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Subject: The government wants your advices on biofuels

Letter 1: General suggestions on biofuels decision making
Letter 2: Out-of-the-box solutions for the bio-economy

To Whom It May Concern,

In response to the open call from OSTP, this letter is my second letter. In Letter One, I introduce myself and provide some opinions about decision making for biofuels and bioeconomy. In Letter Two, I would like to share our vision and provide technical solutions.

The USA has been entering a technological plateau since 1980s. As a result, the USA is losing its ability for creating a large number of high-pay manufacturing jobs. The bioeconomy will be a savior for creating numerous jobs that cannot be outsourced. The Office of Science and Technology Policy makes a right decision for the future of USA but how to achieve it is another key question.

We need a paradigm shift. Since the USA had picked up all low-hanging (technological) fruits, only a new paradigm shift will allow us to discover a new world. Synthetic biology is receiving wide attention. But classic (in vivo) synthetic biology is not a game changer in the bioeconomy. The reason is its low production efficiency relying on living microorganisms. In fact, living entities keep duplicating themselves rather than producing the desired products only. For thousands of years, we are used to using living microorganisms for fermentation, e.g., beer, cheese, wine, ethanol, etc. In fact, living biocatalysts is not necessary. Most persons cannot think outside the box due to their habit of reasoning. We propose the use of cascade enzyme biocatalysis replacing traditional fermentations. Several important reactions have been accomplished by this new system, while they cannot be done by micro-organisms, for example, a low-cost and high-efficiency conversion of cellulose to starch, the production of 12 mol of hydrogen from one mol of glucose. The latter example is highlighted by the Royal Society of the UK as a good example of synthetic biology in 2007.

We envision a future carbon-neutral carbohydrate economy (below figure). Both natural and this newly-designed artificial photosynthesis are responsible for fixing CO₂ by utilizing solar energy; while the degradation of carbohydrate and its derivatives will release CO₂ to the atmosphere. Carbohydrate, which is renewable, carbon-neutral, and evenly distributed, will replace oil because of lower costs (\$/GJ), better performance in

the transport sector, better safety, and more applications (e.g., hydrogen carrier and electricity storage compound).

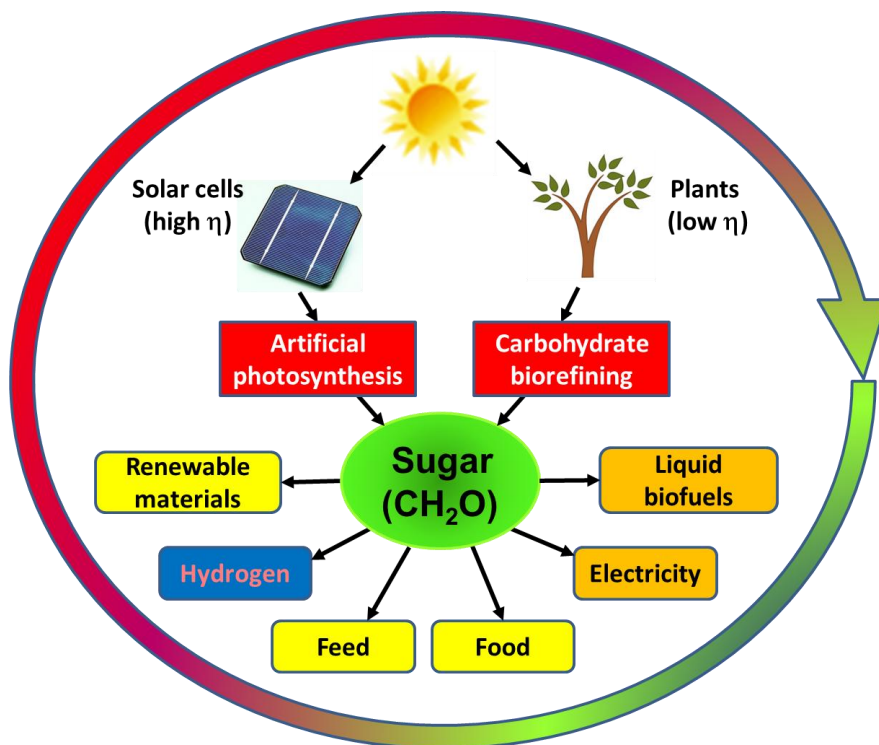


Figure. The carbon-neutral cycle based on carbohydrates as food, feed, a source of renewable material precursors (e.g., lactic acid, isoprene, succinic acid), an electricity storage carrier (e.g., ~10-14 MJ electricity output/kg), and a hydrogen carrier with a hydrogen storage capacity of 8.33-14.8 H₂ mass%.

In this package, please find five papers representing our key points:

Paper 1 (PONE 2007) – a seminal paper – sweet hydrogen generation from sugar. It is highlighted by the Royal Society of UK, ACS, and ASM. It is very terrible for most US funding agencies not to fund it because it is outside the box. (We submitted 10 DOE proposals and 5 NSF proposals. All were rejected). *Now German and Chinese governments are funding similar R&D efforts.* If the USA does not take action now, the USA might lose race in renewable energy because Germany is stronger than USA in the industrial enzyme field. Since Germany scientists knows right directions proposed by me, they could utilize their advantage and achieve the bioeconomy before the USA. As a result, *the USA invention does not equal the USA innovation.*

Paper 2 (EES sugar car 2009). We clearly explain why this technology is an out-of-the box solution to the hydrogen economy and bioeconomy. This vision is against interests of most H₂ R&D persons.

Paper 3 (carbohydrate is H₂ carrier, 2010). The use of biomass sugars as a high density hydrogen carrier, better than methanol and others. *This is against general interests of hydrogen storage persons.*

Paper 4 (PONE fuel independence 2011). Our analysis clearly suggests that it is possible to replace all gasoline by using a small fraction of biomass resource if we can increase biomass utilization efficiency. Clearly, our solution can be scaled up easily than other solutions. *This is against general interests of most biofuels experts.*

Paper 5 (ACS Cat. Simpler 2011). This perspective clearly explains that our technology SyPaB is an incremental technology, but its impact will be revolutionary. The implementation of this technology is doable based on ready knowledge and technology. Its impacts would impact a lot of fields, such as biomass, hydrogen, fuel cells, batteries, CO₂ fixation, water, agriculture, and vehicles.

