and processing of high-moisture-content biomass. Moreover, direct combustion systems may not be a clean technology per se, as toxic emissions are potentially released from certain contaminated wastes (e.g. municipal solid wastes). Accordingly, future research and development should be geared towards improving energy outputs, broadening the range of usable feedstock, and reducing the release of harmful pollutants.

*3.2.2. Gasification*

Gasification is a thermo-chemical process where biomass is converted into a combustible gaseous mixture (e.g. syngas) under partial oxidation at high temperatures (800–900 °C) with gasification media such as air, oxygen or steam [29]. The process is optimized to increase combustible gaseous components of CO, $H_2$, $CH_4$, and other gaseous hydrocarbons while minimizing char and tar formation [30]. Four types of gasifiers are currently available for commercial use, i.e. fixed bed (counter-current and co-current), fluidized bed, and entrained flow. The performance of gasification processes is affected by different operation conditions, such as biomass flow rate, biomass properties, gasifying agent flow rate, and gasification temperature profile [31,32]. The generated gas mixtures are intermediate energy carriers that are either combusted for heat and power generation or processed further to synthesize transportation fuels [33]. The conversion of syngas to liquefied fuels is referred as Fisher–Tropsch Synthesis (FTS) and dates back to the 1920s when coal syngas was used to produce hydrocarbons (e.g. gasoline and diesel). Syngas can be also used as a feedstock for the production of high-value chemicals (e.g. olefins and formaldehyde). Products derived via FTS vary greatly, depending on the catalyst types and process conditions [30,34]. One obstacle that limits large-scale application of gasification conversion technologies is the formation of tars and other undesired byproducts, thus gas cleaning is indispensable to prevent catalyst poisoning before fuel synthesis [35].

*3.2.3. Pyrolysis*

Pyrolysis is a thermal process for biomass decomposition in the absence of oxygen with temperatures ranging from 350 °C to more than 800 °C [36]. Temperature and residence time are key factors to control the composition of pyrolysis products. Three types of pyrolysis are applied, i.e. slow pyrolysis, fast pyrolysis, and flash pyrolysis [37], depending on the operation parameters such as heating rate, temperature, particle size, and residence time. Slow pyrolysis (also referred as conventional pyrolysis) of wood has been used to produce wood charcoal, whereas fast and flash pyrolysis are employed to produce bio-oils with various reactor schemes [37,38]. The major composition of bio-oils produced via pyrolysis are organic acids, esters, alcohols, ketones, phenols, aldehydes, alkenes, furfurals, sugars and some inorganic species [38]. They are easier to transport and store than solid biomass and can also be converted into valuable chemicals, fuels, and distillates used in engines and turbines for power generation. However, there are numerous technical bottlenecks associated with the utilization of bio-oils as transportation fuels because of their crude and inconsistent nature, thermal instability, and corrosive properties. As a result, several strenuous upgrading steps are required to ensure the applicability of these bio-oils as transportation fuels. Hydrodeoxygenation, catalytic cracking, emulsification, steam reforming, and chemical extraction are relevant techniques developed to improve the bio-oil quality [39].

*3.2.4. Liquefaction*

Liquefaction is a conversion process under a liquid phase with a low temperature (250–350 °C) and a high pressure (10–20 MPa), whereby biomass is catalytically broken down into fragments of light molecules in the presence of hydrogen. These unstable and active light fragments are subsequently re-polymerized into heavier oily compounds with appropriate molecular weights [40,41]. The process and products are analogous to pyrolysis except the use of lower temperatures and higher pressures. To prevent undesired side reactions and heavy solid char formation during re-polymerization, hydrogen and organic solvents are added into the reaction system [41,42]. Catalysts (e.g. alkaline hydroxides and carbonates) are crucial to lower the solid residue and improve the yield of bio-oils [42]. To date, technological advances in liquefaction are still in its infancy and its economic feasibility is uncertain due to the high cost associated with the complex reactor and feeding system [43,44].

*3.3. Biochemical conversion routes*

Biochemical conversions include a variety of chemical reactions catalytically mediated inside microorganisms as whole-cell biocatalysts and/or enzymes to convert fermentable feedstock substrates (e.g. monosugars) into fuels or other high-value commodities [23]. They are one of the few conversion technologies that enable energy production in an environmentally friendly manner. While biochemical conversions are generally slow (taking days to weeks or even months) in comparison to the rapid thermo-chemical reactions (taking minutes to hours), these reactions produce less byproducts and pollutants. Thermo-chemical reactions, on the other hand, lack specificity and generally yield multiple and complex products. If implemented for large-scale biofuel production, biochemical conversions are considered more sustainable than thermo-chemical conversions, as these processes can be operated at a lower temperature with the use of a broader range of biomass feedstock. Feedstock for thermo-chemical processes often contain a low moisture content, whereas biological-derived processes can utilize both dry feedstock as well as those with a high moisture content such as herbaceous sugar and starch plants or livestock manures [13,23]. The two main biochemical processes for harnessing chemical energy from biomass are anaerobic digestion and microbial/enzymatic processes.

*3.3.1. Anaerobic digestion*

Anaerobic digestion (AD) is a biological process in which various bacterial species mediate in the decomposition of organic matters under anoxic conditions. The product from this process is biogas, which is a gas mixture containing mainly methane (60–70%) and carbon dioxide (20–40%). This process also occurs in many natural anoxic environments, such as watercourses, soils, animal intestines, and landfills. Currently, biogas is naturally produced in landfills and contributes greatly to accruing greenhouse gases in the troposphere. Such an environmental issue can be greatly ameliorated if naturally emitted biogas from anoxic reservoirs is efficiently harvested and processed. The crude biogas from AD can be burned for heat generation, and it is an indispensable and inexpensive energy source particularly in developing nations [45,46]. In addition to heat generation, purified methane can also be directly used in gas turbines for electricity generation or for use as a transportation fuel, similar to natural gas. In addition, AD produces a solid and liquid residue known as digestate, which can be used for soil conditioning and fertilizing [47].

