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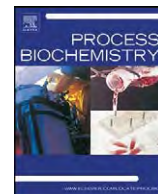
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Highlights

What is vital (and not vital) to advance economically-competitive biofuels production*Process Biochemistry xx (2011) xxx–xxx*

Y.-H. Percival Zhang*

- ▶ We are flooded by a wave of biased or even false information about biofuels.
- ▶ Most advanced biofuels cannot be economically produced compared to ethanol and H₂.
- ▶ Thermodynamics determines economics.
- ▶ Cellulosic ethanol and butanol are the best short- and middle-term biofuels.
- ▶ Sugary hydrogen would be the best ultimate biofuel.

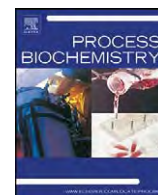
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Review

What is vital (and not vital) to advance economically-competitive biofuels production

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ABSTRACT

Since biofuels is a hot topic, many researchers new to this field are eager to propose different solutions while they often seem not to have full understanding of the current status of technologies and numerous (hidden) constraints. As a result, the general public, policymakers, academic researchers, and industrial developers have been assaulted by a wave of biased, misinterpreted, or outright false information. In reality, only a small fraction of exploding biofuels R&D teams are addressing vital rather than trivial challenges associated with economically production of advanced biofuels. Biofuels R&D is not a completely basic science project; instead, it is a typical goal-oriented (engineering) project because so many constraints prevent economically competitive production of most advanced biofuels and are expected to do so in the future. In this opinion paper, I present some basic rules and facts in thermodynamics, physical chemistry, and special constraints in the transport sector, sort through and challenge some claimed breakthroughs or new directions, and identify vital topics to advance biofuels in the short and long terms. Simply speaking, energy efficiency is the most important long-term criterion whereas cost is the most important short-term criterion; eventually thermodynamics determines economics. For light-duty passenger vehicles, which consume ~60% transportation fuels, cellulosic ethanol and butanol are the best short- and middle-term biofuels, whereas sugary hydrogen would be the ultimate biofuel in the long term. The top three priorities of biofuels R&D are (i) cost-effective release of sugars from lignocellulose, (ii) co-utilization of lignocellulose components for the production of value-added compounds that subsidize whole biorefineries, and (iii) enhancing the biomass-to-kinetic energy efficiency from conversions to prime movers through a potential evolutionary scenario from ethanol or butanol/internal combustion engines (ICE) to ethanol/hybrid diesel-like ICE to sugar hydrogen fuel cell vehicles.

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Nomenclature

BE	battery electric motor
BEV	battery electric vehicle
BTW	biomass-to-wheel CH ₂ O carbohydrate
C _n H _m	hydrocarbons
COSLIF	cellulose solvent- and organic solvent-based ligno-cellulose fractionation
FC	fuel cell
FCV	(hydrogen) fuel cell vehicle
GT	gas turbine
ICE	internal combustion engine
ICE-gas	ICE based on gasoline
ICE-diesel	ICE based on diesel
PEMFC	proton electron membrane fuel cell
SE	steam engine
SFCV	sugar fuel cell vehicle
ST	steam turbine
SyPaB	Synthetic (enzymatic) pathway biotransformation
TTN	total turn-over number

1. Introduction

Energy is the only universal currency. Energy systems are comprised of three basic components: primary (natural) energy sources, their conversion to secondary energies (i.e., energy carriers), and the use of secondary energies (Fig. 1). Primary energy sources include renewable sources, such as solar energy and biomass, and non-renewable sources, such as crude oil, coal, natural gas, nuclear energy, and methane hydrate [1,2]. The nine major types of renewable energies are geothermal energy, tidal energy, solar radiation, and the six transformations of solar radiation: running water (hydro energy), wind, wind-generated ocean waves, ocean currents, thermal differences between the ocean's surface and deep waters, and biomass photosynthesis (primary production) [1,2]. Not all primary energy sources can be economically utilized through available technologies. For example, non-point primary energies that have very low energy concentrations in terms of W/m², such as ocean thermal differences, currents, and methane hydrate, are difficult to harness economically.

Major energy applications include transportation, residential, industrial, commercial, food, and feed applications (Fig. 1). Energy conversion systems bridge primary energy sources with specific applications or needs through secondary energies, such as electricity and transportation fuels (Fig. 1). In the past, the simplest systems utilized a small number of primary energy sources (e.g., plant biomass) through one or two kinds of inefficient energy conversion for meeting basic needs. In contrast, modern societies can utilize numerous primary sources (e.g., fossil fuels, solar insolation, biomass, and wind energy), convert them to energy carriers in many ways with enhanced energy efficiencies, and apply carriers in a myriad of ways to power complex high-energy societies [3,4].

Transport is the movement of people and goods from one location to another. Mobility usually reflects the level of civilization

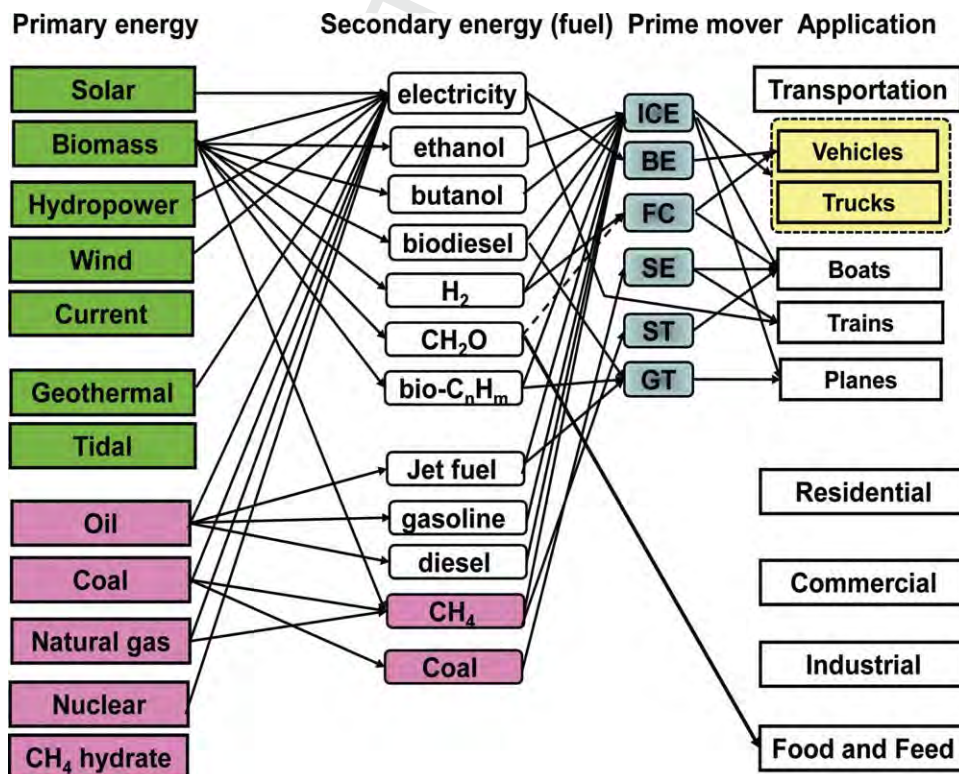


Fig. 1. Energy systems from primary energies to secondary energies to transportation applications. The lines represent energy conversion pathways.

[5,6]. The transport sector consumes approximately 20% of the global energy produced [7]. For example, the average transportation energy consumption for a British citizen is approximately 70 kWh/d (i.e., 40 kWh/d for car and 30 kWh/d for jet flight), which is 4.67 times and 23.3 times higher than the energy consumption for food production (15 kWh/d) and food-only consumption (2600 kcal/d = ~3 kWh/d), respectively [4]. Including the energy needed to transport items (12 kWh/d), transportation energy consumption per person in typical developed countries accounts for approximately 40% of the total energy consumption of 195 kWh/d [4].

Human societies have passed through two transportation revolutions: from animal forces to external combustion engines to internal combustion engines [7–10]. Currently, a combination of high-energy density liquid fuels as well as high-power density and low-cost internal combustion engines provides a convenient and affordable transportation solution (see Section 2).

Biofuels are defined as transportation fuels produced from biological resources or through biological processes [7,11–14]. Biofuels can be either liquid (e.g., ethanol, butanol, long-chain alcohols, fatty acid esters, biodiesel, algal diesel, alkanes, methanol), gas (e.g., hydrogen, methane), solid (e.g., wood chips, sawdust, grass cuttings, agricultural waste, manure), or electrical. They can be produced from diverse primary energy sources, such as plant biomass, algae, and solar energy, as well as CO₂ plus secondary energies, such as hydrogen and electricity [14]. They can be produced through biological approaches mediated by microbes or cascade enzymes, chemical catalysis (e.g., gasification, pyrolysis, and aqueous phase reforming), or a combination of these two [15–17]. *Advocates of a particular biofuel often highlight the advantages of their favorite biofuel while belittling competitor biofuels.*

When biofuels is a popular topic, many supposed “instant bio-fuels experts” are eager to proffer various solutions. In reality, however, only a relatively small group of people understand transportation fuel affairs well enough and recognize how much is unknown about the structure and dynamics of complex energy systems and how perilous it is to prescribe any lasting course of action [3]. As a result, the general public, policymakers, academic researchers, and industrial developers have been assaulted by a wave of biased, misinterpreted, or outright false information. A vast collection of myths, misplaced hopes, and uninformed fervor is creating today’s “biofuels bubble”, which is damaging biofuels’ bright future.

The goals of this opinion paper are to sort through the numerous breakthroughs and new biofuels directions, to challenge some of the most unrealistic ones, and to refocus on vital elements to advance economically-competitive biofuels production in the short and long terms. Although energy techniques and choices are affected by many external factors, we introduce scientific and engineering arguments, give quantitative estimates for important aspects of biofuels production, and provide some suggestions and predictions. In the short and middle terms, cellulosic ethanol/butanol remain among the best biofuels; in the long term, sugar-fuel cell vehicles would be an ultimate winner because they have the highest energy efficiency in addition to high energy storage densities, good safety, and low environmental burdens as compared to battery electric vehicles [6,7,10].

2. Basis of energy conversions and constraints in the transport sector

Evaluating future transportation systems is always challenging because it involves multiple factors (metrics): direct, indirect, and hidden costs of fuels; prime mover performance; costs of transportation vessels; resource availability and scalability; cradle-to-grave energy efficiency; societal, economic, environmental, resource, and safety concerns; technology maturity; and potentials for technology improvement. To simplify the above matrix, we suggest that thermodynamics (i.e., energy efficiencies) determines economics (i.e., costs) in the long term whereas costs determine market shares in the short term.

2.1. Thermodynamics and energies

The principle of conservation of energy states that energy cannot be created or destroyed. The first law of thermodynamics teaches us “the increment in the internal energy of a system is equal to the increment of heat supplied to the system minus the increment of work done by the system on its surroundings”. Clearly, not all forms of energy are equal. Typical energy forms include electro-magnetic, chemical, thermal, kinetic, electrical, nuclear, and gravitational energy (Table 1). Energy in a particular form can be converted into another; conversion efficiencies vary greatly in a range of <1 to ~99%. During conversions, a fraction of the energy is usually dissipated to the environment in the form of thermal energy. It is nearly impossible to convert low-temperature ther-

Table 1
Matrix of energy conversions.