I appreciate your interests and reading. If you have any question, please feel free to contact me via email at biofuels@vt.edu, or by telephone, at 01-540-231-7414.

Yours sincerely

A handwritten signature in black ink, appearing to read 'Y. H. Zhang', with a long horizontal line extending to the right.

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BTW: Please find five papers for your information.

High-Yield Hydrogen Production from Starch and Water by a Synthetic Enzymatic Pathway

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Background. The future hydrogen economy offers a compelling energy vision, but there are four main obstacles: hydrogen production, storage, and distribution, as well as fuel cells. Hydrogen production from inexpensive abundant renewable biomass can produce cheaper hydrogen, decrease reliance on fossil fuels, and achieve zero net greenhouse gas emissions, but current chemical and biological means suffer from low hydrogen yields and/or severe reaction conditions. **Methodology/Principal Findings.** Here we demonstrate a synthetic enzymatic pathway consisting of 13 enzymes for producing hydrogen from starch and water. The stoichiometric reaction is $C_6H_{10}O_5(l) + 7 H_2O(l) \rightarrow 12 H_2(g) + 6 CO_2(g)$. The overall process is spontaneous and unidirectional because of a negative Gibbs free energy and separation of the gaseous products with the aqueous reactants. **Conclusions.** Enzymatic hydrogen production from starch and water mediated by 13 enzymes occurred at 30°C as expected, and the hydrogen yields were much higher than the theoretical limit (4 H₂/glucose) of anaerobic fermentations. **Significance.** The unique features, such as mild reaction conditions (30°C and atmospheric pressure), high hydrogen yields, likely low production costs (\$~2/kg H₂), and a high energy-density carrier starch (14.8 H₂-based mass%), provide great potential for mobile applications. With technology improvements and integration with fuel cells, this technology also solves the challenges associated with hydrogen storage, distribution, and infrastructure in the hydrogen economy.

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INTRODUCTION

Photosynthesis is the biological process that converts light energy to chemical energy and stores it in carbohydrates as “ $6 CO_2 + 6 H_2O \rightarrow C_6H_{12}O_6 + 6 O_2$ ”, and fixes atmospheric carbon into biomass (living carbon). Before the industrial revolution, the global economy was largely based on carbon extracted directly or indirectly (via animals) from plants; now the economy is mainly dependent on fossil fuels (dead carbon). At the dawn of the 21st century, a combination of economic, technological, resource, and political developments is driving the emergence of a new carbohydrate economy [1,2].

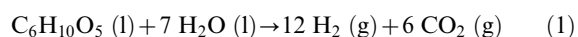
Climate change, mainly due to CO₂ emissions from fossil fuel burning, and the eventual depletion of the world's fossil-fuel reserves, are threatening sustainable development [2–4]. Abundant, clean, and carbon-neutral hydrogen is widely believed to be the ultimate mobile energy carrier replacing gasoline, diesel, and ethanol; a high energy conversion efficiency (~50–70%) can be achieved *via* fuel cells without producing pollutants [3]. Four main R&D priorities for the future hydrogen economy are: 1) decreasing hydrogen production costs *via* a number of means, 2) finding viable methods for high-density hydrogen storage, 3) establishing a safe and effective infrastructure for seamless delivery of hydrogen from production to storage to use, and 4) dramatically lowering the costs of fuel cells and improving their durability [5–7]. Hydrogen production from less costly abundant biomass is a shortcut for producing low-cost hydrogen without net carbon emissions [8–15].

Synthetic biology is interpreted as the engineering-driven building of increasingly complex biological entities for novel applications, involving the steps of standardization, decoupling, abstraction, and evolution [16]. One main goal of synthetic biology is to assemble interchangeable parts from natural biology into the systems that function unnaturally [17]. The simplest synthetic biology example is to assemble enzymes to implement an unnatural process, in which the gene regulatory systems do not exist. Here we apply the principles of synthetic biology to

implement an important reaction by using 13 well-known enzymes, which form an unnatural enzymatic pathway. The most obvious advantage of this process is that the hydrogen yield is far higher than the theoretical yield (4 H₂/glucose) of biological hydrogen fermentations [9,15,18]. This novel enzymatic high-yield hydrogen production method is anticipated to have great impacts on the future hydrogen and carbohydrate economy.

RESULTS

We designed a new enzymatic method for producing hydrogen from starch and water,



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Competing Interests: YHPZ and JRM are the co-inventors of this enzymatic hydrogen production process, which is covered under provisional patent application.

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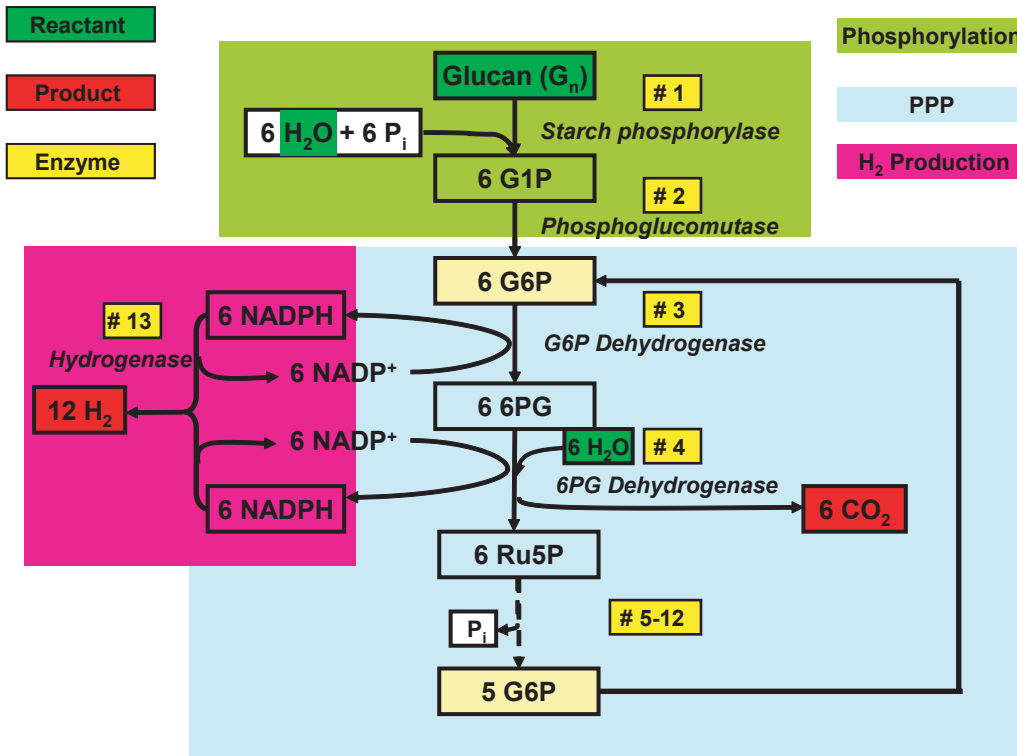
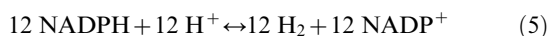
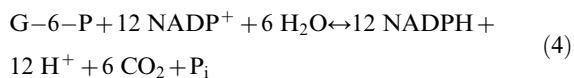
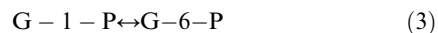
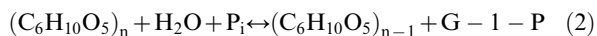