A wide range of biodegradable waste materials can be applied to the versatile AD process [48,49], such as agricultural waste, industrial waste, animal manure, sewage sludge, leftover food, municipal solid waste, pulp and paper residues, even microalgae waste after oil extraction [50]. However, wood residues are less favorable in this process due to the difficulty in lignin degradation. Many of these feedstock are processed in anaerobic containers known as digesters, where feedstock and water are mixed. Digesters can range from 1 $m^3$ for domestic units to as large as 2000 $m^3$ for large-scale industrial installations [26,46]. Many considerations are crucial for optimization of AD [51], including reactor design, pretreatment, mixing, temperature, pH, buffering capacity, fatty acid concentrations, number of stages, and monitoring and control systems. AD is a well-established technology widespread in numerous countries, such as China which is the largest biogas producer and user in the world [52,53]. While in Europe and North America, AD is less common, certain countries like Germany and

UK hold several thousand operation units [54]. Ultimately, the sustainability and reliability of AD will greatly depend on the transportation costs of feedstock, the energy production efficiency, and the accessibility of biomass feedstock.

### 3.3.2. Microbial/enzymatic processes

*3.3.2.1. Pretreatment.* Feedstock costs often represent the largest portion (∼40–70%) of the selling prices of biofuels. Although lignocellulosic feedstock are cheap and abundant, these recalcitrant feedstock contain complex chains of polysaccharides and other carbonaceous polymers that must be depolymerized prior to enzymatic hydrolysis. Depolymerization of lignocellulosic materials can be carried out physically (e.g. steam treatment), chemically (e.g. hydrolysis by acid or alkali), enzymatically or via a combination of these methods [55]. Enzymes employed for the degradation of lignocelluloses include cellulase, hemicellulase, accessory enzymes (debranching enzymes), and lignin modifying enzymes. After the hemicellulose and lignin barriers to cellulose microfibrils are mitigated by physical and chemical pretreatments, crystalline cellulose is exposed for hydrolysis by cellulase enzymes, which generally include three classes of endo-cellulase, exo-cellulase, and cellobiase [56]. The cellulases derived from cellulose-utilizing microorganisms are divided into two major categories: i.e. individual non-complex cellulases produced by aerobic bacteria and fungi and complex cellulase (or cellulosome) secreted by anaerobic bacteria and fungi [57]. The efficiency of cellulose hydrolysis relies largely on the synergistic coordination of these enzyme activities to produce soluble sugar substrates.

*3.3.2.2. Enzymatic hydrolysis.* The production of cellulases is rather costly and thus has been identified as a potential bottleneck limiting the commercialization of lignocellulose biorefineries. Most commercially available cellulases are produced by *Trichoderma* or *Aspergillus* species. Being widely regarded as a model strain and industrial source of cellulases and hemicellulases [58,59], *Trichoderma* has a high protein secretion ability and its genome has been sequenced recently [60]. To enhance industrial biodegradation of cellulosic raw materials, recent research initiatives in cellulase-engineering have focused on improving specificity, catalytic activities, temperature and pH stability, and environmental tolerance. Rational design and directed evolution are two genetic strategies widely applied to improve cellulase activity [61]. Since the information of the protein structure and catalytic mechanisms of cellulases remains limited, random mutagenesis followed by elaborate screening has been commonly employed to identify novel lignocellulose-degrading enzymes [62,63]. Recombinant cellulosomes, in which various complexes of heterologous cellulases are artificially assembled as scaffolding constructs, may also prove to be a breakthrough for cellulosic conversion [64]. Advances arising from these genetic and protein engineering approaches have led to a great improvement in the enzymatic hydrolysis of lignocelluloses, reflected by a significant reduction in the cellulase cost associated with lignocellulosic ethanol production from more than \$5 to approximately \$0.2 per gallon ethanol with more cost-effective expectation of less than \$0.1 per gallon ethanol [65].

*3.3.2.3. Microbial fermentation.* Monosugars derived from the hydrolysis of lignocellulosic materials or agricultural crops can be converted to various biofuels or high-value commodities via different fermentative and/or synthetic pathways using microbial cell factories. The first-generation feedstock are still the major feedstock source because of numerous unresolved technical issues associated with the utilization of lignocellulosic biomass [22]. As a result, no industrial-scale microbial fermentation plant currently exists for the production of lignocellulosic biofuels. On the other hand, sugarcane- and corn starch-based bioethanol production plants were widely implemented in the United States and Brazil during the stagflation of the 1970s [55]. Other clean biofuels produced based on microbial fermentation include methane, butanol, and hydrogen. These biofuels and the microorganisms associated with their production are detailed in Section 4.

Enzymatic hydrolysis and microbial fermentation are carried out either sequentially, i.e. Separate Hydrolysis and Fermentation (SHF) process, or in parallel as a single-stage operation, i.e. Simultaneous Saccharification and Fermentation (SSF) process. While the enzymes and microorganisms can function at their own optimal conditions in SHF processes, the operation is laborious and enzymatic hydrolysis may be incomplete due to the inhibition from the end products. As a result, the strategy of combining the two stages via SSF is adopted to reduce process complexity and overall cost and to increase process yield [66]. Recently, a novel strategy has been proposed by combining cellulosic enzyme production and SSF, leading to a so-called consolidated bioprocessing (CBP) technology for simultaneous cellulase production, cellulose breakdown, and fermentation in a single bioreactor [67].

### 3.4. Physiochemical conversion routes

Physiochemical conversion (also known as agrochemical conversion or mechanical extraction) broadly refers to conversion processes in which oils are extracted from various oilseed feedstock, such as oilseed rape, cotton, and groundnuts, for direct fuel use or biodiesel production via transesterification. Among a wide variety of crops, rapeseed has garnered tremendous interests for high energy content and ease of cultivation. This process also produces the byproduct of glycerin and a residual 'cake', which can be further processed into energy sources via a glycerin platform and animal fodder, respectively. Although physicochemical conversion is an environmentally amicable and clean transformation technology with an excellent energy security for the future, its biorefinery strategy remains relatively immature and is particularly hindered by the cost of raw materials. Consequently, there are only ∼85 biodiesel plants currently operating around the world [26,29].

## 4. Clean energy carriers

### 4.1. Bioethanol

A major impetus for ethanol production through fermentation was initiated largely in response to the oil embargo of 1970s. Currently, two major fermentation platforms for ethanol production exist, i.e. the corn-ethanol program in the United States and the sugarcane-ethanol program in Brazil, with annual production of ∼13 and ∼7 billion gallons, respectively. Attractiveness of bioethanol as a transportation fuel stems from its high production efficiency, high octane rating (108), and GHG benefits. However, ethanol possesses several applicative limitations, i.e. the relatively low energy density and vapor pressure, the corrosive nature as a result of its hygroscopicity, and the incompatibility with existing fuel transportation infrastructures. Hence, bioethanol is not targeted as a key contender to petroleum-derived fuels per se, but rather as a gasoline extender and an octane enhancer [3,23].