To	From						
	Electro-magnetic	Chemical	Thermal	Kinetic	Electrical	Nuclear	Gravitational
Electro-magnetic		Chemi-luminescence	Thermal radiation	Accelerating charge	Electro-magnetic radiation	Gamma reactions	
				Phosphor	Electro-luminescence	Nuclear bombs	
Chemical	Photosynthesis	Chemical reactions	Dissociation	Dissociation by radiolysis	Electrolysis	Radiation catalysis	
	Photochemistry					Ionization	
Thermal	Solar adsorption	Combustion	Heat exchange	Friction	Resistance heating	Fission	
						Fusion	
Kinetic	Radiometers	Metabolism	Thermal expansion	Gears	Electric motors	Radioactivity	Falling objects
		Muscles	ICE		Electro-striction	Nuclear bombs	Hydro turbines
Electrical	Solar cells	Fuel cells	Thermo-electricity	Electric generators		Nuclear batteries	
	Photo-electricity	Batteries	Thermionics				
Nuclear	Game-neutron reactions						
Gravitational				Rising objects (pumps)			

mal energy into other useful energy forms, such as electricity or chemical energy. However, there are always some exceptions. For example, the sugar-to-hydrogen reactions mediated by cascade enzymes have energy efficiencies of ~122% because these reactions are spontaneous ($\Delta G < 0$) and endothermic ($H > 0$) [18–20]. As a result but not against thermodynamics, extra chemical energy of hydrogen higher than carbohydrates in the aqueous reactants is converted from thermal energy from the environment without a cold sink [18,19,21].

The second law of thermodynamics states that one-point low-temperature thermal energy (i.e., without a temperature gradient between two sinks) cannot generate useful kinetic energy or in any cyclic process the entropy either increases or remains the same. (Note: kinetic energy, a form of potential energy, is the energy of an object due to its motion). In other words, the efficiency of an ideal heat engine cannot be higher than $(T_h - T_l)/T_l$, where T_h and T_l are the high and low temperatures for both sinks. The temperature difference ($T_h - T_l$) of any heat engine cannot be increased indefinitely for several reasons. First, materials in heat engines cannot tolerate extremely high temperatures for a long period of time. Second, simple combustion using air cannot reach very high temperatures due to the nitrogen dilution effect. Third, temperature of low-temperature sinks cannot be below ambient temperature. As a result, the best large heat engines (e.g., gas turbines) have efficiencies of ~60% [1]. In the transport sector, the maximum efficiencies of gasoline Otto (spark plug firing) internal combustion engines (ICE) and diesel (compression ignition) ICEs are ~32%, due to low compression ratios, and ~45%, due to high compression ratios, respectively [1,5,10].

Some energy forms (e.g., chemical, electrical, gravitational) can be stored, whereas others (e.g., electromagnetic) cannot be easily stored. The gravimetric energy densities of storable energies vary by several orders of magnitude (Table 2). Clearly, liquid fuels, such as diesel, gasoline, and jet fuels, are the most energy-dense fuels, followed by liquid biofuels, such as biodiesel, butanol, and ethanol (Fig. 2a), followed by hydrogen storage approaches and rechargeable batteries (Fig. 2b).

Some energy forms (e.g., chemical, electrical, nuclear) can be transported easily, whereas others (e.g., thermal, electric, kinetic) cannot. Among storable primary (chemical) energies, such as crude oil, natural gas and coal, crude oil has the highest gravimetric energy density. Because it is liquid, crude oil can be easily transported by numerous methods, including tankers, pipelines, trains and vehicles. As a result, the oil age replaced the coal age and approximately 95% of transportation fuels are made from crude oil.

In the transport sector, the prime mover is a device that transforms stored energies to kinetic energy in the transport vehicle. Liquid fuels (e.g., gasoline, diesel, and kerosene) and biofuels (e.g.,

Table 2

Gravimetric energy densities of chemical compounds, batteries, capacitors and flying wheels.

Name	Gravimetric energy density (MJ/kg)
H ₂ without container	143
Gasoline	~46.4
Diesel	~46.2
Jet fuel	~42.8
Ethanol	30.0
Methanol	19.7
Butanol	36.6
Wood	~18.0
Starch/cellulose	17.0
Corn kernel	~14.5
8% H ₂ mass including container	~11.4
Cryocompressed H ₂ including container	~9.3
4% H ₂ mass including container	~5.7
Compressed H ₂ (350 bars) including container	~5.0
Compressed H ₂ (700 bars) including container	~6.0
Lithium ion rechargeable battery	~0.56
NiMnH rechargeable battery	~0.36
Lead acid rechargeable battery	~0.14
Ultra-capacitor	~0.02
Super-capacitor	~0.01

ethanol, butanol, and biodiesel) are good choices in the current transport sector because they have high gravitational energy densities (Fig. 2). Although hydrogen is widely believed to be a clean and high-efficiency energy carrier, its low storage densities are the primary deterrent for its widespread use in the transport sector [7,11,22,23]. Although electricity can be stored in rechargeable batteries and capacitors, their energy storage densities are two orders of magnitude and three to four orders of magnitude, respectively, lower than those of liquid fuels (Fig. 2), limiting their potential application in the transport sector, especially in the USA with long-distance driving needs [7,10,24].

2.2. Energy costs related with non-point and point energy sources

Comparing the direct costs of available primary energy sources and secondary energy carriers is relatively easy (Table 3), although their prices may vary by several-fold. Among the three fossil fuels, coal is the least costly at ~\$50/ton (\$1.54/GJ), and crude oil is the most costly at \$80/barrel (\$15.04/GJ). Clearly, coal and natural gas are two good primary energy sources for heating. In contrast, most crude oil is upgraded to other high-end applications (e.g., transportation fuels and materials). With regard to renewable biomass resource, the collection and transportation costs of cellulosic waste (e.g., corn stover, wheat straw, rice straw) may be as low as \$35/ton (\$2.10/GJ), but this is still more costly than coal based on energy

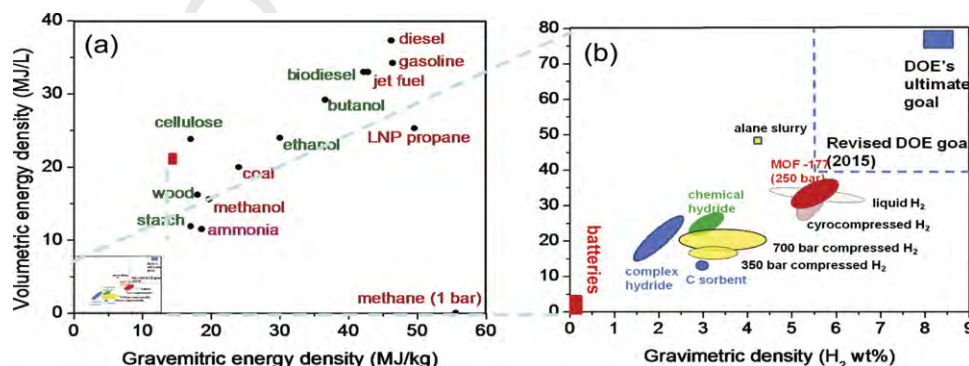


Fig. 2. Comparison of gravimetric and volumetric energy storage densities of liquid fuels, biofuels, solid energy carriers, hydrogen storage techniques, and rechargeable batteries.

Table 3
Direct costs of primary energies and secondary energy carriers.

Name	Price (\$)	Unit	Price (\$/GJ)	Energy density (MJ/kg)
Primary energy source				
Crude oil	80	barrel	15.04	~41.8
Natural gas	4	mbtu	3.79	~53.6
Coal	50	metric ton	1.54	~32.5
Cellulosic waste	35	metric ton	2.10	~16.7
Cellulosic crop	60	metric ton	3.60	~16.7
Soy oil	0.23	lb	12.00	42.2
Secondary energy carrier				
Electricity	0.10	kWh	27.78	
Diesel	2.7	gallon	19.49	~46.2
Gasoline	2.5	gallon	17.63	~46.4
Hydrogen	2.0	kg	13.99	143
Methanol	0.35	kg	17.77	19.7
Carbohydrate	0.22	kg	12.94	17.0
Ethanol	2.5	gallon	27.56	30.0
Biodiesel	3.5	gallon	27.43	42.2

content. Therefore, without considering carbon credits and potential environmental benefits, burning of biomass residues may not be economically competitive with coal. Dedicated cellulosic crops may be more costly at ~\$60/ton (\$3.60/GJ). Therefore, burning dedicated cellulosic crops is not economically competitive with use of coal. Alternatively, a promising application of cellulosic material is converting it to relatively-high value products – biofuels and value-added chemicals.

Secondary energies converted from primary energy sources are designed for specific applications. Because of some energy losses during the conversions, secondary energies are usually more costly than primary energies (Table 3). Carbohydrate isolated from cellulosic materials is the least costly secondary energy (\$0.22/kg and \$12.94/GJ). Further conversions would upgrade low-cost carbohydrate to other high-value fuels, such as ethanol, butanol, and hydrogen. In the transport sector, gasoline and diesel are two predominant reference liquid fuels (\$ ~ 18–25/GJ). Direct costs in Table 3 provide some information for judging any promising biofuels that would be economically produced from primary (e.g., biomass and coal) or secondary energies (e.g., carbohydrate, hydrogen and electricity).

Primary energies can be classified as point or non-point energy sources (Fig. 3). The three primary chemical energies – crude oil, coal, and natural gas – can be regarded as typical point primary energy sources (e.g., >10,000 W/m²) [5]. Because they are storable energies and their collection and storage costs are minimal, they had and have been used in the transport sector (Fig. 3). On the contrary, typical non-point primary energy sources, such as insolation, wind, and tidal energy, are being utilized much later. For these types of non-point energy sources, high hidden collection, conversion, and storage costs may prohibit their utilizations. For example, solar energy, the most abundant renewable primary energy on Earth, is not really free [25,26]. When non-point insolation with an average power density of ~170 W/m², is converted to electricity through photovoltaic panels, electricity can be collected through wires and grids and then be enriched to a point energy source. However, electricity storage on a large scale remains challenging [27–29]. When non-point insolation is converted to solar fuels, the processes of collecting the low-concentration secondary chemical energies must be solved [25,26,30,31]. Although wind power densities are low, ~2–4 W/m² electricity generated by wind turbines can be easily collected through wires [4]. There-

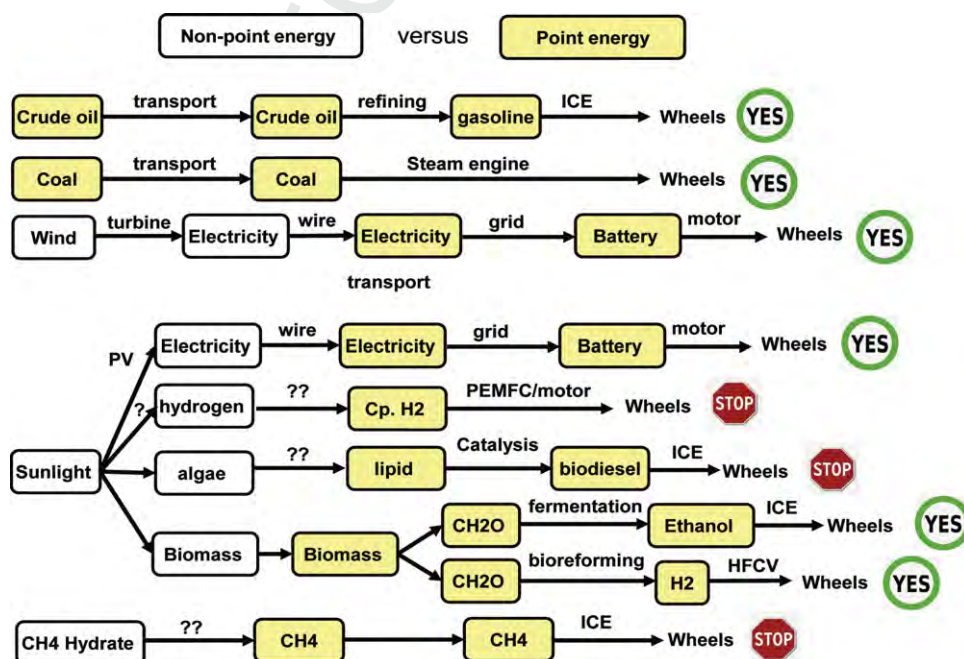


Fig. 3. Comparison of several parallel competing technologies based on their scalability potentials from non-point energies to point energies.