Figure 1. The synthetic metabolic pathway for conversion of polysaccharides and water to hydrogen and carbon dioxide. The abbreviations are: PPP, pentose phosphate pathway; G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; Ru5P, ribulose-5-phosphate; and P_i , inorganic phosphate. The enzymes are: #1, glucan phosphorylase; #2, phosphoglucomutase; #3, G-6-P dehydrogenase; #4, 6-phosphogluconate dehydrogenase; #5 Phosphoribose isomerase; #6, Ribulose 5-phosphate epimerase; #7, Transaldolase; #8, Transketolase; #9, Triose phosphate isomerase; #10, Aldolase; #11, Phosphogluco isomerase; #12, Fructose-1, 6-bisphosphatase; and #13, Hydrogenase. doi:10.1371/journal.pone.0000456.g001

Figure 1 shows the synthetic enzymatic pathway that does not exist in nature. It is comprised of 13 reversible enzymatic reactions: a) a chain-shortening phosphorylation reaction catalyzed by starch phosphorylase yielding glucose-1-phosphate (Equation 2) [19]; b) the conversion of glucose-1-phosphate (G-1-P) to glucose-6-phosphate (G-6-P) catalyzed by phosphoglucomutase (Equation 3) [20]; c) a pentose phosphate pathway containing 10 enzymes (Equation 4) [21]; and d) hydrogen generation from NADPH catalyzed by hydrogenase (Equation 5) [22].



We first validated the reaction scheme of Woodward et al. [23], in which hydrogen was produced from G-6-P via 11 enzymes, based on the reaction of $G-6-P + 6 H_2O \rightarrow 12 H_2 + 6 CO_2 + P_i$ (top curve in Fig. 2). The proof-of-principle experiment was then

conducted to validate whether hydrogen can be produced from starch and water at 30°C using 13 enzymes (see Materials and Methods). Clearly, hydrogen was produced as expected (bottom curve in Fig. 2). As compared to using G-6-P as the substrate,

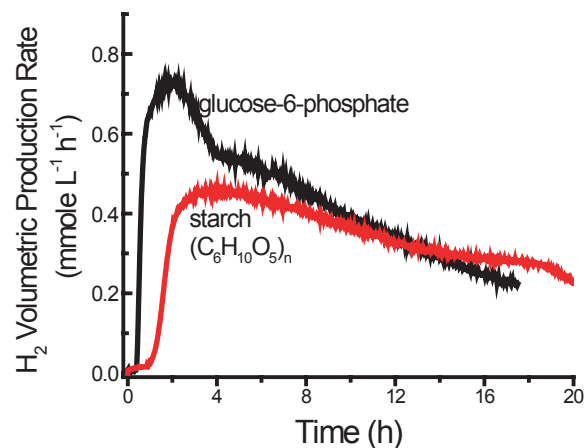


Figure 2. Hydrogen production from either 2 mM G-6-P or 2 mM starch (glucose equivalent). The reaction based on G-6-P contained the pentose phosphate cycle enzymes (#3-12, 1 unit each), ~70 units of *P. furiosus* hydrogenase (#13), 0.5 mM thiamine pyrophosphate, 2 mM $NADP^+$, 10 mM $MgCl_2$, and 0.5 mM $MnCl_2$ in 2.0 ml of 0.1 M HEPES buffer (pH 7.5), at 30°C. The reaction based on starch rather than G-6-P was supplemented by 10 units of α -glucan phosphorylase (#1), 10 units of phosphoglucomutase (#2), and 4 mM phosphate at 30°C. doi:10.1371/journal.pone.0000456.g002

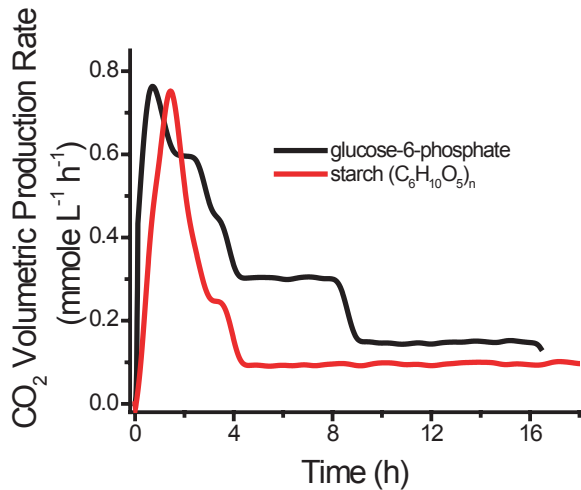


Figure 3. Carbon dioxide production from either 2 mM G-6-P or 2 mM starch (glucose equivalent). The experimental conditions were the same as those in Figure 2.
doi:10.1371/journal.pone.0000456.g003

hydrogen production from starch exhibits a) a longer lag phase, b) a lower peak production rate (0.44 mmol/h/L), and c) an extended reaction time, all of which are consistent with the reaction mechanism (Fig. 1). The CO_2 production for both cases was measured at the same time (Fig. 3). Clearly, CO_2 was produced before H_2 generation, which was in a good agreement with the mechanism in Figure 1. The integrated yields (mol/mol) of hydrogen and CO_2 , based on substrate consumption of G-6-P and starch, were 8.35 H_2 /G-6-P and 5.4 CO_2 /G-6-P, and 5.19 H_2 /glucose unit and 5.37 CO_2 /glucose unit, respectively. The yields of hydrogen and CO_2 from G-6-P were approximately 70% and 86% of theoretical yields. The corresponding value for hydrogen from starch was lower (43%) although the CO_2 yield was the same. The lower hydrogen yield was anticipated and its causes, such as the unfinished reaction, batch operation, and accumulation of metabolites (e.g., NADPH), are currently under study.