Common feedstock harnessed for ethanol production comprise of the first-generation feedstock derived from sugar and starch crops and the second-generation lignocellulosic feedstock. While it is advantageous to convert lignocelluloses to ethanol, this production scheme is presently unrealistic because of the limited substrate spectrum for most microbial species and the recalcitrant nature of lignocellulosic materials. The genetically tractable baker yeast, *Saccharomyces cerevisiae*, has become the preeminent choice to convert sugars derived from biomass for the production ethanol based on its robust growth, high ethanol yield, and ethanol tolerance. Like most microbial species, wild-type *S. cerevisiae* is only capable of fermenting mono- and disaccharides of hexose sugars, such as glucose, sucrose, maltose, and fructose via glycolysis (Fig. 3B), but does not possess enzymes for hydrolyzing cellulose/hemicellulose or for fermentation of pentose sugars present in hemicellulose (i.e. xylose and arabinose) [68]. Consequently, the first-generation feedstock are presently used for industrial production of bioethanol [55] with three primary operating stages: (1) mono- and disaccharides are released through either chemical or enzymatic hydrolysis, (2) ethanol fermentation using microbial cell factories such as *S. cerevisiae* and other yeast, fungi or bacteria, (3) distillation for ethanol separation and concentration [55,68].

The ethanologenic bacterium *Zymomonas mobilis* is another attractive cell factory for industrial production of ethanol. While *Z. mobilis* also lacks the ability to ferment pentose sugars, it has several appealing properties, including the ability to anaerobically metabolize glucose via the Entner–Duodoroff (ED) pathway (Fig. 3C), as opposed to glycolysis, and high tolerance to ethanol (∼120 g/L). As a result, the bacterium produces ethanol with minimal byproduct formation, leading to ∼5–10% higher in ethanol yield in comparison to the traditional yeast-based microbial platform. Because the ED pathway has a lower ATP yield than glycolysis, *Z. mobilis* constitutively maintains a high glucose flux and produces less biomass than yeasts [68].

Enteric bacteria (e.g. *Escherichia coli*) and certain types of yeast (e.g. *Pachysolen tannophilus* and *Pichia stipites*) are potentially capable of metabolizing pentose sugars. However, pentose-fermenting yeasts are not suitable for large-scale bioethanol production due to the organisms' low ethanol yield, heightened sensitivity to ethanol (∼40 g/L), inability to ferment xylose in acidic environments, and strict requirement for microaerophilic conditions [68,69]. Enteric bacteria and yeasts possess different metabolic pathways for xylose dissimilation. In bacteria, xylose is first converted into xylulose by xylose isomerase (XI). In xylose-utilizing fungi and yeasts, xylose is converted to xylulose through a two-step conversion by xylose reductase (XR) and xyitol dehydrogenase (XDH). In both cases, xylulose is phosphorylated and dissimilated via the pentose phosphate pathway (PPP) (Fig. 3A) [55,68–70].

Over the past two decades, metabolic engineering and genetic engineering strategies have played a pivotal role in broadening the substrate range of *S. cerevisiae*, *Z. mobilis*, and *E. coli* for more effective dissimilation of the pentose sugars and ethanol production (Table 3). Popular strategies that have been explored include: heterologously grafting the xylose catabolic pathway from *P. stipitis* into *S. cerevisiae*, incorporation of various pentose dissimilation genes from *E. coli* into *Z. mobilis*, and enhancing the ethanol competence of *E. coli* via knocking out various diverting pathways (e.g. lactate and formate formation pathways) and displacing the native fermentation pathway with the homo-ethanol pathway of *Z. mobilis* [24,55,68,69]. Other microbial candidates that may prove to be efficient ethanol producers in the future include genetically modified *Klebsiella oxytoca* strains and various *Clostridium* species (e.g. *C. thermocellum* and *C. thermosaccharolyticum*) that possess the ability to metabolize treated or even untreated lignocellulosic substrates [24,55].

For more sustainable production of bioethanol in the future, it is imperative to displace the first-generation feedstock with lignocel-