Table 4
Energy conversion efficiencies for numerous kinetic energy-generation devices.

Conversion	Efficiency (%)	Input
Large electrical motor	90–97	Electricity through grid
Small electric motor	60–75	Electricity in battery
FEMFC/electric motor	40–55	Hydrogen
Large steam turbine	40–45	Steam
Large gas turbine	35–40	Gas
Diesel ICE	30–35	Diesel
Best large steam engine	20–25	Coal
Gasoline ICE	15–25	Gasoline
Mammalian muscle	15–20	Glucose
Steam locomotive	3–6	Coal

fore, the conversion and collection of electricity from non-point insolation and wind energy is economically feasible while its low-cost storage remains challenging (Fig. 3). Methane hydride is the most abundant fossil fuel resource [14,32]. It is more abundant than crude oil, coal, and natural gas combined [14,33]. However, its high collection costs to a point chemical energy currently prohibit its use even though its separation and storage technologies are mature. In another case, although microalgae with theoretically high photosynthetic efficiencies [34,35] can convert non-point insolation to chemical energies, such as lipids, carbohydrates, and proteins [36], the consequent collection of non-point low-cell density wet algal cells to point dried cells followed by extraction are energy-intensive [20,35,36]. As a result, it is difficult to economically produce algal biodiesel compared to biodiesel made from plants [20,36]. In addition to the above collection challenges, algal biofuels also face scientific (e.g., low lipid contents and insulation of its grown from lipid accumulation) and engineering challenges (e.g., contamination, reaction configuration, mixing, and insolation density optimization). The separation costs of solar (gas) hydrogen produced by microalgae are less than those of extracting algal diesel from wet algae cells. However, a lack of low-cost, high-transparency, low-hydrogen diffusivity and durable materials, along with very slow algal hydrogen production rates and low photosynthesis efficiencies, prohibits algal hydrogen production from economically viable [37]. Terrestrial phyto biomass can be regarded as a nearly non-point primary chemical energy resource, but its drying, collection, and transportation costs are economically reasonable within a radius of ~50 miles [38,39]. Therefore, non-point biomass resources can be economically converted to a point biomass resource (i.e., storable chemical energy) for local biorefineries.

The concept of non-point and point primary and secondary energies is vital to evaluate and develop future (primary) energy utilization systems (Fig. 3). Without systematic consideration of cascade processes among primary energy sources (point vs. non-point), harvesting, conversion, storage, distribution, and utilization at end user sites plus economically viable solutions to address all of these issues, we will not be able to change from the current fossil fuel-based energy systems to any hypothetical new energy system.

2.3. Transport modes, prime movers, and their constraints

Modes of transport include vehicles on roads, planes in the air, trains on rails, boats in the water, electricity by cable, oil/gas by pipelines, and rockets in space. For some applications, the best choice is often very clear. For example, fixed-route trains are preferentially powered by electric motors/electricity lines along their routes; jet planes must be powered by high-density liquid fuels through light-weight gas turbines; pipelines are the most energy-efficient approach for transport of goods, but they are limited to

Table 5
Weight/power output ratios (power densities) for prime movers related with their applications.

Name	Power density (g/W)	Application
Steam engine	250–300	Trains, boats, stationary
Steam turbine	50	Boats, stationary
Direct methanol fuel cell	60	Portable electronics
Diesel ICE	3–4	Tractors, trucks, tanks
Gasoline ICE	1	Light-duty passenger vehicles, some planes (at the beginning)
PEM fuel cell	1	Hydrogen fuel cell vehicles
Electric motor	0.5–4	Any places
Gas turbine	0.02–0.07	Jet planes
Rocket engine	0.01	Rocket, missile, space shuttle

liquid fuels and natural gas; electricity is and will be mainly distributed by electric grids/cables [2,9].

In this paper, we focus on transportation biofuels for passenger vehicles on roads because passenger vehicles account for the largest fraction of transportation needs and represent the largest challenges in the transport sector. Light-duty passenger vehicles in the USA consume approximately 60% of the transportation energy [11,40]. Driving a vehicle without a fixed route requires the prime mover to convert stored energy to kinetic energy on wheels. Because passenger vehicles, in contrast to boats, are small, they result in several special constraints, such as, high energy storage capacities in a small container (e.g., ~50 L), high power outputs (e.g., ~20–100 kW per vehicle), affordable fuel costs (e.g., \$ ~20–30/GJ), affordable vehicles, low costs for rebuilding the relevant infrastructure, fast charging or refilling of the fuel (e.g., several minutes per time), and safety concerns [2,7,10,11].

Table 4 presents the typical energy conversion efficiencies of numerous prime movers. The most efficient system is large electric motors, with efficiencies of 90–97%, which is good for (high-speed) electric trains; while small electric motors have much lower efficiencies of 60–75%. Large steam turbines in power stations usually have efficiencies of 40–45%. Typical gasoline ICEs and diesel ICEs have efficiencies of 15–25% and 30–35%, respectively. Steam engines have much lower efficiencies of 3–6% so that ICEs eventually replaced steam engines. It is expected that high-efficiency electric motors and proton exchange membrane (PEM) fuel cells will replace low-efficiency ICEs if high-density electricity or hydrogen storage in vehicles is addressed [9,10,41].

Another important constraint is the weight to power output ratio (g/W) for prime movers (Table 5). Steam engines having weight to power ratios of 250–300 g/W are too big and heavy for passenger vehicles. As a result, they were widely used in stationary applications, trains, and boats until they were replaced with more compact and more efficient ICEs. Although steam turbines are one-fifth the size of steam engines, they are still too heavy and large for passenger vehicles; however, they are appropriate for stationary power stations and large-size boats. Very low weight-to-power ratio gas turbines and rocket engines are too costly and do not fit for regular vehicles with an expected running time of 5000 h. Therefore, gas turbines powered by high-energy density liquid fuels are widely used in jet planes whereas single-use rocket engines are used in rockets and missiles. Clearly, diesel ICEs, gasoline ICEs, and electric motors are viable choices for passenger vehicles.

The last constraint is energy storage density in vehicles. An ideal transportation fuel must be stored in both high gravimetric and volumetric densities for a sufficient driving range (e.g., 300 miles or 480 km) before refilling. Clearly, high-energy density liquid (bio)fuels have several advantages such as long travelling

distance, fast refilling (MJ/min), easy storage, and convenient distribution of liquid fuels. Although hydrogen has a very high energy density based on mass (143 MJ/kg), it has very low volumetric densities because it is gas at ambient conditions. It is very challenging to find a practical solution to meet the US Department of Energy's ultimate hydrogen storage goals – $>8 \text{ H}_2$ mass % and $>70 \text{ kg of H}_2/\text{m}^3$ (Fig. 2b). The use of high energy density hydrogen carriers, such as hydrocarbons, biodiesel, methanol, ethanol, and carbohydrates may solve hydrogen storage challenge [7,42–44]. In this approach, end users must have an on-board reformer that can convert hydrogen carriers to hydrogen and purify the hydrogen stream before entering the PEM fuel cells. Significant efforts were conducted to produce hydrogen from biodiesel or sulfur-free hydrocarbons in the 1990s, but the bulky size of reformers, difficult and complex controls, and a need for hydrogen purification prohibited their potential applications in small-size passenger vehicles [6]. Currently, some research efforts are being conducted to convert ethanol to hydrogen through chemical catalysis [43], but it may be not economically competitive because ethanol is more costly than hydrogen (Table 3). In addition, hydrogen made from ethanol is not pure enough to enter PEM fuel cells directly. Methanol can be converted to hydrogen easily *via* reforming or can be converted to electricity through a direct methanol fuel cell (DMFC) [44]. Because DMFCs have relatively low power densities and low energy conversion efficiencies [45,46], they may work well as a power source for low-power portable electronics [47], but not for high-power vehicles (Table 5).

The use of renewable carbohydrates as a high-density hydrogen carrier and energy source could address high-density energy storage and low-cost sustainable energy production due to emerging synthetic biology technology – cell-free synthetic pathway biotransformation (SyPaB) [11,14,48–50]. The assembly of numerous enzymes and co-enzymes *in vitro* can create a complex set of biological reactions or pathways that microorganisms or chemical catalysts cannot achieve, for example, $\text{C}_6\text{H}_{10}\text{O}_5(\text{aq}) + 7 \text{ H}_2\text{O}(\text{l}) \rightarrow 12 \text{ H}_2(\text{g}) + 6 \text{ CO}_2(\text{g})$ [18,19]. Because enzymes are 100% selective, work under moderate reaction conditions, and generate highly pure hydrogen, carbohydrates are a promising hydrogen carrier with a gravimetric density of carbohydrates/water of 8.33 H_2 mass% [6,7,11].

Current rechargeable lithium batteries have energy densities of $\sim 150 \text{ Wh/kg}$ (i.e., 0.54 MJ/kg) [51,52], far lower than those of liquid fuels, solid fuels and available hydrogen storage technologies (Fig. 2), even accounting for higher efficiencies in electric motors (Table 4). As a result, battery electric vehicles (BEVs) have a very short driving distance per charge. If the energy densities of rechargeable batteries could be increased by 10-fold, as some have claimed [53], safety concerns would prohibit their use in personal vehicles. Based on the basic configuration of batteries and the combustion chemical energy in lithium (i.e., 43.1 MJ/kg), future energy densities of rechargeable lithium batteries are expected to increase by two-fold [51,52], but not ~ 10 -fold as some cheaters claimed [53]. Rechargeable batteries require a long recharging time and have limited recharging cycles. Although ultra-fast charging batteries have been claimed [54], these capacitor-like batteries have been achieved at the cost of decreasing energy storage densities [55]. If the obstacles to ultra-fast recharging and battery life-time were solved, a huge infrastructure investment would be needed to upgrade the electrical grid, install sockets for fast recharge, and build power stations [3]. Disposing and recycling a large number of used rechargeable batteries would be another environmental challenge [3]. In addition, costly batteries may restrict their broad implementation in the transport sector even if production costs could be decreased by several-fold. It is not realistic to assume that battery costs would be significantly reduced following Moore's Law because it is impossible to exponentially both decrease material

consumption in batteries and increase battery performance according to the basic physical limits of materials. A national BEV fleet would offer no overall primary energy savings and no carbon emission reduction compared to a highly efficient gasoline car fleet or the large-scale adoption of hybrid vehicles unless all of the electricity consumed by the BEVs were generated by renewable primary energy sources [3]. The lifetime of rechargeable batteries is two or three years of service (check your batteries for cellular phones and laptops), while major car components are now designed to last at least ten years. Furthermore, the capacity loss rates of rechargeable batteries depend on temperature; for example, standard capacity loss rates are 6% at 0°C , 20% at 25°C , and 35% at 40°C , per year [3]. The last question is whether there is enough low-cost lithium for BEVs.

The principal challenges facing the development of rechargeable batteries for electric vehicles are cost, safety, cell energy density (voltage \times capacity), rate of charge/discharge, and service life [56]. The future of electric cars may repeat Edison's frustrating experience with electric cars when better technologies (e.g., sugar fuel cell vehicles and hybrid ethanol-diesel ICEs) appear. Because of the above questions and special requirements for the transport sector, the International Energy Agency along with numerous experts in big auto companies, predict that hydrogen fuel cell powered train systems will dominate in the future [24,57]. Therefore, a complete switch to all battery electric cars is utterly unrealistic [3] but BEVs may remain popular in some special markets, for example, in golf courts.

In summary, a combination of liquid fuels and ICEs is a logical and historic choice for passenger vehicles. Advanced biofuels associated with powertrain systems must be economically competitive with current systems and new future systems. All estimates should be realistic without breaking the basic laws of thermodynamics and physical chemistry.