Thermodynamic analysis (Fig. 4) shows that the overall reaction (Equation 1) is a spontaneous process (i.e., $\Delta G^\circ = -48.9$ kJ/mol) and is a weakly endothermic reaction (i.e., $\Delta H^\circ = 595.6$ kJ/mol), based on data elsewhere [21,24]. Since the gaseous products (H_2 and CO_2) are simultaneously removed from the liquid reaction solution, the real Gibbs free energy at 30°C and atmospheric pressure is much less than -48.9 kJ/mol, according to Le Chatelier's principle. The fairly large negative values of Gibbs free energy suggest a complete conversion. Sugar chain-shortening substrate phosphorylation (Eq. 2) utilizes the energy stored in the glucosidic bonds of polysaccharides (15.5 kJ/mol glucosidic bond) to produce the activated phosphorylated monosaccharide (G-1-P) without ATP consumption [20,25] and avoids using expensive substrates such as glucose-6-phosphate [23]. The endothermic reaction suggests that some low-temperature heat energy from the environment is used to produce high quality energy carrier hydrogen, an extra 22% net energy gain. Although photosynthesis efficiency from solar energy to chemical energy is not so high as that of solar cells [26], hydrogen production based on inexpensive abundant biomass will be a shortcut to realization of the hydrogen economy without net carbon emissions, will avoid large capital investments for the hydrogen infrastructure, and will save the huge energy consumption currently required for production of solar cells [3].

DISCUSSION

There are four other means converting biomass to hydrogen: 1) direct polysaccharide gasification [8,13]; 2) direct glucose chemical catalysis after polysaccharide hydrolysis [10,11]; 3) anaerobic fermentations [9,15,18]; and 4) polysaccharide- or glucose-ethanol fermentations [27–29] followed by ethanol chemical reforming [12]. The chemical methods have low hydrogen yields (50~57%) due to poor selectivity of catalysts and requires high reaction temperatures (e.g., 500~900 K) [8,10,11,13]. Anaerobic hydrogen fermentation is well known for its low hydrogen yield of 4 H_2 /glucose [9,15,18]. The combination of ethanol fermentation and ethanol-to-hydrogen reforming has a theoretical yield of 10 H_2 /glucose unit (e.g. 83% of the maximum). Allowing 5~10% fermentation loss [30] and ~5% reforming loss [12], the practical hydrogen yield through ethanol could be ca. 75% of the maximum yield. Assembly of the

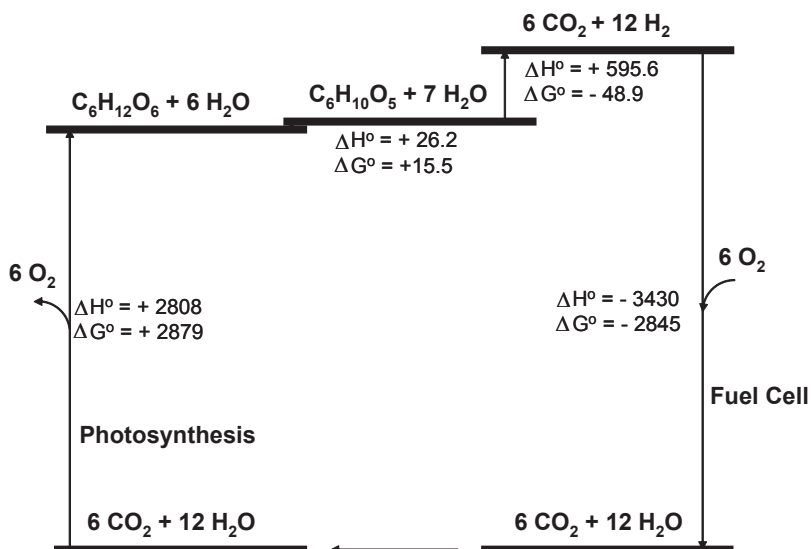


Figure 4. An energy diagram showing the standard enthalpy (ΔH°) and free energy changes (ΔG°) in kJ/mol for the reactions in a renewable energy cycle operating among H_2O , CO_2 , glucose, and starch.
doi:10.1371/journal.pone.0000456.g004

high-substrate-selectivity enzymes results in an artificial cascade enzymatic pathway, accompanied by a high hydrogen yield (12 H₂/glucose), three time higher than the theoretical yield (4 H₂/glucose) from biological hydrogen fermentations [9,15,18] and much higher than those from chemical catalysis [8,10,11].

Distinct from the severe reaction conditions of chemical catalysis [8,10–14], the mild reaction conditions mediated by enzymes (~20–100°C, depending on the enzymes employed) provide two obvious benefits: 1) easy implementation in a small space, especially for mobile applications, and 2) simple process configurations due to easy separation of the gaseous products (H₂ and CO₂) from the reactants (starch and water).

Costs of hydrogen production from less-costly starch (e.g., \$~0.15/kg) would be ~\$2/kg H₂, assuming that feedstock costs account for half of overall costs and enzymes and co-enzyme account for another half. In general, approximately 40–75% of prices of commodities, such as gasoline from crude oil, hydrogen from natural gas, and ethanol from corn kernels, come from feedstock costs [31]. For example, current crude recombinant enzyme production costs are estimated to range ~\$10/kg; commercial cellulase production cost is as low as \$1–2/kg [29]. Based on the rule of thumb for commodity production costs, the likely hydrogen-producing costs (~\$2/kg H₂) could meet or exceed the hydrogen cost goals (\$2–3/kg H₂), established by the US DOE [32]. For example, the soaring prices of natural gas drove hydrogen costs from \$1.40/kg H₂ in 2003 to \$2.70/kg H₂ in 2005. We improve the method first described by Woodward [23] by starting with a less costly and abundant substrate—starch. Thus we avoid several major shortcomings of Woodward’s method: 1) costly glucose-6-phosphate, 2) accumulation of phosphate, which is a strong inhibitor of fructose-1,6-bisphosphatase, 3) increasing ionic strength in the buffer, which slows down overall reaction rates, and 4) a pH shift in the buffer.