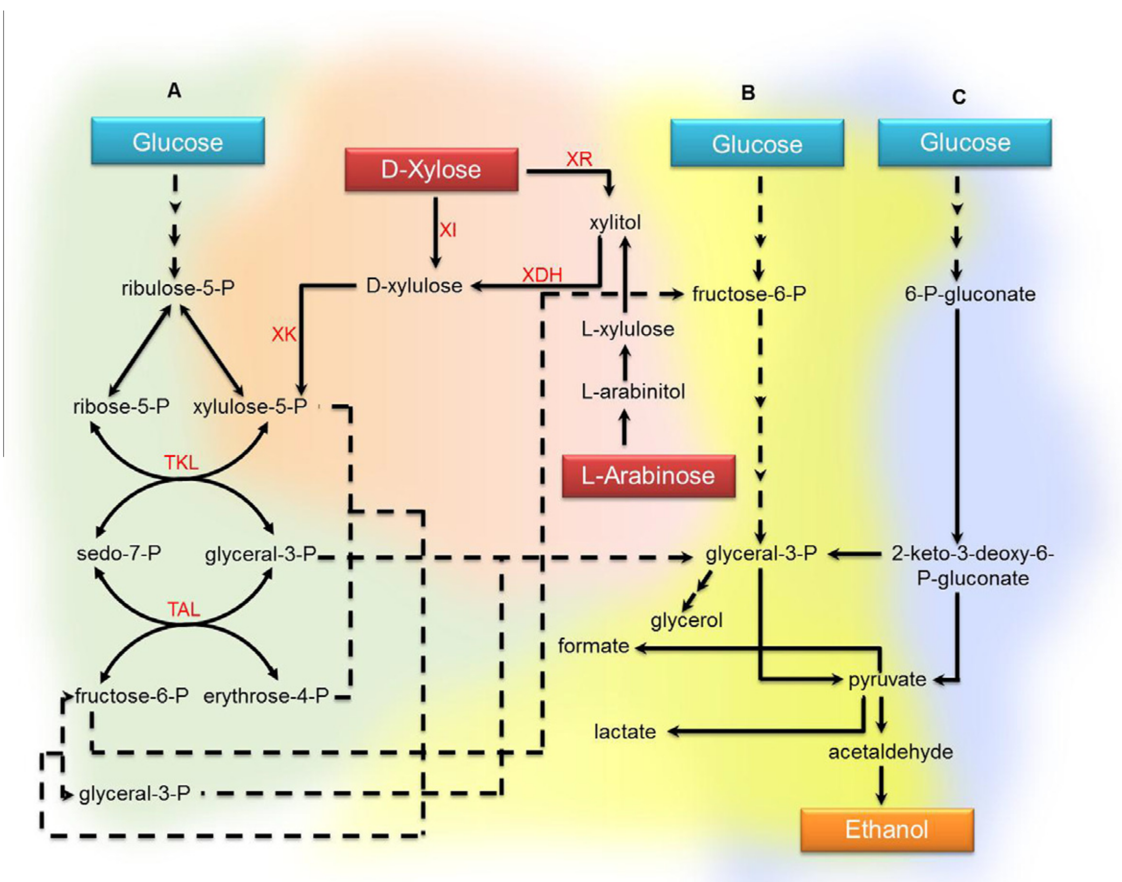


**Fig. 3.** Major metabolic pathways for ethanol production from hexose and pentose sugars: (A) pentose phosphate pathway (PPP) with the inclusion of the xylose and arabinose dissimilation pathways, (B) glycolysis; and (C) Entner–Doudoroff (ED) pathway [69]. Abbreviations: sedo-7-P, sedoheptulose-7-P; glyercal-3-P, glyceraldehyde-3-P; TKL, transketolase; TAL, transaldolase; XI, xylose isomerase; XK, xylulokinase; XR, xylose reductase, XDH, xylitol dehydrogenase.

**Table 3**
Major metabolic engineering approaches to enhance the production of bioethanol.

| Cell factory | Carbon source | Genetic approach | Maximum ethanol titer ($g\,l^{-1}$) | References |
|---|---|---|---|---|
| *S. cerevisiae* NRRL Y-50463 | Glucose ($50\,g\,l^{-1}$) and xylose ($50\,g\,l^{-1}$) | Overexpression of key genes responsible for xylose utilization from *P. stipitis* | ~38 | Ma et al. [128] |
| *S. cerevisiae* MT8-1XS | Glucose ($50\,g\,l^{-1}$) and xylose ($50\,g\,l^{-1}$) | Overexpression of key genes responsible for xylose uptake and utilization from P. stipitis | ~40 | Katahira et al. [129] |
| *Z. mobilis* CP4 | Glucose ($25\,g\,l^{-1}$) and xylose ($25\,g\,l^{-1}$) | Overexpression of two genes responsible for xylose catabolism from *E. coli* | ~24 | Zhang et al. [130] |
| *Z. mobilis* A3 | Glucose ($25\,g\,l^{-1}$) and xylose ($25\,g\,l^{-1}$) | Overexpression of four *E. coli* xylose metabolic genes; strain further enhanced for xylose utilization via adaptive evolution | ~50 | Agrawal et al. [131] |
| *E. coli* KO11 | Xylose ($10\,g\,l^{-1}$) | Replacement of the native fermentation pathway with a homo-ethanol pathway from *Z. mobilis* | ~45 | Tao et al. [132] |
| *K. oxytoca* M5A1 | Glucose ($20\,g\,l^{-1}$) or xylose ($20\,g\,l^{-1}$) | Replacement of the native fermentation pathway with a homo-ethanol pathway from *Z. mobilis* | ~46 | Ohta et al. [133] |
| *K. oxytoca* P2 | Microcrystalline cellulose ($100\,g\,l^{-1}$) | Chromosomally integrated genes responsible for homo-ethanol production from *Z. mobilis* | ~36 | Golias et al., 2002 [134] |

losic biomass or other cheap non-food materials. Major operating stages for lignocellulosic ethanol production are similar to those for starch- or sugarcane-based ethanol production except lignocellulosic feedstock require tedious pretreatment prior to chemical/enzymatic hydrolysis. Barriers limiting industrial-scale production of lignocellulosic ethanol include the technical difficulties associated with the pretreating and hydrolytic steps as well as the ineptness of most microbial species for the assimilation of the pentose sugars. The pretreatment issues can be addressed by optimizing the operating conditions for effective breakdown of the lignocelluloses structure whilst minimizing the release of byproduct inhibitors. Also, the catalytic efficiency of cellulolytic and saccharolytic enzymes should be enhanced with the enzyme production cost being minimized.

### 4.2. 2. Biodiesels

Biodiesels have properties closer to gasoline and petrodiesel so that they can be blended at high levels up to 30% (v/v) or even completely displace petrodiesels in certain vehicles. Currently, biodiesel-powered flexible-fuel vehicles are widely available in many countries [71]. Similar to bioethanol, the production cost of biodiesel varies significantly, depending on the feedstock source and the scale of the plant. Biodiesel production from the first-generation feedstock (i.e. oilseeds which are abundant) is technically mature and commercially viable. The conversion is conducted through two main routes, i.e. transesterification, which is a simple catalytic process with oils and short-chain alcohols as reactants and hydrogenation, which is a process resembling oil refining. While hydrogenation produces renewable diesels of superior quality and free of particulates and byproducts (such as glycerol, which is a byproduct associated with the transesterification process), this process is technically limited by the degradation of hydrogenation catalysts [72]. In addition to oils, fatty acids can serve as a potential reactant for biodiesel production. Since fatty acid biosynthesis is a natural pathway for energy storage in microorganisms, fatty acyl coenzyme A or fatty acyl carrier protein can be used as a starting molecule for the intracellular accumulation of fatty acids, which can be further esterified in vivo to form fatty acid ethyl esters (FAEEs; Fig. 4) known as microdiesels with similar properties to biodiesels [73]. Such a production pathway has been demonstratively implemented in *E. coli* for novel biodiesel production in a pilot scale [74,75].