3. What is vital to advance biofuels

3.1. Biofuels overview

Numerous biofuels are produced and/or could be produced in the future, including grain/sugar ethanol, biodiesel, green diesel (oils and fats blended with crude oil), cellulosic ethanol, pyrolysis biooils, syngas liquids, algae diesel/jet fuel, *n*-butanol, iso-butanol and/or long-chain alcohols [58,59], bioelectricity [60,61], bioalkanes [62], fatty acid esters [63,64], enzymatic hydrogen [18,19], hydrocarbons [16,17], and waxes [63]. Which biofuels will become short-, middle- and long-term transportation fuels is a subject of debate.

Analyzing biofuel production and conversion systems is very complex. This process is similar to circuit network analysis because it involves many different conversion elements among numerous cascade nodes and/or parallel circuits (Fig. 4a). Between any two nodes, the most economically feasible conversion would have the highest energy efficiency if there were no technological obstacles. In principle, the highest energy efficiency is like the lowest resistance between any two nodes in the network so that it would allow the most energy flux to pass through the highest efficiency conversion combinations and to pass by un-competitive conversions. For example, a comparison of the energy efficiency of ethanol, butanol, fatty acid esters, and hydrogen from sugars clearly suggests that hydrogen production by SyPaB is the most promising followed by ethanol and butanol by considering three factors: thermodynamics in biological conversion pathways, bioenergetics for microbial fermentation, and product separation (Fig. 4b) [20]. The production of fatty acid esters [63] or bioalkanes [62] through semi-aerobic fermentations may be not economically competitive with available

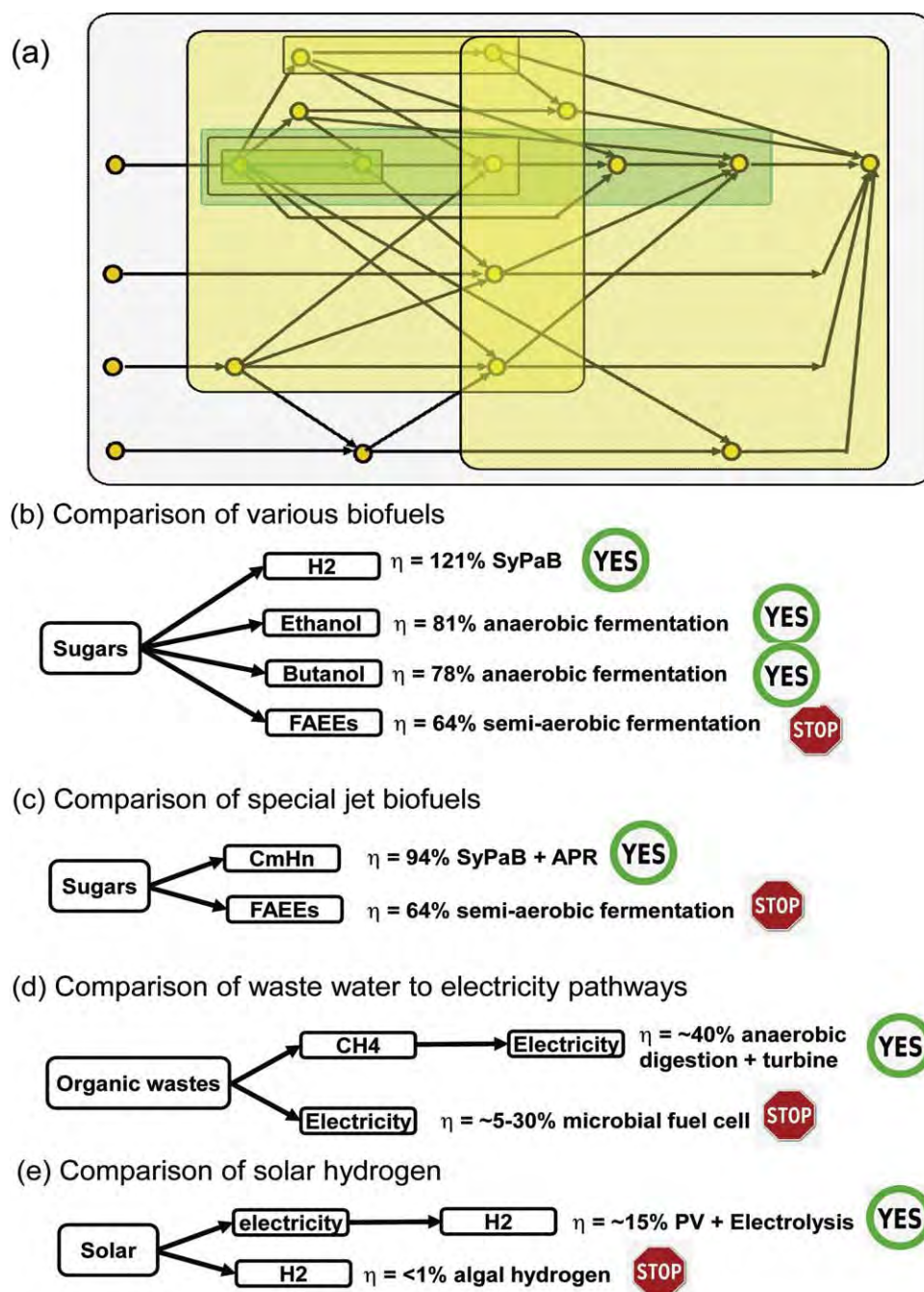


Fig. 4. Analysis of complex energy utilization and conversion network (a) and comparison of several parallel pathways sharing the same input and same/similar output (b-e).

ethanol fermentation even after significant improvements [20]. Some advocates for the production of microbial fatty acid esters or bioalkanes may argue that these products could be used as jet fuel, for which ethanol and hydrogen are not appropriate. At this case, the production of jet fuel through a very high-energy efficient hybrid of biocatalysis and aqueous phase reforming ($\eta = 94\%$) would be better than that of fatty acid esters ($\eta = 64\%$) (Fig. 4c) [17]. Similarly, microbial fuel cells (MFCs) cannot compete with anaerobic digestion followed by methane power stations [65] due to lower energy efficiencies and high capital investments (Fig. 4d). In the future, MFCs may have few applications as micro-power sources for remote areas and treat low biochemical oxygen demand (BOD) waste water. Similarly, solar hydrogen through solar cells and elec-

trollysis is far more energy efficient than algal hydrogen (Fig. 4e) and has no problem associated with hydrogen collecting (see Section 2).

3.2. Non-food biomass-based biorefineries

Relatively-dry lignocellulose delivered at the door of biorefineries can be regarded as a point chemical energy source. In this section, I focus on the key conversions from delivered lignocellulose to wheels of passenger vehicles (i.e., sugar release, co-utilization of lignocellulose components, and biomass-to-wheel efficiency (Fig. 5)), discuss what is vital to advance economically competitive biofuels production, and provide perspective suggestions.

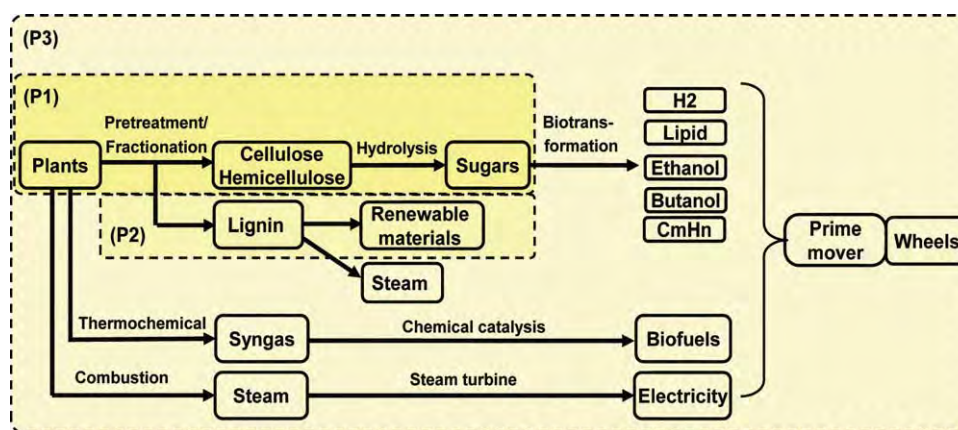


Fig. 5. Biomass conversions and utilizations with three blocks: (i) cost effective sugar release, (ii) co-utilization of lignocellulose components, and (iii) enhancing biomass-to-kinetic energy efficiency.

3.2.1. Priority 1: Cost-effective sugar release from non-food biomass

The largest economical and technical obstacle to non-food biomass refinery is the cost-effective release of fermentable sugars [8,66,67]. When fermentable sugars are isolated at prices of \$~0.22/kg [6,8], further conversion to cellulosic ethanol or other value-added biofuels/chemicals would not be prohibitive to economically viable biorefineries. Natural cellulose molecules are closely associated with other structural polymers, such as hemicellulose, lignin, pectin and/or proteins, making them difficult to degrade [8,66,68].

Biomass saccharification usually involves two sequential steps: biomass pretreatment/fractionation and enzymatic hydrolysis [68–70]. Lignocellulose pretreatment may be the most costly step [71,72], accounting for approximately 40% of total processing costs [73]. Also, it impacts the cost of most other operations, including the reduction in lignocellulose size prior to pretreatment [74] and enzymatic hydrolysis and fermentation after pretreatment [68]. Pretreatment is strongly associated with downstream costs involving enzymatic hydrolysis rates, enzyme loading, mixing power consumption, product concentration, detoxification if inhibitors were generated, product purification, power generation, waste treatment, and other process variables [8,71,75].

The goal of pretreatment/fractionation is to alter the physical features and chemical composition of lignocellulose to make it more digestible by high-selectivity cellulase. The root causes of the recalcitrance to degradation are thought to be attributed to a number of factors, such as substrate accessibility, degree of polymerization, pore volume, presence of lignin and hemicellulose, and particle size. Also, a synergy among various actions of depolymerizing enzymes is needed [67,68,76–79]. To address the above factors, pretreatment improves substrate accessibility and effectiveness by

- De-crystallizing cellulose fibers to increase cellulose accessibility to cellulase,
- Decreasing the degree of polymerization of cellulose to suitable lengths (Note: too short DP water-soluble cellulose fragments are not preferred because of costly separation of soluble sugars from solvents or water),
- Removing hemicellulose,
- Removing lignin, and
- Decreasing acetyl groups from remaining hemicellulose if a significant amount of hemicellulose remains.

Nearly all lignocellulose pretreatments can be divided into five main categories: (i) physical, including dry milling (chipping, ball

milling and comminuting), wet milling, irradiation, and cellulose swelling/dissolution reagents (e.g., concentrated phosphoric acid, ionic liquids, Cadoxen, ZnCl₂, DMAc/LiCl, and so on); (ii) chemical, including dilute acids (e.g., dilute H₂SO₄, H₃PO₄, HCl, acetic acid, formic acid/HCl), alkalis (e.g., NaOH, lime, ammonia, amine, etc.), organosolv, and oxidizing agents (e.g., O₃, NO, H₂O₂, NaClO₂); (iii) physio-chemical methods, including steam explosion with or without catalysts, CO₂ explosion, ammonia fiber expansion (AFEX), hot water, hot water with flow-through, cellulose- and organic solvent-based lignocellulose fractionation (COSLIF), and supercritical fluid extractions (e.g., CO₂, CO₂/H₂O, CO₂/SO₂, NH₃, H₂O); (iv) biological methods (e.g., white rot fungi); and (v) combinations of the methods described above (e.g., SO₂ explosion followed by H₂O₂ delignification). Most pretreatments share one or several common shortcomings: (i) severe pretreatment conditions, except AFEX and COSLIF, resulting in some sugar degradation and inhibitor formation [80–82]; (ii) low or modest cellulose digestibility due to the presence of residual lignin and hemicellulose and incomplete disruption of highly ordered hydrogen bonds in cellulose fibers [70,76,83]; (iii) high cellulase loading required (e.g., 15 filter paper units of cellulase per gram of glucose); (iv) slow hydrolysis rates (e.g., 72–120 h); (v) large utility consumption; and (vi) less co-utilization of other major components of lignocellulose, except organosolv and COSLIF, resulting in huge capital investments (e.g., \$~8 per annual gallon ethanol capacity) [8].