Solid starch has a relatively high energy density, with a mass-storage density of 14.8 H₂-mass % and a volume-storage density of 104 kg H₂/m³. These densities are higher than most of the solid hydrogen storage technologies [7], as well as exceeding the DOE

goals of 4.5 mass%, 6 mass%, and 9 mass% in 2005, 2010, and 2015, respectively [5]. Replacement of conventional solid hydrogen storage technologies by the on-board starch-H₂ converter and starch container will also solve several problems for solid hydrogen storage devices, e.g., energy loss for hydrogen compression or liquefaction, durability of reversible adsorption/desorption materials, high temperatures for desorption, and a long refilling time [5,7]. Easy and safe storage and distribution of solid starch will address many issues of the hydrogen economy infrastructure. For example, setting up the infrastructure to store and distribute gaseous hydrogen to vehicles might cost hundreds of billions in the USA alone [33].

This robust synthetic enzymatic pathway that does not function in nature was assembled by 12 mesophilic enzymes from animal, plant, bacterial, and yeast sources, plus an archaeal hyperthermophilic hydrogenase. The performance (e.g., reaction rate and enzyme stability) is anticipated to be improved by several orders of magnitude by using the combination of (a) enzyme component optimization *via* metabolic engineering modeling [34], (b) interchangeable substitution of mesophilic enzymes by recombinant thermophilic or even hyperthermophilic enzymes [23], (c) protein engineering technologies, and (d) higher concentrations of enzymes and substrates. We have increased the hydrogen production rates by nearly 4 times greater than Woodward’s results [23] through a) decreasing the ionic strength of the buffer and b) substituting one mesophilic enzyme (#11). This research approach will naturally benefit from on-going improvements by others in synthetic biology systems that are addressing cofactor stability [35], enzyme stability by additives [36], and co-immobilization [37], and development of minimal microorganisms [38] that can be built upon to create an *in vivo* enzyme system that produces H₂ in high yields.

The concept of cell-free synthetic enzymatic pathway engineering is anticipated to be applied to other commodity chemical production because of its unique benefits: high product yields (i.e., no formation of by-products and cell mass), modest reaction conditions as compared to chemical catalysis, no toxic chemicals

Table 1. The enzymes used for hydrogen production from starch and water, and their reaction mechanisms, sources, and amounts used in the reaction.

E.C.	Enzyme Name	Reaction	Vender	Origin	Unit
2.4.1.1	glycogen phosphorylase	(C ₆ H ₁₀ O ₅) _n +P _i +H ₂ O→(C ₆ H ₁₀ O ₅) _{n-1} +glucose-1-P	Sigma	rabbit muscle	10
5.4.2.2	phosphoglucomutase	G-1-P→G-6-P	Sigma	rabbit muscle	10
1.1.1.49	glucose-6-phosphate dehydrogenase	G-6-P+NADP ⁺ →6-phosphogluconate+NADPH	Sigma	<i>S. cerevisiae</i>	1
1.1.1.44	6-phosphogluconic dehydrogenase	6-phosphogluconate+H ₂ O+NADP ⁺ →ribulose-5-phosphate+NADPH+CO ₂	Sigma	<i>S. cerevisiae</i>	1
5.3.1.6	ribose 5-phosphate isomerase	ribulose-5-phosphate→ribose-5-phosphate	Sigma	spinach	1
5.1.3.1	ribulose-5-phosphate 3-epimerase	ribulose-5-phosphate→xylulose-5-phosphate	Sigma	<i>S. cerevisiae</i>	1
2.2.1.1	transketolase	xylulose-5-phosphate+ribose-5-phosphate→sedoheptulose-7-phosphate+glyceraldehyde-3-phosphate xylulose-5-phosphate+erythrose-4-phosphate→fructose-6-phosphate+glyceraldehyde-3-phosphate	Sigma	<i>E. coli</i>	1
2.2.1.2	transaldolase	sedoheptulose-7-phosphate+glyceraldehyde-3-phosphate→fructose-6-phosphate+erythrose-4-phosphate	Sigma	<i>S. cerevisiae</i>	1
5.3.1.1	triose-phosphate isomerase	glyceraldehyde 3-phosphate→dihydroxacetone phosphate	Sigma	rabbit muscle	1
4.1.2.13	aldolase	glyceraldehyde 3-phosphate+dihydroxacetone phosphate→fructose-1,6-bisphosphate	Sigma	rabbit muscle	1
3.1.3.11	fructose-1,6-bisphosphate	fructose-1,6-bisphosphate+H ₂ O→fructose-6-phosphate+Pi	[41]	<i>E. coli</i>	1
5.3.1.9	phosphoglucose isomerase	fructose 6-phosphate→glucose-6-P	Sigma	<i>S. cerevisiae</i>	1
1.12.1.3	<i>P. furiosus</i> hydrogenase I	NADPH+H ⁺ →NADP ⁺ +H ₂	[22,42]	<i>P. furiosus</i>	~70