In addition to the land oil crops, algae represent a nascent platform to be actively exploited for biodiesel production as its harvested oils can be extracted for conversion into biodiesels. This production scheme is particularly attractive on the basis of the microorganisms' rapid growth rate, high photosynthetic efficiency, and high biomass production. The use of algal oils as a feedstock appears to be more effective in biodiesel production than land oil crops [22]. The cultivation of algae can be conducted in either open (e.g. ponds) or closed systems (e.g. bioreactors). Open systems are advantageous in that they are economical to operate and are scalable for mass cultivation. However, the risk of contamination allows the growth of only a few hardy algal strains with a low lipid content. In addition, the open process can suffer from evaporative losses, low photosynthetic efficiencies, and inadequate mixing, leading to low biomass yields. Closed systems, on the other hand, are expensive to establish and operate though they offer far superior biomass productivities. The three main types of closed systems are flat plate bioreactors, vertical bioreactors and tubular bioreactors [76,77]. A technical limitation for algal cultivation is that the high cell density often compromises the growth rate due to reduced illumination. To extract oil, algae cells are first harvested and disrupted through various mechanical and chemical treatments, which represent a major portion of the production costs. There are still many technical challenges to be overcome for the large-scale production of algal biofuels. In particular, genetic tools may lead to the construction of strains with desired characteristics, such as high oil contents. Nevertheless, the economic feasibility of algal biofuels might be achieved progressively by combining the fuel production with high-value byproducts for food and feed ingredients to hopefully meet the growing energy demand in the future [78,79].

### 4.3. Biomethane

Biogas, with methane as the major component, is produced via anaerobic digestion based on the use of a wide range of feedstock, including agricultural wastes, municipal wastes, food wastes, and industrial and municipal waste waters. The conversion of methane from organic waste residues is carried out by a mixed community of microbes capable of catabolizing complex biopolymers and polysaccharides to form acetate, hydrogen, and formate via acetogenesis. Acetate is further converted to methane by methanogenic archaea, such as *Methanosarcina* spp. and *Methanosaeta* spp. (Fig. 4) [55,80]. Apart from being a combusting source for heat and electricity generation, biogas can also be upgraded to refined biomethane, which can be injected into the natural gas networks for various alternative uses [81]. While economical production of biogas is often limited by inconsistent quantity and quality of the feedstock, this conversion route has been experiencing significant development and deployment, particularly in light of more common use of biogas as a vehicle fuel in many countries like Sweden, Germany, India, China, USA [82]. Nevertheless, the incentives

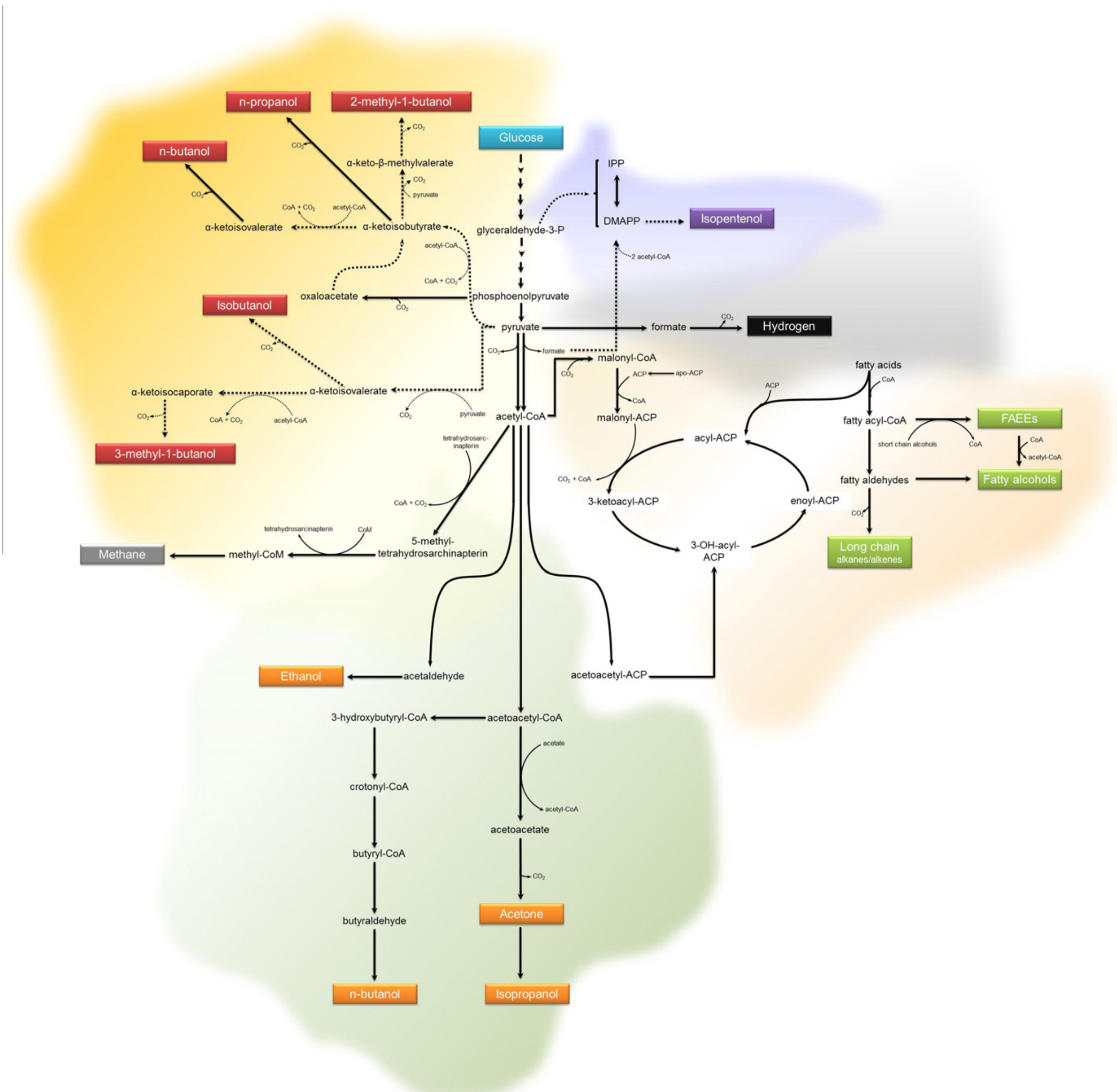


**Fig. 4.** General pathways for the production of several advanced liquid and gaseous biofuels, adapted from [126,127]. (1) 2-ketoacid metabolic pathway for the production of various fuel alcohols (highlighted in red); (2) methanogenesis for the production of methane (highlighted in gray) from acetyl-CoA; (3) clostridial pathway for the production of ethanol, and several fuel alcohols (highlighted in orange) from acetyl-CoA; (4) fatty acid pathway for the biosynthesis of FAEEs, fatty alcohols, and long chain alkanes and alkenes (highlighted in green); (5) hydrogen evolution (highlighted in black) from formate, an aspect of microbial dark fermentation; (6) isoprenoid (highlighted in purple) biosynthesis pathway. Abbreviations: ACP, acyl carrier protein; CoA, Coenzyme A; CoM, Coenzyme M; DMAPP, dimethylallyl pyrophosphate; FAEEs, fatty acids ethyl esters; IPP, isopentenyl pyrophosphate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for biogas as a vehicle fuel can be strengthened by reducing the production cost, improving the pertinent technology, and building the industry and commercial standards.