Ideal pretreatment/fractionations have the following features:

- Preserve cellulose and hemicellulose (i.e., minimize sugar degradation);
- Have low processing costs, including low use of costly cellulase plus its recycling, little formation of toxic inhibitors, removal of acetate before fermentation if possible, low energy and water use, low waste treatment, and few pollutants emitted;
- Lower overall capital investment;
- Effective for addressing diverse lignocellulosic feedstocks;
- Separate cellulose and hemicellulose for better revenues; and
- Isolate high-quality lignin for making other value-added products.

An ideal pretreatment with all of the above features may be costly. Few biomass pretreatments, such as organosolv [84,85] and COSLIF [86], take in account co-utilization of lignocellulose components at the beginning. Organosolv pretreatment, developed from organosolv pulping, was studied for biorefineries in the 1980s [87,88]. Organosolv pulping was designed to replace the Kraft pulping method due to its environmentally benign ben-

efits, small capital investment, and co-product utilization [89–93]. It uses a lignin-extracting solvent blend containing catalysts and water/organic solvents, such as ethanol or methanol, to extract lignin in high temperature and pressure digesters [87,88,94,95]. The lignin extraction step is conducted at 180–200 °C and ~400 psi in a blend of ethanol/water in the range of approximately 50:50 (w/w) plus approximately 1% H₂SO₄ for ~30–90 min [93,95]. After organosolv pretreatment, a black liquor containing sulfur-free lignin, furfural, hemicellulose sugars, and other natural chemicals, such as acetic acid [92], is further processed to separate lignin, recycle ethanol, distill acetic acid and furfural, and separate hemicellulose [95]. An economic analysis report suggests that revenues from the multiple co-products, particularly the lignin, ethanol, and xylose fractions, ensure the economic viability of a small plant (approximately ~100 metric tons per day) [95], which is one-fifteenth the size of a typical ethanol-only lignocellulose biorefineries.

A functionally-based kinetic model predicts that the major cause of the recalcitrance of lignocellulose is low cellulose accessibility, which restricts cellulase activities [96]. As a result, cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) featuring moderate reaction conditions was invented in 2005 and published in 2007 [86,97]. The original basic ideas of COSLIF were (i) to disrupt orderly hydrogen bonds in cellulose fibers so to enhance substrate accessibility to cellulase; (ii) to remove lignin and hemicellulose based on their different solubilities in organic solvent and water; (iii) to fractionate lignocellulose components under moderate conditions to avoid sugar degradation, to decrease inhibitor formation, and to lower utilization consumption; and (iv) to fractionate lignocellulose components for higher revenues [86]. COSLIF can separate amorphous cellulose from hemicellulose and lignin based on their different solubilities in concentrated phosphoric acid, the organic solvent, water and their mixtures. COSLIF not only enables the highest sugar yield at the lowest enzyme loadings (e.g., 1–3 filter paper units per gram of glucan) [98,99] but also separates lignocellulose components (e.g., lignin and acetic acid) for potential higher revenues [86]. This technology can be applied to a variety of biomass feedstocks, such as corn stover, switchgrass, poplar, common reed, hemp hurds, elephant grass, energy cane, and bamboo [86,98–101]. Therefore, it could be regarded as a feedstock-independent pretreatment. This feature may be important to build biorefineries that utilize diverse feedstocks and save feedstock transportation costs. Furthermore, our comparative studies between COSLIF and other pretreatments suggest that increasing cellulose accessibility to cellulase is more important than removing lignin [76,100], which contradicts a common belief in biomass pretreatment research [102]. This discovery would be important to guide bioenergy plant development [76]. Since removing lignin is not as important as increasing cellulose accessibility, more stable and less corrosive ethanol can replace acetone in COSLIF, which would decrease organic solvent recycling costs and capital investments [98,99].

Cellulase costs may be not a show stopper to second generation biofuels since Genencor and Novozymes claimed a 20–30 fold reduction in cellulase production costs [78]. However, they still represent one of the costly factors (e.g., from 0.50 to 1.00 US\$ per gallon of cellulosic ethanol). Typical cellulase uses are nearly 100–200 g of cellulase per gallon of ethanol produced based on an enzyme loading of 15 filter paper units per gram of glucan [77,103,104], where specific activities are approximately 0.6–1.5 filter paper unit per mg of fungal cellulase [105]. Most industrial enzyme companies (e.g., Novozymes, Genencor, Iogen, Dyadic) produce commercial cellulases from *Trichoderma* or its derivative strains except Dyadic's *Chrysosporium lucknowense*. Commercial fungal fermentations can currently produce more than 100 g of crude cellulase (weight) per liter of broth, which is much higher

than bacterial enzyme producers [106,107]. As a result, current fungal enzyme production costs based on protein weight have decreased to very low levels (e.g., ~\$5/kg of dry weight cellulase). Since it is relatively difficult to further decrease cellulase production yields based on sugars (i.e., 0.24g cellulase per g glucose consumed), we must decrease cellulase usage per substrate.

To further increase a cellulose/enzyme ratio from ~50 (i.e., 15 filter paper units per gram of glucan) to 100 or higher [49,77,103,104,108], cellulase use can be decreased by increasing its mass-specific activity by enhancing individual components using rational design or directed evolution [108–111], reconstituting different action-mode cellulase components plus new components (cocktail development) on cellulosic substrates [67,112–115], and recycling enhanced thermostability cellulases [104,116]. Directed evolution is becoming an important tool for enhancing cellulase performance because it is independent of protein structure and the complex relationship between heterogeneous cellulose and cellulase components [77]. It is noted that cellulase activities on artificial substrates have no relation to their activities on natural substrates, making directed cellulase evolution difficult [77,105]. For example, a glycoside hydrolase family 5 endoglucanase (Cel5A) is displayed on the surface of *E. coli* by using the *Pseudomonas syringae* ice-nucleation protein as an anchoring motif to identify Cel5A mutants with improved thermostability on carboxymethyl cellulose (CMC). Only some of more stable mutants displayed on the cell surface are more stable than wild-type Cel5A when expressed in a free form. Cellulose-binding module (CBM)-containing wild-type Cel5A and mutants show much longer half lifetimes of thermo-inactivation when they are pre-bound to substrates before thermo-inactivation, especially to insoluble substrates [109]. The addition of either a CBM on the N- or C-terminus does not influence cellulase activity on soluble substrate CMC but drastically decreases its activity on insoluble substrates – regenerated amorphous cellulose and microcrystalline cellulose [109]. The best Cel5A mutant has similar activity to wild-type but an increased half lifetimes of thermo-inactivation on CMC, RAC, and Avicel by approximately 90%, 36% and 46%, respectively. All of the results suggest the complexity of cellulase engineering [109]. A real breakthrough in directed cellulase evolution was achieved by using *B. subtilis* as a host for identifying secretory cellulase mutants on natural insoluble cellulose [110]. A new protocol with high transformation efficiencies of ~10⁷ transformants per μg of PCR product is developed based on super-competent cells prepared from a recombinant *B. subtilis* strain SCK6 and multimeric plasmids. Both protein expression levels and specific activity of glycoside hydrolase family 5 endoglucanase on regenerated amorphous cellulose have been improved through this new system [110]. In this way, the activity of the *C. phytofermentans* processive endoglucanase Cel9 has been enhanced, too (manuscript in preparation). Among three key bacterial cellulase components – family 5 endoglucanase, family 9 processive endoglucanase and family 48 cellobiohydrolase [67], family 9 cellulases are believed to be the most important cellulase components in bacterial cellulase mixtures [67,117,118]. Furthermore, it is found that linking different cellulase components as complexes through scaffoldins enables to increase mass-specific cellulase activity due to enzyme proximity effects [119–121]. Cell-bound cellulase complexes exhibited several-fold higher hydrolysis digestibilities than free mini-cellulosomes on insoluble cellulosic materials [122]. The synergy among such cellulosome-microbe complexes was not completely explained by fast removal of hydrolysis products from the bulk fermentation broth, and appeared due to fast substrate channeling of soluble hydrolysis products in the boundary layer on the surface of solid cellulosic materials to adjacent cells [121,122] (manuscript in preparation).

Table 6

Simple economic analysis of biomass (corn stover)-based biorefineries based on their major intermediate outputs – sugars, lignin, and acetate. Co-utilization 1: all sugars for biofuels production, a half of lignin for the production of high-value polymers and another half for burning, as well as acetate isolation; and co-utilization 2: all glucose and a half of hemicellulose for the production of biofuels, a half of rare sugars isolated from hemicellulose for the production of high-value products, all lignin as burning fuels, as well as 80% acetate separation.

Composition	Weight (kg/dry ton)	Simple utilization		Co-utilization (1)		Co-utilization (2)	
		Price (\$/kg)	Price ^a	Price (\$/kg)	Price	Price (\$/kg)	Price
Glucan	361	\$0.22	\$ 79.42 ^a	\$ 0.22	\$ 79.42	\$ 0.22	\$ 79.42
Hemicellulose	240	\$0.22	\$ 52.80 ^a	\$ 0.22	\$ 52.80	\$ 1.10 ^e	\$ 132.00
Lignin	208	\$0.05 ^b	\$ 10.40	\$ 1.10 ^c	\$ 114.40	\$ 0.05	\$ 10.40
Acetyl group	32			\$ 0.04 ^b	\$ 5.20		
Sum			\$142.62	\$ 1.00	\$ 25.60 ^d	\$ 1.00	\$ 25.60
					\$ 277.42		\$ 273.82

^a 90% sugar yield.

^b **Burning** as white coal in the pulping and paper industry, equaling \$50/ton of coal.

^c **Lignin** used as polymeric substitute for petroleum's based plastics.

^d 80% separation efficiency.

^e 50% of rare sugars in hemicellulose is used for the formation of high-value products.

3.2.2. Priority 2: Co-utilization of lignocellulose components

Simple economic analyses between biomass feedstock prices and potential intermediates (for example, sugars and lignin) suggest that co-utilization of lignocellulose components increase overall revenues by several-fold (Table 6). The profitability of a whole process plant can be estimated by its return on investment (ROI) [123].

$$ROI = \frac{R_M + R_C - C_F - C_P - C_O - C_D - TAX}{TCI} \quad (1)$$

where R_M is the revenue of the main products; R_C is the revenue of co-products; C_F is the cost of feedstock; C_P is the cost of processing; C_O is the cost of operation; C_D is the cost of capital depreciation, and TCI is the total capital investment. According to the USDA Biofuels Strategic Production 2010 Report, a typical cellulosic ethanol biorefinery with a capacity of 40 million gallons per year (i.e., ~500,000 dry tons of biomass per year or **1500 tons** per day) needs a cellulosic capital investment of \$8 per gallon or \$ ~ 1.1 per kg of sugar.

Table 6 shows simple economic analyses for a sugar biorefinery that can produce sugars, lignin and/or acetate as major products rather than biofuels. The margins between expenditures for purchasing biomass feedstock and revenues of sugars only are very small [8,86]. Such small margins (\$ ~ 50–100/ton of dry biomass) have to pay for processing costs for pretreatment (e.g., utilities, chemicals consumed or recycled, detoxification), bioconversion (e.g., cellulase, media, utilities), waste treatment, and utilities plus capital investment and depreciation, taxes, and labor. Therefore, the overall revenues of whole biorefineries must be increased, for example, by isolating value-added lignocellulose components (e.g., high quality lignin), producing other valuable residues [8], and/or reducing costly inputs (e.g., cellulase, medium nutrients) [107].