doi:10.1371/journal.pone.0000456.t001

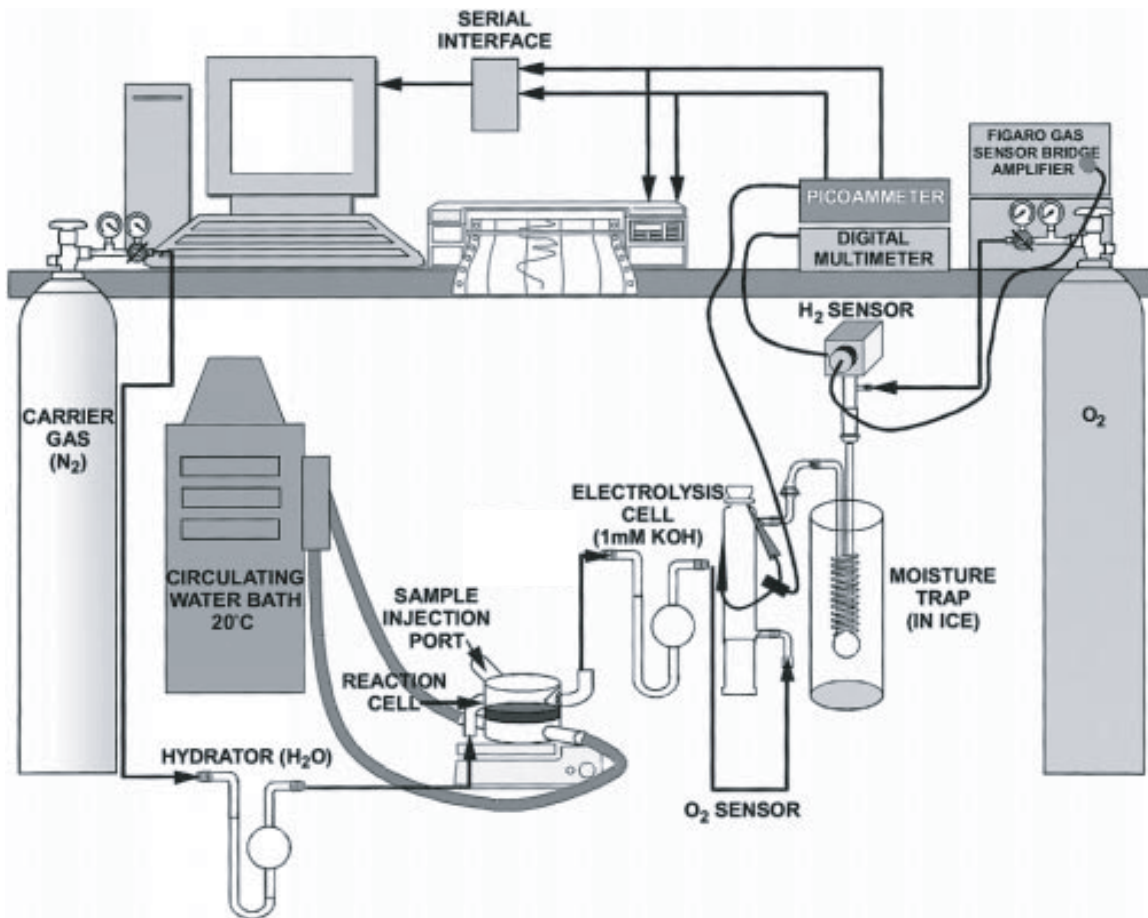


Figure 5. The hydrogen cell system configured for monitoring H₂ with the ORNL in-house sensor based on the Figaro TGS 822 and O₂ with a modified Hersh galvanic cell [43]. The CO₂ analyzer (not shown) is attached between the reaction cell and the electrolysis cell. doi:10.1371/journal.pone.0000456.g005

required or produced, broad reaction conditions (e.g., high temperature and low pH) as compared with microorganisms, and easy operation and control. For example, it has been argued that cell-free ethanol fermentation systems would replace microbe-based ethanol fermentation someday [39].

With technology development and integration with PEM fuel cells, the starch-to-hydrogen conversion technology is anticipated to have wide mobile applications. We envision that future mobile appliances will store solid starch, produce hydrogen from starch and water via this reaction, and then generate electricity by hydrogen fuel cells at the same compact place.

MATERIALS AND METHODS

All chemicals and enzymes were purchased from Sigma Co, unless otherwise noted. All enzymes and their catalysis reactions are listed in Table 1.

The experiments were carried out in a continuous flow system as described previously [23], with the modification that the moisture traps were cooled with ice instead of liquid nitrogen, and that oxygen as well as hydrogen and carbon dioxide were monitored in the gas stream [23] (Fig. 5). The working volume of the custom reactor was 2 mL. The system was continuously purged with helium at a flow rate of 50 mL/min. The temperature of the jacketed reaction vessel was maintained at 30°C with a Polyscience (Niles, IL 60714) circulating water bath. Hydrogen evolution was measured with a Figaro TGS 822 tin oxide sensor connected over

a bridge amplifier to a Keithley Model 2000 multimeter (Keithley Instruments, Cleveland, OH). Oxygen concentration was monitored with a modified Hersh galvanic cell using 24% KOH as the electrolyte connected to a Keithley autoranging picoammeter. Carbon dioxide production was measured with a LI-COR CO₂ Analyzer Model LI-6252 connected to a Keithley 2000 multimeter. The multimeters and picoammeter were connected to a 486 computer through IEEE 488 general-purpose interface boards. Electrolysis for calibration of hydrogen and oxygen by Faraday's law of electrochemical equivalence was carried out with a Keithley 220 programmable current source connected to an in-line electrolysis cell. Calibration for carbon dioxide was carried out with an analyzed gas mixture consisting of 735 ppm carbon dioxide and 1000 ppm oxygen in helium (Air Liquide America Corp., Houston, TX 77056). Data collection and analysis was carried out with ASYST 4.0 software (ASYST Technologies, Inc., Rochester, NY).

The integrated molar/molar yields of hydrogen (Y_{H_2}) and carbon dioxide (Y_{CO_2}) are calculated as

$$Y_{H_2} = \frac{\int r_{H_2} dt}{12 * \Delta GE}$$

$$Y_{CO_2} = \frac{\int r_{CO_2} dt}{6 * \Delta GE}$$

in which r_{H_2} and r_{CO_2} are the volumetric production rates in terms of mmole of H_2 or CO_2 per liter of reaction volume per hour, as shown in Figs. 2 and 3; AGE is the net consumption of glucose equivalent in terms of mM. Residual G-6-P can be measured using Sigma glucose HK kit [40]. The mixtures were incubated at 35°C for 5 minutes and the change in absorbance at 340 nm was determined. In the case of starch, the residual starch, G-1-P, and G-6-P were hydrolyzed to glucose by addition of dilute H_2SO_4 and hydrolysis at 121°C for 1 hour. The neutralized glucose solutions were measured by a glucose HK kit [40].

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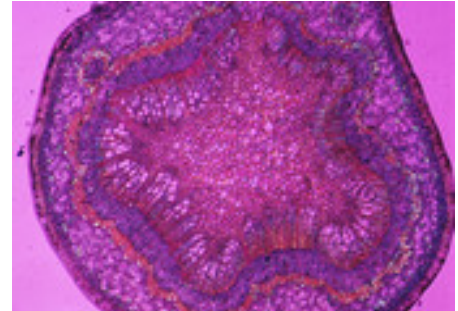
We thank Dr. Larson at Virginia Tech for supplying the strain containing the recombinant fructose-1,6-bisphosphatase.

Author Contributions

Conceived and designed the experiments: YZ JM. Performed the experiments: YZ BE. Analyzed the data: MA YZ BE. Contributed reagents/materials/analysis tools: YZ RH. Wrote the paper: MA YZ BE JM.