### 4.4. Biohydrogen

In addition to an important material in the chemical industries, hydrogen is also an excellent and clean energy carrier with a high heating content (i.e. 141.8 kJ/g, which is almost 3 times that of gasoline) and with no $CO_2$ emission upon burning. Currently, more than 95% of the hydrogen is derived from fossil fuels and electrolysis. The use of abundant biomass feedstock, including dedicated energy crops and organic wastes, for hydrogen production has arnered tremendous interests [83,84]. Transformation for biohydrogen production is often carried out via biophotolysis (in green algae and cyanobacteria), photo-fermentation (in purple non-sulfur bacteria), and dark fermentation (in anaerobic bacteria, Fig. 4) [85,86]. Though the biological platforms are considered more environmentally friendly and less energy intensive for hydrogen production, they are not technically mature and economically feasible to compete with traditional chemical or electrochemical processes [87]. So far, gasification and fermentation of waste biomass are two practical systems for biohydrogen production and further development is needed to overcome the efficiency and economic challenges, particularly in the aspect of identifying cheaper feedstock [83].

### 4.5. Biobutanol

While bioethanol appears to be the most popular and successful biofuel in the market, it has numerous unfavorable attributes such as low energy content, incompatibility with the existing storage and distribution infrastructures, and hygroscopicity. Hence, various liquid biofuels, in particular C3–C8 fuels, are recently under exploration and n-butanol seems to be an attractive alternative among them. n-Butanol is a linear C4 alcohol potentially superior to ethanol as a transportation fuel due to its immiscible property, higher energy content, lower volatility, low hygroscopicity, and low corrodibility [88]. While n-butanol is primarily produced through chemical processes in commercial scales, biological routes based on microbial fermentation have been actively investigated over the past few decades. Microbial anaerobes, such as *Clostridium acetobutylicum* and other solventogenic *Clostridia*, are native n-butanol producers owing to the microorganisms' unique pathway for ABE (acetone–butanol–ethanol) fermentation (Fig. 4). ABE fermentation by *C. acetobutylicum* was previously explored as a potential production platform in the early 20th century, but was determined to be economically unfavorable as compared to chemical processes. In light of recent biotechnological advances and growing attention on biofuels, the applicative potential of this biological route is being revaluated with the following major disadvantages to be overcome. First, similar to bioethanol production, the ABE fermentation platform suffers from the high cost of biomass feedstock. Second, conducting anaerobic cultivation is tedious, inconvenient, and expensive, particularly for large-scale production, and the associated n-butanol recovery (e.g. distillation) is energy-intensive and costly. Third, *Clostridium* species often have a complex physiology that is not well understood and genetic tools and strategies for improving the productivity of these species are still under development [88–90].

Technological advances in genetic engineering and metabolic engineering have offered a promise to genetically tailor *Clostridium* species to overcome the aforementioned limitations. Among various solventogenic *Clostridia*, *C. acetobutylicum* and *C. beijerinckii* strains have served as model microorganisms for metabolic engineering because of the establishment of key genetic tools, such as transformation techniques, integrative and shuttle vectors, and targeted gene disruption methods [88]. Rational metabolic engineering approaches (Table 4) include the disruption of pathways diverting the n-butanol flux (e.g. butyrate, acetone, lactate, and acetate formation pathways), overexpression of genes encoding key enzymes to enhance n-butanol yield, genetic manipulation to improve n-butanol tolerance, and lastly the introduction of exogenous genes to broaden substrate specificity [88,89].

To circumvent the innate limitations of *Clostridium* species, numerous synthetic biology strategies based on heterologously grafting the n-butanol production pathway into the genetically amenable host of *E. coli*, which is a non-native n-butanol producer. These approaches appear to be powerful enough, particularly in tandem with metabolic engineering strategies, to develop novel production strains with n-butanol titers up to 30 g/L [91–93]. On the other hand, reconstructing the clostridial n-butanol pathway in other non-native host producers, such as *Pseudomonas putida* [94], *Bacillus subtilis* [94], *Lactobacillus brevis* [95], and *S. cerevisiae* [96], often leads to low titers.

### 4.6. Biomethanol

Similar to other conventional biofuels such as bioethanol and biobutanol, biologically-derived methanol has also garnered tremendous interest. Although traditionally methanol is produced via a non-sustainable and cost-intensive chemical process involving catalytic steam reforming of natural gas, it is possible to produce this fuel in an environmentally benign manner using biomass resources [97,98]. Biomethanol can be produced through either the distillation of woody materials via pyrolysis, or the synthesis using gaseous products (i.e. biohydrogen and CO) derived from bio-oil, or syn-gas derived from cheap waste biomass and woody materials. Nonetheless, given that the yield obtained from these resources is quite low (particularly biohydrogen), biomethanol production processes are not economically viable at an industrial scale. Biomethanol can be an indispensable fuel with multiple applications. First and foremost, it can be used as a motor fuel in conventional engines, in its pure form or as a blend with gasoline, with an excellent emission profile. It is also possible to directly covert methanol to gasoline [97–99]. Second, it can be converted to MTBE (methyl tert-butyl ether), an additive to gasoline. While MTBE is a formidable fuel additive and enhancer, its production process involves using isobutylene, a product derived from fossil fuels. Third, it can be dehydrated to produce DME (dimethyl ether), a suitable replacement for natural gas. Lastly, it can be used as a raw material in the production of biodiesels (as FAMEs, fatty acid methyl esters) [97,98].