The history of oil refineries and corn biorefineries teaches us two important lessons [8,124]. First, co-utilization of complex raw material is vital to the profitability of the whole process. The corn-to-ethanol industry, regardless of whether it is dry-milling and wet-milling, must generate revenues from co-products, such as animal feed and corn oil. Ethanol production alone cannot guarantee a net profit for the whole process. Similarly, oil refineries were developed from single-product (e.g., kerosene for lighting) plants to multiple-product complex refineries [125]. Second, revenues generated from co-products can offset process complexity (capital investment) and higher processing costs. For example, although wet milling is a more complex bioprocess and requires higher initial capital investment and higher processing costs than dry milling, the former is the dominant process [124,126] because of greater revenues from co-products, such as gluten feed, gluten meal, and corn

oil [127]. Such co-products in wet milling biorefineries account for approximately a half of total revenues [128], whereas co-products of dry milling biorefineries account for only 20% of total revenue [124].

Potential revenues from high-value co-products would not only provide economic opportunities for the first several biorefineries with relatively small markets for co-products [8] but also for a small biorefinery that could achieve economies of scale by saving feedstock transportation costs and fully utilizing scattered biomass resources [95,101,124]. After several successful operations, investment risks and processing costs for lignocellulose biorefineries would be reduced greatly through further technology improvements [8,71,124]. Similar scenarios have occurred in the commercialization of bioethanol from corn kernels and sugarcane [124,128], wind power [129,130], and oil extraction from oil shale [131,132]. However, processing costs cannot decrease to as low as wished because most unit operations have been well developed for more than 100 years, and their energy consumption cannot be decreased any further due to physical limitations.

Hemicellulose and its derivatives have much higher inherent values than cellulose. They could have large potential markets because limited supplies of hemicellulose sugars restrict their potential applications and associated research [133]. Hemicellulose has been used as plant gum for thickeners, adhesives, protective colloids, emulsifiers, and stabilizers [8,127]. A new promising application for hemicellulose is making low-oxygen diffusion biodegradable films [134–136]. Partially hydrolyzed hemicellulose oligosaccharides may be higher value co-products, such as animal feed additives [137,138]. Monomeric xylose from hemicellulose has at least 5-fold the inherent value of glucose from cellulose (e.g., \$1.1/kg xylose). Xylose can be converted to another higher value product, xylitol, which has a large market as a sweetener and nutritional additive or jet fuel precursor [17,139–141]. Furfural, a chemically-degraded product of xylose, is used to produce lubricants, coatings, adhesives, plastics, or polytetramethylene ether glycerol for the production of Lycra and Spandex with a selling price of ~\$1/kg [95,127,142]. Another potentially huge market for furfural is a precursor of Nylon 6,6 and Nylon 6. Early production of Nylon 6,6 started with hemicellulose-derived furfural, but it was abandoned in 1961 due to competition of low-cost petroleum products [8,127].

Lignin applications depend primarily on the development of the production of sulfur-free high-quality lignin [143]. Most technical lignins – Kraft sulfur-containing lignin and lignosulfonate – are not suitable for further conversion to value-added products [144]. Sulfur-free organosolv lignin and COSLIF lignin, which are extracted using organic solvents, may have various potential applications: (i)

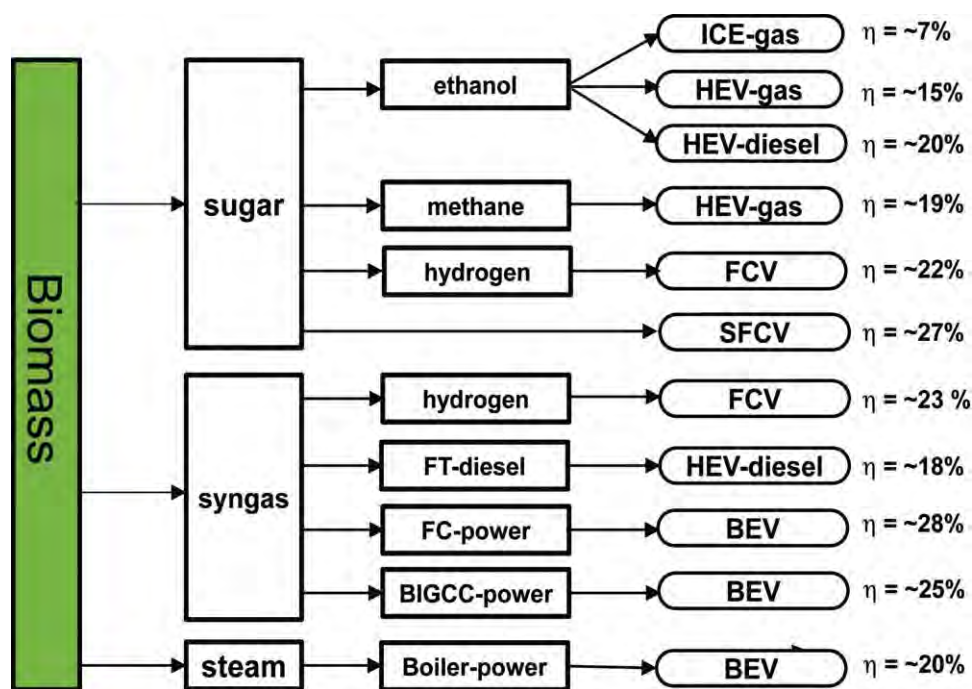


Fig. 6. Comparison of biomass-to-kinetic energy (BTW) efficiencies through different scenarios.

lignin-based bio-dispersant for slow-release fertilizers and pesticides [145], (ii) substitution of polymeric materials for phenolic powder resins, polyurethane, polyisocyanurate foams, and epoxy resins, and (iii) soil conditioners, humus formation aid [144,146]. The important role of lignin co-utilization in economically viable biorefineries is raising interests for discovering new applications for these products [147–151].

3.2.3. Priority 3: High-efficiency biomass-to-kinetic energy

In addition to the sugar-to-biofuel energy efficiency analysis [20], it is more interesting to calculate energy efficiency from biomass to kinetic energy on wheels (Fig. 6) because this information would help make informed decisions for future biomass-based transportation systems. To avoid many assumptions and uncertain inputs in life cycle analysis, a simple, straightforward, and transparent biomass-to-wheel (BTW) analysis is suggested [10]. The biomass-to-wheel efficiency (η_{BTW}) is a ratio of the kinetic energy on wheels to the chemical energy in the harvested biomass delivered to biorefineries, involving three sequential elements – biomass-to-fuel production, fuel transport and distribution, and a powertrain system responsible for fuel-to-wheel. The η_{BTW} value can be calculated as below:

$$\eta_{BTW} = \frac{W}{E_B} = \eta_{BTF} * (1 - \eta_{TDL}) * \eta_{FTW} \quad (2)$$

where, W is kinetic energy transferred to wheels; E_B is the chemical combustion energy in biomass, where dry corn stover as a typical biomass contains $\sim 65\%$ carbohydrates (cellulose and hemicellulose), $\sim 18\%$ lignin, $\sim 5\%$ ash, $\sim 12\%$ other organic molecules [152,153]; and the E_B value is 16.5 MJ low heating value/kg of dry corn stover [154]; η_{BTF} is the biomass-to-fuel (BTF) efficiency through biorefineries or power stations without a significant input or output of other energy; η_{TDL} is the loss in efficiency of the fuel during its transport and distribution; and η_{FTW} is the fuel-to-wheel (FTW) efficiency from the fuel to kinetic energy in the powertrain system.

Eleven scenarios are analysed based on BTW efficiency from the same input – the chemical energy in the delivered biomass – to

the same output of kinetic energy on wheels (Fig. 6). Through the sugar platform, we analyse four different biofuels: liquid ethanol, gas methane, hydrogen, and solid sugar. Ethanol can be used in regular ICE vehicles based on a gasoline ICE (ICE-gas), hybrid electric vehicles based on a gasoline ICE (HEV-gas), and hybrid electric vehicles based on a diesel-like ICE (HEV-diesel). (Note: ethanol can only be blended with gasoline at certain percentage below 10% (v/v) in most regular ICEs while high ethanol blends can be used in flex-fuel vehicles). Methane and hydrogen can be used in HEV-gas and fuel cell vehicles (FCV), respectively. Solid sugar or sugar/water slurry can power high-efficiency sugar fuel cell vehicles (SFCV) [6,7]. On the syngas platform, we considered three secondary energies: hydrogen, liquid Fischer-Tropsch diesel, and electricity from fuel-cell power and biomass integrated gasification combined cycle (BIGCC)-power. The last platform is electricity generated by simple boiler-power, fuel cell (FC)-power and BIGCC-power. Electricity can be distributed through electric grids and charge BEV.

Because infrastructure and vehicles for liquid ethanol are currently available, it is clear that ethanol-ICE-gas is the best short-term solution. Considering great potential in enhancing powertrain systems from ICE-gas to HEV-gas, BTW efficiencies based on ethanol would double from $\sim 7\%$ to $\sim 15\%$. Furthermore, the adoption of HEV-diesel (compression-ignition engine) for ethanol would enhance the BTW efficiency to $\sim 20\%$, due to the high-octane ethanol. Although a number of advanced biofuels, such as, fatty acid esters, bioalkanes, hydrogen, and bioelectricity, have been proposed and investigated, they show lower BTW efficiencies compared to ethanol and butanol (see Fig. 6 and reference [10]) as well as have more technical [62–64,155] or infrastructure challenges [3]. Therefore, the production of cellulosic ethanol and butanol from renewable sugars would be a short- or middle-term solution for the transport sector. It is worth noting that although high-energy density liquid biofuels are compatible with current internal combustion engines and can be distributed easily and safely, energy efficiencies of internal combustion engines are restricted by the second law of thermodynamics (see Section 2).

Sugar fuel cell vehicle (SFCV) is a hypothetical hybrid powertrain system based on a hydrogen FCV in which sugar is used as a hydro-

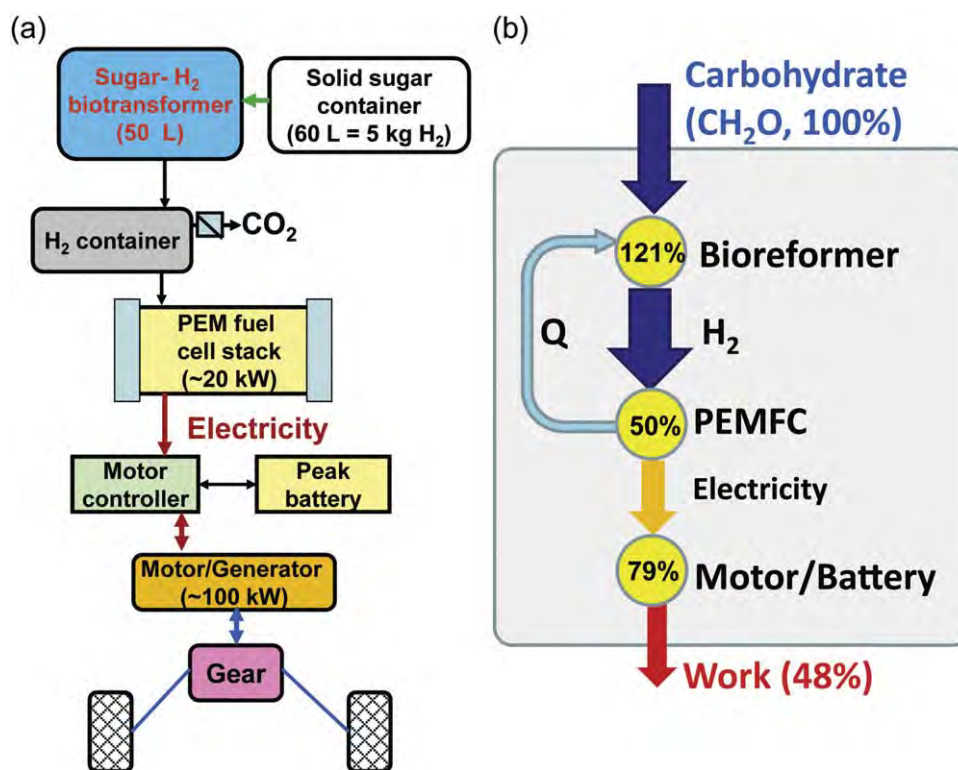


Fig. 7. Scheme of a sugar fuel cell vehicle (a) and energy conversion analysis for the primer mover (b).

gen carrier and is converted to high-purity hydrogen on-board [6,7] (Fig. 7a). Compared to FCV, SFCV can solve problems associated with hydrogen production, storage, distribution, and safety [6,10,19]. Also, this new system has very high efficiencies of ~48% (biotransformer, 122%, note: a combustion energy gain by absorbing waste heat from fuel cells; fuel cell systems, 50%; and electric motor/battery, 79%) (Fig. 7b). As compared to BEV through the FC-power scenario, SFCV would have higher energy storage densities, faster refilling rates, better safety, and fewer environmental burdens than BEV [10].