Call for views: Synthetic biology

The Royal Society seeks your views on the emerging area of synthetic biology. This is your opportunity to shape the focus of the Royal Society's future policy work in this important area. We welcome views from individuals or organisations by 27 August 2007. Please see below for submission details.



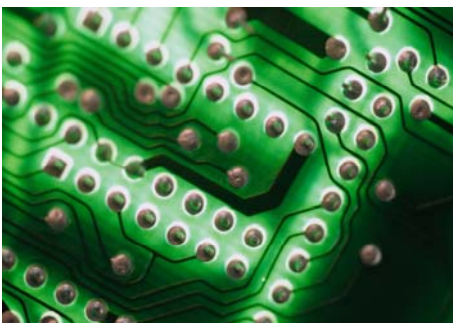
What is synthetic biology?

Synthetic biology is an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems.

Biologists have traditionally sought to understand how life works. In contrast, synthetic biologists seek to design and build new biological systems. The application of engineering principles to the design and construction of complex biological systems is likely to provide a step change from the tweaking of existing genomes, usually described as genetic engineering.

The development of standardised technology and methodology for designing and manufacturing semiconductor chips (electronic components) has transformed information and communications technologies (ICTs) over recent decades. The principles of abstraction and modularisation, which underpinned this transformation, are now being applied to the design and construction of biological systems. Parallels have been drawn between the revolution in ICTs and the potential impact of developments in synthetic biology.

Synthetic biologists are seeking to construct standardised biological parts and instructions for assembling these into biological systems. This could eventually lead to the manufacture of novel biological systems and devices that could have applications in a range of areas such as healthcare, energy and the environment.



Synthetic biologists are also constructing a bacterium with the minimal genome required for life. Genes could be inserted to this genome to build biological pathways with functions that have commercial applications. Research is also seeking to extend and rewrite the genetic code to enable the production of proteins that do not occur naturally, but that could have industrial and medical applications. A few potential applications of synthetic biology are outlined below.

Synthetic biology has developed from the convergence of knowledge and tools from other disciplines such as systems biology, genetic engineering, mechanical engineering, electrical engineering, information theory, physics, nanotechnologies and computer modelling. Like most emerging technologies, the boundaries between synthetic biology and other technologies and scientific disciplines are blurred.

Potential applications of synthetic biology

Potential applications of synthetic biology range widely due to the interdisciplinary nature of the field. It could have implications for agriculture, engineering and processing, energy production and the pharmaceutical industry. A few examples of potential applications include:

- **Development of a cheap anti-malarial drug** – The plant derived drug has a high success rate in treating malaria, but has been impractical and costly to produce by standard chemical methods. By building a new metabolic pathway in yeast and *E coli* with genes from three separate organisms, researchers have created a bacterial strain that can produce amorphadiene. This precursor can then be converted into artemisinin. It is hoped that the drug could be available in the next few years.
- **The beginning stages of a cheap and green, high yield hydrogen production** – Hydrogen could become an important alternative to fossil fuels. A novel synthetic pathway consisting of 13 enzymes derived from five different organisms has been developed to produce hydrogen from starch and water. This pathway is being developed further with the aim of producing hydrogen from cellulose, a more abundant sugar, which could provide hydrogen for fuel cells cheaply and easily.
- **Looking for an answer to environmental contamination** – Communities of micro organisms are responsible for most naturally occurring biodegradation. The metabolic and genetic control mechanisms of these organisms could provide clues to create and develop novel micro organisms to decontaminate the most potent environmental contaminants.
- **Programmable cells for use in gene therapy** – Pathogenic bacteria and viruses are able to identify and manipulate cells to produce harmful affects. Programming a bacterium or virus that can identify malignant cells and deliver a therapeutic agent could have major benefits for treating cancer and similar illnesses.

Call for views

This is your chance to shape the focus of the Royal Society's future policy work in this area. This work could take a number of forms, such as a substantial policy study or a stakeholder workshop.

Synthetic biology has the potential to lead to a wide range of useful applications, but it also raises a number of uncertainties including its possible impact on society. There has been some discussion around the social, ethical and legal issues that synthetic biology may present and the Society is keen to encourage a wider constructive discussion and debate about these issues. We are hoping to receive comments and information from a range of stakeholders on both the opportunities and uncertainties that could accompany the development of synthetic biology.

We would like to receive submissions commenting on any aspects of synthetic biology and would be pleased to hear suggestions on particular areas or issues that the Society should focus on when deciding what work to undertake in this area. Broad topics that you may wish to comment on are listed below. There is no need to comment on all these areas, and we welcome comments on subjects other than those listed:

- Potential developments and applications
- Current research capacity and geographical distribution
- Societal implications
- Ethical concerns
- Biosecurity risks
- Implications for the environment
- Research support and funding
- Implications for human health
- Legal issues and implications for regulation (national and international)
- Ownership, sharing and innovation frameworks (including intellectual property)
- Biosafety concerns
- Education and training
- Governance and oversight of research
- Economic considerations for developed and developing countries

We would be happy to receive electronic copies, links to electronic copies, or hard copies of relevant reports and references.

Submissions

The deadline for submissions is **27 August 2007**, either electronically (preferred format) or by post to:

E-mail synthetic.biology@royalsoc.ac.uk

Post Kate O'Shea, Science Policy, The Royal Society, 6-9 Carlton House Terrace, London SW1Y 5AG, UK

Responses are likely to have the greatest impact if they are restricted to four pages, plus appendices if appropriate.

Confidentiality

A list of organisations and individuals who have submitted views will be listed in our website and the submissions may be published. Please inform us if you **do not** want your name or your submission to be made public. If you are submitting information on behalf of an organisation, please include details of the relevant person to contact should we wish to discuss issues raised in your submission.

If you would like to submit your views but are unable to meet the deadline, or if you have any questions, please contact us on the details above.

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The Royal Society is the independent scientific academy of the UK and the Commonwealth, dedicated to promoting excellence in science. As well as providing an authoritative voice and leadership for UK science, it aims to ensure that policies on key issues are influenced by the best independent science and it provides advice for policymakers on science and its relationship with society.