### 4.7. Other biologically derived fuels

Other synthetic biology strategies based on biocatalytic rearrangement of 2-keto acid intermediates from the amino acid biosynthetic pathways (Fig. 4) via decarboxylase and dehydrogenase have been applied to engineer *E. coli* strains for the production of non-native short-chain alcohols, including n-butanol, isobutanol, 3-methyl-1-butanol, and 2-methyl-1-butanol [100]. Similar strategies have also been implemented in other microbial cell factories, such as *Corynebacterium glutamicum*, *Clostridium cellulolyticum*, and *Synechococcus elongatus*, for the production of longer chain alcohols [100–102]. Isoprenoid compounds are generally synthesized from isoprenyl pyrophosphate and dimethylallyl pyrophosphate (Fig. 4) [103] and isoprenoid-derived fuels or precur-

**Table 4**
Major metabolic engineering approaches to enhance the production of n-butanol in clostridial strains.

| Cell factory | Genetic approach | Maximum n-butanol titer ($g\,l^{-1}$) | References |
|---|---|---|---|
| *C. acetobutylicum* EA 2018 | Disruption of the acetone pathway via Targetron gene knockdown system | ~14 | Jiang et al. [135] |
| *C. acetobutylicum* M5 | Overexpression of several key genes responsible for butanol production in a solvent-negative strain | ~11 | Lee et al. [136] |
| *C. acetobutylicum* ATCC 824 | Thiolase/alcohol dehydrogenase overexpression and down-regulation of key gene involved in acetone-formation pathway | ~13 | Sillers et al. [137] |
| *C. beijerinckii* NCIMB 8052 | Overexpression of two exogenous glycoside hydrolases to broaden substrate specificity | ~5 | López-Contreras et al. [138] |
| *C. acetobutylicum* ATCC 824 | Overexpression of several heat-shock proteins to improve butanol tolerance | ~17 | Tomas et al. [139] |

sors, such as branched-chain and cyclic alkanes, alkenes and alcohols, could be produced in *E. coli* through isoprenoid biosynthesis pathways [101,103]. Several clostridial species are also natural producers of isopropanol (e.g. *C. isopropylicum*) (Fig. 4), but these microorganisms are not suitable for large-scale production due to the low isopropanol yield. Akin to the above synthetic biology strategies, recent efforts have concentrated on heterologously transplanting the clostridial isopropanol pathway into *E. coli* to enhance the production of isopropanol with reported titers as high as 140 g/L [101,104–106].

## 5. Future challenges and sustainability

While biologically derived energy carriers offer an alternative to traditional petroleum-derived fuels, these biofuels may be uneconomical, energy insufficient, and environmentally deleterious [107]. Accordingly, several arduous challenges in sustainable development must be overcome before commercial-scale production of biofuels can be realized. In this section, four common dimensions for sustainable biofuel production are addressed: (1) economic viability and policy implementations, (2) scalable efficiency (land availability and valuation of natural resources), (3) social concerns and socioeconomic impacts, (4) environmental aspects.

### *5.1. Policy initiatives and biofuel production economics*

Although the renewable energy encompasses a myriad of different environmentally benign forms (e.g. wind, solar, geothermal, and biohydrogen), it has been perceived that liquefied biofuels (e.g. bioethanol, biodiesel, and biobutanol) seem to be the most realistic options for large-scale production within the foreseeable future primarily because these fuels can be readily blended with petroleum-derived fuels and more or less compatible with the existing fuel transportation and refueling infrastructures [88,108,109]. Nevertheless, the profitability of these biofuels is heavily reliant on the prices of petroleum and the feedstock, which tend to fluctuate considerably. Additionally, biofuel commercialization is largely dependent on the policy measures and economic governances of national governments, which include tax exemptions, investment subsidies, and compulsory blending of biofuels with petroleum-derived fuels [110,111].

To date, at least 23 nations (including the United States, Brazil, and several countries in the European Union and Asia) have formulated policies and regulations for supporting biofuel production and utilization. For example, as of 2006, the European Union and the United States have provided a total of ∼US$12 billion to support the bioethanol and biodiesel industries, with increased global annual production by 43% and 23%, respectively [111,112]. However, a vast majority of these biofuels are derived from the first-generation feedstock and further increases in their production are projected to cause significant impact on global food prices. In a recent study [113], prices of maize, soybean, and other agricultural commodities are subject to increases by as much as 17% by 2020. Nevertheless, it is expected that most nations, especially in Asia, will continue to promote the first-generation feedstock until the second-generation feedstock become commercially viable. Market analysts argue that in order to prevent an unparalleled increase in the number of hungry people, governments should accelerate transition from the first-generation feedstock to the second-generation through policy and regulatory actions [110,114]. Policy instruments associated with the second-generation feedstock should focus on improving the production and conversion technologies as well as increasing the livelihood of rural farmers in developing nations through various subsidies and tax benefits [111,115]. Presently, only farmers in large nations, such as the United States and Brazil, benefit from agricultural subsidies, creating an unbalanced market for the second-generation feedstock [111,116].

Economic and sustainable development of biofuel technologies is greatly dependent on various costs associated with feedstock, capital, and plant operating and maintenance. It is estimated that feedstock often represents ∼40–80% of the total production cost of biofuels [111,115]. Although the applicative potential of several liquid biofuels has been extensively explored, all biofuels cost much higher than fossil fuels primarily due to expensive feedstock, limiting their economic feasibility. However, the problem can potentially be alleviated through either producing biofuels in developing nations or developing production schemes utilizing the second-generation feedstock and their associated waste residues or even the third-generation feedstock such as microalgae. Recent data show that the second-generation feedstock, such as woody and herbaceous energy crops and agricultural residues, cost between US$19–84/dry tone and may yield significantly higher titers of bioethanol and other liquid biofuels if cultivated in a large scale [115]. Economic production of biobutanol via the clostridial ABE fermentation is also hindered by the unavailability of inexpensive feedstock. Recent economic assessments of ABE fermentation [117,118] suggest that biobutanol production from certain dedicated energy crops with a high content in cellulose or starch (e.g. switch-grass) can potentially lead to economic production at US$0.59–0.75/kg of butanol. On the other hand, the feedstock cost of biodiesel may be reduced by large-scale cultivation of Jatropa, a relatively inexpensive oilseed that can be cultivated in both the tropics and subtropics, as well as waste oils, grease and animal fat [111]. In addition, with an oil content up to 75%, genetically engineered microalgae may produce up to 30 times more oil than traditional agricultural crops, effectively reducing the costs for biodiesel production [108,119].