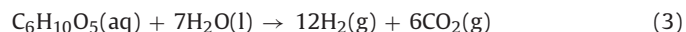
3.3. Resource analysis and environmental impacts

Approximately 150 billion tons of lignocellulosic materials are produced by photosynthesis yearly [68,69,156], compared to 1.5×10^8 tons of synthetic polymers [157]. The United States produces approximately 8.5 billion tons of net primary production and is expected to have the potential to produce more than 1.3 billion dry tons of biomass, including agricultural resources (933 million tons/year) and forest resources (368 million tons/year) [158]. Among such a large amount of biomass resource, a small fraction can be economically utilized, called annual biomass reserves. It is estimated that approximately one billion tons of dry biomass could replace at least 30% or more of the USA's present petroleum consumption through the biomass-to-ethanol/ICE scenario [158]; while the most optimistic estimate would be 100% replacement through high BTW efficiency SFCV [10]. In a word, the role of biomass in the transport sector highly depends on BTW efficiencies.

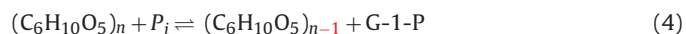
4. A silver bullet: Sugary hydrogen bridging the hydrogen and carbohydrate economies

In this section, I present a silver bullet for the future transport sector – sugary hydrogen produced by cell-free synthetic pathway biotransformation (SyPaB). Cell-free SyPaB is the implementation

of complex biochemical reaction networks by assembling a number of purified enzymes and coenzymes *in vitro* [14,48–50]. Its history can be traced back to cell-free ethanol fermentation in the 1890s [17,159,160]. The non-natural synthetic pathways have been designed to split water by using the chemical energy in carbohydrates [18,19]



The non-natural synthetic catabolic pathway is comprised of 13 enzymes in one pot (Fig. 8). The pathway contains four biocatalytic modules: (i) a chain-shortening phosphorylation reaction for producing glucose-1-phosphate (G-1-P) catalyzed by glucan phosphorylase [161–163] (Eq. (4)); (ii) generation of glucose-6-phosphate (G-6-P) from G-1-P catalyzed by phosphoglucomutase [164] (Eq. (5)); (iii) generation of 12 NADPH from G-6-P through a pentose phosphate pathway and four enzymes in the glycolysis and gluconeogenesis pathways (Eq. (6)); and (iv) generation of hydrogen from NADPH catalyzed by hydrogenase (Eq. (7)).



Because nearly all of the reactions except the one mediated by fructose-1,6-bisphosphatase are reversible, the removal of gaseous products – H₂ and CO₂ from the aqueous phase under mild reaction conditions (<100 °C and ~1 atm) favors the unidirectional reaction toward the formation of desired gaseous products. Thermodynamic analysis suggests that the overall reactions from starch or cellulosic materials and water are spontaneous and endothermic (i.e., $\Delta G^\circ = -49.8$ kJ/mol and $\Delta H^\circ = +598$ kJ/mol) [18,19]. Similarly, the biological conversion of acetic acid to methane mediated by methanogenesis microorganisms is spontaneous and endothermic [14,165,166].

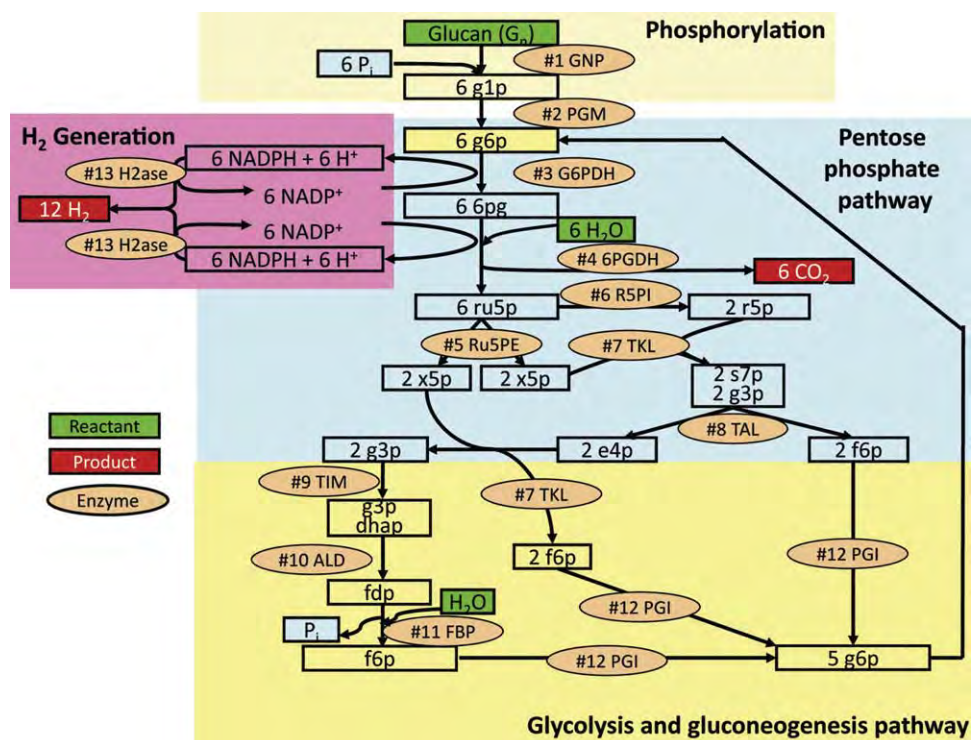


Fig. 8. The synthetic enzymatic pathway for high-yield generation of hydrogen from glucan and water modified based on previous ones [18,19]. The enzymes are GNP, glucan phosphorylase; PGM, phosphoglucumutase; G6PDH, G-6-P dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; R5PI, phosphoribose isomerase; Ru5PE, ribulose 5-phosphate epimerase; TKL, transketolase; TAL, transaldolase; TIM, triose phosphate isomerase; ALD, aldolase; FBP, fructose-1, 6-bisphosphatase; PGI, phosphoglucose isomerase; and H2ase, hydrogenase. The metabolites and chemicals are g1p, glucose-1-phosphate; g6p, glucose-6-phosphate; 6pg, 6-phosphogluconate; ru5p, ribulose-5-phosphate; x5p, xylulose-5-phosphate; r5p, ribose-5-phosphate; s7p, sedoheptulose-7-phosphate; g3p, glyceraldehyde-3-phosphate; e4p, erythrose-4-phosphate; dhap, dihydroxacetone phosphate; fdp, fructose-1,6-diphosphate; f6p, fructose-6-phosphate; and Pi, inorganic phosphate.

Constructing *in vitro* enzyme mixtures is much simpler than modifying living biological entities [14,48–50,167,168]. Living biological entities are complex open systems, and their regulation mechanisms are far beyond our understanding, while we can control and define components *in vitro*. Therefore, *in vitro* systems have been widely used to understand *in vivo* systems [169–174]. Until now, humankind cannot create any machine that can duplicate itself. Why should we expect that living biological entities that evolve to duplicate themselves can be modified to do both self-duplicate and produce at the same time? Cell-free systems can surpass natural biological entities on the basis of product yields and reaction rates, which are vital for large-scale biofuel production [14,50].

The notion that the challenges of SyPaB make this technology impossible for the economically viable production of low-value biofuels is a fixed paradigm for most scientists. These challenges include (i) enzyme deactivation, (ii) costly enzymes, (iii) costly and labile coenzymes, (iv) a lack of a thermoenzyme library, (v) different optimal conditions for different enzymes, and (vi) scalability potential. Respective solutions and supportive examples addressing these challenges are listed in Table 7. For example, enzyme deactivation can be addressed by the use of thermoenzymes, protein engineering through directed evolution and/or rational design, and/or enzyme immobilization. As a result, it is feasible to obtain enzymes with desired TTN values of more than 10,000,000 mol of product per mol of enzyme, for example, *Clostridium thermocellum* phosphoglucumutase (CthPGM) [164], *C. thermocellum* phosphoglucose isomerase (CthPGI) [175], *Thermotoga maritima* 6-phosphogluconate dehydrogenase (Tm6PGDH) [176], and *T. maritima* fructose-1,6-bisphosphatase (TmFBP) [177]. As for bulk enzymes, such as amylase and cellulase, their production costs are approximately \$5–10/kg of dry protein [14,50]. The DOE

Biomass program even predicts that enzyme costs may be reduced to \$0.7/kg of dry protein in the future, similar to the cost of soybean protein. With developments in (i) engineered oxidoreductases that can use biomimetic NAD factors and (ii) stable enzymes as building blocks of SyPaB, we estimate that ultimate hydrogen production costs may decrease to \$1.50–2.00/kg of hydrogen, where carbohydrate (\$0.22/kg) accounts for ~75–95% of production costs in part because hydrogen has very low product separation and purification costs and the other chemicals in the reaction can be recycled [14,48,50]. Currently, the largest obstacle to SyPaB may be a costly cofactor -NADPH. The cofactor issue can be addressed by the use of low-cost more stable NAD biomimetic cofactors [178,179]. Several redox enzymes have been engineered for better performance on biomimetic coenzymes [180–182].

These enzymatic sugar-to-hydrogen reactions have numerous potential applications from biohydrogenation for the synthesis of chiral compounds, to sugar batteries, to local hydrogen generation stations, to electricity generators, to sugar-fuel cell vehicles, in an increasing order of technology ready level (Fig. 9). First, the synthesis of chiral compounds by reducing coenzymes (NAD(P)H) and oxidoreductases is the lowest-cost technology for providing reduced coenzymes (NAD(P)H) for biohydrogenation [17]. Compared to available enzymatic technologies, this new technology has the lowest substrate costs, has a simple reactant removal (CO₂), and is based on renewable feedstock: sugars [17]. Second, enzymatic fuel cells can directly convert sugar to electrons [47,183–185]. Their power densities can achieve up to 2–5 mW/cm² of anode [186–188] so that they would be good for portable electronics and could compete with direct methanol fuel cells or rechargeable batteries in the future [47,188]. Third, the production of hydrogen based on local renewable resources is a valuable alternative for supplying hydrogen to local end users for hydrogen fuel cell vehicles or other

Table 7

The challenges of SyPaB technology as well as their respective solutions and supportive examples.