### *5.2. Scalability*

Land availability, land use practices, and water availability are also key limiting factors for the large-scale production of biofuels. Land-use change for the cultivation of energy crops will have significant consequences on the employment and income of regional populations (see Section 5.3), global food security, and the biodiversity of ecological communities (see Section 5.4). In addition to these concerns, some analysts are also pessimistic about the availability of arable land in the future since utilizing existing agricultural lands for energy crop cultivation might result in food shortage. Nevertheless, it may be feasible to convert abandoned, idle, and marginal lands into usable lands for energy crop cultivation [110,116,120].

Since fresh water is a critical limiting resource (only ∼0.6% of the Earth's surface is covered by fresh water), irrigation associated with the extensive farming practices for the production of energy crops may adversely affect the aquatic systems of fresh water. In addition to polluting water bodies (see Section 5.4), there are also concerns over the availability of fresh water for sustainable production of energy crops. This problem is exacerbated by the fact that most biofuel production schemes are extremely water-demanding, such as corn- or molasses-based bioethanol production and microalgae cultivation. Problems arising from water-shortage could potentially be alleviated by selecting energy crops requiring less irrigation, cultivating crops in high-rainfall zones, and utilizing feedstock that can be cultured in saline water (e.g. marine microalgae) [120,121].

### *5.3. Social and socioeconomic issues*

The emergence of biofuel markets is expected to directly affect the livelihood and economy of rural communities, given that almost all feedstock are cultivated in rural areas [107]. Most economists support the notion that global biofuel programs will generally contribute to the sustainable livelihood of agricultural laborers by increasing employment rates in most rural communities since a large portion of feedstock cultivation and refinery processing involves manual labor. A recent editorial [112] suggests the optimism of the economic development and employment generation from the cultivation of biofuel crops in Asia. It was estimated that the Malaysian biodiesel industry is projected to employ ~1 million people, while the Indian sugarcane-based ethanol industry is expected to employ ~45.5 million people. Biofuel programs may also provide economic benefits for auxiliary service sectors, such as animal husbandry and milk production [107]. Nevertheless, as mentioned in Section 5.1, biofuel programs may only offer developmental opportunities to farmers if regional and national governments work to synergistically integrate economic policies and regulatory frameworks, particularly providing agricultural subsidies for rural sections in developing nations. Additionally, while developing nations can be major producers of energy crops, only developed nations possess the technological and agro-processing capacity to convert feedstock into biofuels. Therefore, future industrial development of biofuels can be substantially broadened if the conversion technologies are also established in developing countries [111,116].

### 5.4. Environmental concerns

Although prevalent application of biofuels, in principle, should mitigate the environmental impacts associated with the use of petroleum fuels, most studies indicate that large-scale cultivation of biofuel crops may potentially damage the natural environment. To meet the global demand of biofuels, major strategies should be implemented to significantly increase agricultural productivity without the following environmental impacts: i.e. loss of biodiversity, introduction of invasive energy crops, release of agro-contaminants (e.g. pesticides, herbicides and fertilizers) into aquatic systems, and increase in global emissions of $NO_x$ and $CO_2$ [107,110,111,116,120,121].

First and foremost, cultivation of these biofuel crops should not displace much land currently used for growing food crops. Moreover, an outsized introduction of a monoculture may affect the eco-balance of certain regions, especially if these energy crops are invasive. Without proper nutrient management, mono-cropping a single energy crop on an annual basis may severely deplete essential nutrients in the soil. Using corn as model crop, acceleration of energy crop cultivation may deteriorate water quality of nearby rivers, lakes, and oceans through nutrient pollution (e.g. release of excess nitrogenous and phosphorous compounds), salinization from extensive irrigation, hyper-eutrophication, erosion, and may be harmful to aquatic structures such as coral reefs [107,110,111,116,120,121]. Some of the concerns associated with mono-cropping can however be mitigated through agro-technical innovations for improving agricultural practices. For instance, competition with food crops can be avoided through the cultivation of certain second-generation energy crops with low agrochemical demands on marginal lands, such as Jatropha [121]. Large-scale cultivation of microalgae in open ponds and raceways is another strategy devoid of land competition. Additionally, microalgae can be cultivated in polluted aquatic wastewater to remove environmental pollutants (i.e. phycoremediation) [122,123].

Biofuels derived from certain energy crops may also have emissions similar to petroleum fuels (i.e. $NO_x$ and $CO_2$), thus questioning their greenhouse gas benefits. Some studies [124] suggest that the use of bioethanol produced from the first-generation feedstock corn may result in overall greenhouse gas emissions similar to or even higher than gasoline. Nevertheless, bioethanol and other biofuels derived from the second-generation cellulosic feedstock may potentially reduce greenhouse gas emissions by ~85%. The use of crop residues is projected to decrease greenhouse gas emissions by up to 50% [125]. While some studies indicate that petro-diesel and biodiesel fuels may contribute equal amounts of $NO_x$, it is generally believed that biodiesels and their associated blends with petro-diesel possess cleaner emission profiles overall. According to a recent study [116], the usage of biodiesels in engines may decrease emissions of $NO_x$ by ~10%, CO and particulate matter by ~45%, hydrocarbons by ~65%, and sulfur oxides by ~100%.

## 6. Conclusions

The utilization of biomass feedstock appears to be a genuine solution to sustainable production of clean energy carriers in the future. However, various technical issues remain to be addressed; in particular the recalcitrant nature of the second-generation feedstock and the limited substrate range and inherently low yield associated with biological conversion technologies. While well-established thermo-chemical technologies may seem convenient and advantageous to apply, microbial conversion systems can offer more effective ways to generate single and well-defined energy products with minimum byproducts and pollutants. The biorefinery process should be systematically analyzed, modeled, and optimized based on a number of factors, such as feedstock selection, pretreatment method, reaction and separation process, energy integration, water recycling, and co-product production, to ensure its economical efficacy. Tremendous progress has been made in the last two decades to overcome inherent biotechnological limitations associated with the microbial conversion platform, particularly through the development of novel systems biology, synthetic biology, and metabolic engineering strategies. However, further process improvements are required in light of the still sub-optimal product titers. Future research and development should focus on a more system-level understanding of the metabolism and physiology of microbial cell factories as well as functional analysis of key regulatory elements and mechanisms to hopefully tailor more effective and robust production strains.

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