Challenge	Solution	Example	Ref.
Enzyme deactivation	Utilization of thermoenzymes Protein engineering (directed evolution, rational design, and their combination) Enzyme immobilization	Taq DNA polymerase, amylase Subtilisin, cellulase	[106,192] [77,108,109,193]
Costly enzymes	Enzyme TTN values > 10 ⁷ –10 ⁹ High-cell density microbial fermentation production Recombinant enzyme production Simple scalable purification techniques (e.g., heat precipitation, (NH ₄) ₂ SO ₄ precipitation, adsorption/desorption) Cofactor immobilization and recycling	Glucose isomerase, CthPGI, <i>Thermobifida fusca</i> polyphosphate glucokinase CthPGI, CthPGM, Tm6PGDH, TmFBP Cellulase (\$ ~ 5/kg), amylase (\$ ~ 10/kg), Tm6PGDH Taq polymerase, hydrogenase Heat precipitation of Tm6PGDH one-step purification and immobilization of CthPGI	[50,194–198] [164,175–177] [11,77,176] [199] [11,175,176,200,201]
Costly and labile cofactors	Stable and low-cost biomimetic cofactor replacement	Chiral alcohol synthesis in bio-pharmaceutical industry P450, horse peroxidase, alcohol dehydrogenase	[202,203] [178,179,181,182]
Lack of thermoenzyme library	Meta-genomics, bioinformatics tools, robotic automation, and high throughput cloning	>1400 <i>T. thermophilus</i> HB8 thermoenzyme library, screen ~500 recombinant enzymes in one biocatalysis reaction	[11]
Different optimal conditions for different enzymes	Compromise reaction conditions Obtain numerous enzymes from one source or modify them	Sugar-to-hydrogen, biohydrogenation >1400 <i>T. thermophilus</i> HB8 thermoenzyme library	[17–19]
Scalability potential	Production of 75 million tons of hydrogen replacing 150 billion gallons of gasoline/ethanol	250,000 tons of enzyme mixtures (i.e., 300 kg H ₂ per kg enzymes) ^a	[14]

^a Each enzyme in the cocktail has total turn-over number of >30,000,000 mol of product per mol of enzyme [11].

1026 applications. Local satellite hydrogen generation stations would
1027 produce hydrogen based on this sugar-to-hydrogen approach, store
1028 the hydrogen in tanks, and refill hydrogen-fuel cell vehicles. The
1029 solid sugar powders produced locally would be easily collected and
1030 distributed based on available solid goods delivery systems. Finally,
1031 the most ambitious application is sugar fuel cell vehicles (Fig. 7). A
1032 typical compact 5-person vehicle requires ~20-kW for constant-
1033 speed on highways. Giving the efficiencies of PEM fuel cells of 50%,
1034 the sugar car would consume approximately one kg of hydrogen
1035 per h. Assuming that the on-board biotransformer has a hydro-
1036 gen generation rate of 23.52 g H₂/L/h, the biotransformer tank size
1037 could be 42.8 L, which is feasible to put in a small car. To meet
1038 requirements such as accelerating and start-up, rechargeable bat-

1039 teries are needed, and the maximum output of the motor could be
1040 approximately 80 kW [6]. For driving long distances, the sugar car
1041 needs to store approximately 5 h of fuel, i.e., approximately 5 kg
1042 of hydrogen or 33.8 kg of sugar. Such an amount of sugar occu-
1043 pies a tank of ~48 L or 12.8 gallons, which is comparable to current
1044 gas tank sizes in a compact car. Each SFCV that runs 400 h (i.e.,
1045 12–15,000 miles) per year would consume 2700 kg of sugars and
1046 1.3 kg of enzyme mixtures. The enzyme mixtures in the biotrans-
1047 former would be replaced every hundreds of hours, similar to how
1048 motor oil is changed every 3000–5000 miles. There are currently
1049 thermoenzymes with a half lifetime at 80 °C of more than 100 h
1050 [176]. Dry sugar powder from local sugar stations may be added to
1051 sugar vehicles by nitrogen blowing with fast refilling rates. The run-

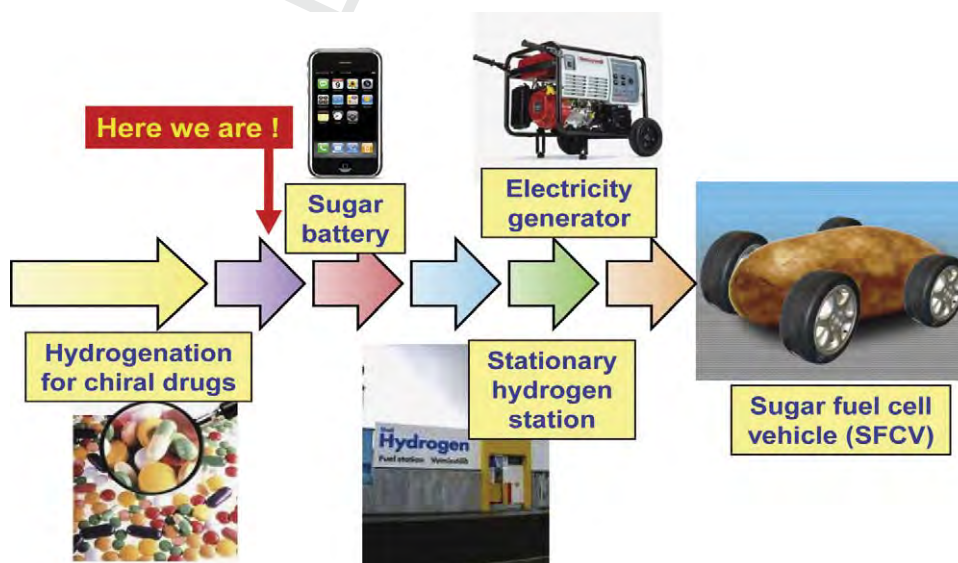


Fig. 9. Roadmap of SyPaB from the synthesis of high-value products (e.g., chiral compound synthesis [17] to ultra-high-energy density biodegradable sugar batteries [183]) to stationary hydrogen stations and electricity generators to sugar fuel cell vehicles (SFCV) [6].

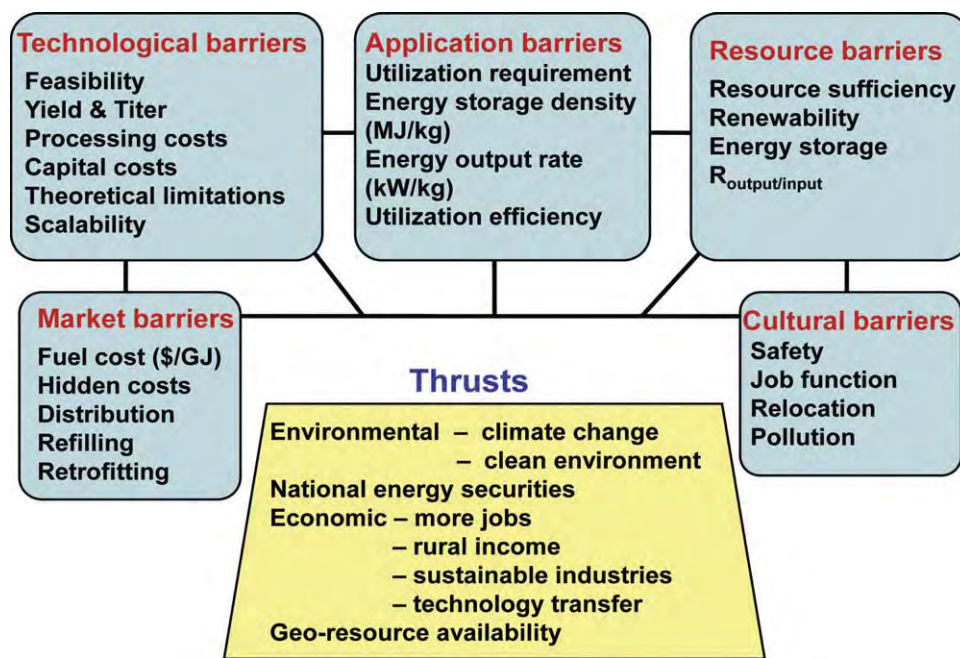


Fig. 10. Scheme of the barriers and incentives for biofuel R&D.

ning costs of SFCV per year may equal \$~700 (including \$600 for 2700 kg of sugars and \$100 for 1.3 kg of the enzyme mixture), lower than gasoline consumption (i.e., ~400 gallons of gasoline = \$1200).

Practical applications of sugary hydrogen systems must be coupled with hydrogen fuel cell technologies [7,14]. The current hydrogen production rate of SyPaB is 3.92 mmol per liter of reactor per h in which the substrate concentration is 8 mM (~1.4 g/L), and the temperature is 32 °C [18]. Numerous approaches can be implemented to accelerate hydrogen reaction rates: (i) increasing reaction temperatures from 30 to 80 °C or higher, (ii) increasing the use of enzymes responsible for rate-limited reactions, (iii) increasing substrate concentrations by 50-fold or higher, (iv) increasing overall enzyme concentrations by 10-fold or higher, (v) accelerating the reaction rates by metabolite (product) channeling, and (vi) increasing the catalytic efficiency of enzymes close to catalytically-perfect enzymes [7,14]. The above analysis may increase rates by 640,000- to 32,000,000-fold. In practice, a conservative estimate is that hydrogen production rates would increase 3000-fold to 23.52 g H₂/L/h. In support of the feasibility of this estimate, the highest biological hydrogen production rate (i.e., 23.6 g H₂/L/h) has been reported by high-cell density microbial fermentation [189]. Because enzymatic reactions are usually faster than microbial fermentations [14,49,185], increasing reaction rates by three or four orders of magnitude would be highly feasible. In support of it, power densities of microbial fuel cells have been improved by greater than 100,000–10,000,000 fold during the past 10 plus years [60].

5. Perspectives of biofuels

Predicting future transportation systems is very challenging and controversial. In addition to direct costs associated with harvesting, collecting, storing, processing, capital depreciation, distribution, and taxes from diverse primary energies to biofuels, it is vital to take in account of indirect costs (e.g., initial costs of prime movers, depreciation of prime movers, disposal costs, and waste treatment), hidden costs (e.g., military expense for securing energy sources, long-term climate change impacts, and infrastructure reconstruction), technological maturity and barriers, application

barriers, market barriers, resource barriers, competing technologies, and cultural barriers (Fig. 10) [2,10,190]. When such metrics are established, these metrics are valid only when used for their intended purpose and are limited by specific constraints and numerous assumptions. Clearly, strong incentives from environmental, national energy securities, economic and geo-resource availability issues will make sustainable transportation a reality (Fig. 10).

A clear understanding of the transportation development history, the needs and (clear and hidden) constraints of transportation systems, and competing fuel means from the point of view of global (i.e., cradle-to-grave) and local optimizations would stop far-from-practical biofuels R&D projects and avoid potential pitfalls. Plenty of biofuels claims published in high-profile journals or by stellar scientists would not come into practice compared to other ready or developing biofuels technologies because nearly all scientists and engineers (including me) are not immune from dabbling in pseudoscience that fits their prejudices and/or knowledge/expertise [191]. Here I would like to present some key information for readers' understanding basic constraints, competing technologies, and related infrastructure, to encourage critical thinking, and present out-of-the-box silver bullets. Clear understanding and careful analysis of a large variety of biofuels R&D projects would help refocus on key R&D topics and avoid unnecessary resource waste. With these in mind, there is no doubt that the USA non-food biomass reserves biomass will replace at least 30% of passenger vehicle fuels [12,78,158] or even achieve transportation fuel independence through high-energy efficiency prime mover systems [10].

I summarize several key points considered in refocusing future biofuel R&D:

1. Transportation needs (such as vehicle, boat, train, and plane) usually determine fuel production, but the reverse is not true;
2. The constraints of specific transportation vessels limit the choices of prime movers and their respective fuels;
3. Economic goals of biofuel R&D projects must be finished within a defined timeframe with numerous clear and hidden constraints plus political and geographic concerns;

4. Thermodynamics (energy conversion efficiency based on mass balance and energy balance) determines economics in the long term;
5. Energy efficiency analysis is simpler and more transparent than life cycle analysis and process economic analysis;
6. R&D can drastically decrease production costs but cannot break the limits set by thermodynamics, energetics, and physical and chemical properties of materials;
7. Every biofuels possibility deserves more research, which means that muddy waters make it easy to catch fish only by lucks. These doings could delay biofuels' wide implementation or even kill biofuels' future. We are in search of clarity on biofuels;
8. Moore's law does not work in the energy field for bother energy efficiencies and production costs due to the above-mentioned limits; and
9. Renewable energy sources could eventually replace most non-renewable fossil fuel sources, but this transition would take at least a half century.

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