The Royal Society is committed to encouraging the responsible development of new and emerging technologies for the maximum benefit of humanity and the environment. It is well placed to provide an expert, independent and realistic assessment of the risks, benefits and impacts that new and emerging technologies could present. The Society has undertaken projects on a wide range of scientific areas, including nanotechnologies. For more information on our policy work, visit www.royalsoc.ac.uk/policy.

Please circulate this document to other interested parties.

A sweet out-of-the-box solution to the hydrogen economy: is the sugar-powered car science fiction?

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Reproduced by permission of the Royal Society of Chemistry from Y.-H. Percival Zhang, *Energy Environ. Sci.*, 2009, 2, 272-282, DOI: 10.1039/B818694D <<link to <http://dx.doi.org/10.1039/B818694D>>> <http://pubs.rsc.org/en/content/articlelanding/2009/ee/b818694d>

The hydrogen economy presents a compelling future energy picture, especially for the transportation sector. The obstacles, such as low-cost hydrogen production, lack of high-density hydrogen storage approaches, costly infrastructure, and safety concerns are prohibiting its large-scale implementation. To address the above challenges, we propose a new solution – use of starch or cellulose (C₆H₁₀O₅) from biomass as a hydrogen carrier. This new solution is based on the invention of complete conversion of glucans (starch and cellulose) and water to hydrogen and carbon dioxide as C₆H₁₀O₅ (aq) + 7H₂O (l) → 12H₂ (g) + 6CO₂ (g). The production of hydrogen from carbohydrates is a nearly carbon-neutral process based on the whole carbon cycle. The use of low-cost renewable carbohydrate as a high hydrogen density carrier (14.8 H₂ mass %) may solve problems such as hydrogen production, storage and distribution, as well as address safety concerns. Increasing hydrogen generation rate (power density) and decreasing costs are two major tasks prior to this technology's wide implementation. Analysis based on past scientific knowledge and technical achievements suggests that sugar-powered vehicles could become real in the future with intensive R&D efforts. Here we are calling for international R&D collaborations to pursue the holy grail of the carbohydrate hydrogen economy.

1. Introduction

Human society has smoothly passed through two transportation energy revolutions from animal forces relying on living plant biomass to external combustion engines (steam engines) driven by solid coal to internal combustion engines (ICE) driven by liquid gasoline and diesel.¹ Transportation ability often reflects civilization level. Without it, cities could not exist; families would have to live close to the land, gathering and growing their own food; materials, medicines, medical cares, manufacturing, and electricity generation all depend on transportation.²

Currently, liquid fuels (gasoline, diesel, and jet fuel), along with internal combustion engines, are widely used to propel vehicles, trains, ships, and jet planes because of several advan-

tages: (1) relatively low fuel prices (until more recently); (2) very high energy storage densities (MJ per kg of fuel and MJ per litre of fuel); (3) high power density (kW per kg of engine); (4) easy storage, distribution, transportation, and refilling for liquid fuels; (5) relatively low costs for ICE (\$ per kW of output); and (6) safety for mass utilization. But the concerns pertaining to soaring prices of crude oil, depleting fossil fuels, net CO₂ emissions, climate change, national energy security, global and local food security, (rural) economic development, energy utilization efficiency, and wealth transfer are motivating the development of sustainable alternative transportation fuels. Second generation biofuels such as cellulosic ethanol, butanol, algae biodiesel, hydrocarbons, and synthetic diesel, can be integrated well with current infrastructures for liquid fuels and ICE systems but the ICE systems have relatively low energy efficiencies, since the efficiencies of heat engines are restricted by the second law of thermodynamics.

In the long term, improving energy utilization efficiency through hydrogen-fuel cell/electricity systems will be vital for sustainable transportation. Distinct from first generation fuels (e.g., solid coal) and second generation fuels (e.g., liquid gasoline,

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Broader context

Synthetic biology is an emerging interdisciplinary area that combines science and engineering in order to design and build novel biological functions and systems. Cell-free synthetic biology through *in vitro* assembly of a number of enzymes and coenzymes has been designed to implement unnatural reactions as C₆H₁₀O₅ (aq, starch or celloextrins) + 7 H₂O (l) → 12 H₂ (g) + 6 CO₂ (g). This new sugar-to-hydrogen technology promises to address several obstacles to the hydrogen economy – cheap hydrogen production, high hydrogen storage density (14.8 H₂ mass%), and costly hydrogen infrastructure, and to eliminate safety concerns about mass utilization of hydrogen. Also, these reactions can produce more chemical energy output as hydrogen than chemical energy input stored in polysaccharides for the first time.

diesel), third generation transportation fuels include hydrogen and electricity, both of which work as energy carriers that can be converted to kinetic work efficiently without the restriction of the second law of thermodynamics. Both hydrogen and electricity will be generated from various primary energy sources, such as biomass, solar energy, wind energy, geothermal energy, tidal energy and so on. The hydrogen-fuel cell-electricity system will play a predominant role because of (1) very high energy conversion efficiency through fuel cells, (2) minimal pollutants generated, (3) much higher energy storage densities than rechargeable batteries alone, and (4) diverse hydrogen-producing means from primary energy resources. But large-scale implementation of the hydrogen economy must break four technological hurdles – low cost hydrogen production from any primary energy resources, high hydrogen density storage means (>9 mass%), affordable fuel distribution infrastructure, and affordable fuel cells throughout the whole life cycle.^{3–5} In addition, hydrogen is a flammable, odorless, colorless gas. Any significant hydrogen explosion accident could prevent the public from accepting hydrogen as a transportation fuel.

Transportation fuels are and will be mainly produced by four primary resources – crude oil, natural gas, lignocellulosic biomass, and starchy crops like corn. Based on energy contents (\$ per gigajoule, GJ), delivered lignocellulosic biomass at \$60 per dry ton (\$3.60 per GJ) is least costly among all primary energy sources – compared to natural gas (\$7.58 per GJ, \$8 per mbtu), crude oil (\$15 per GJ, \$80 per barrel), and corn kernels (\$13 per GJ, \$4.5 per bushel) (Fig. 1). Although coal energy content (\$1.54 per GJ, \$50 per ton) is lower than that of lignocellulosic biomass, the conversion of coal to liquid transportation fuels is economically and environmentally prohibitive, except in special times or areas (e.g., Germany during World War II and South Africa).

Comparison of different current and potential transportation fuels is very complicated, involving a number of factors – fuel costs, resource availability, infrastructure availability, costs and lifetime of the engine/motor, environmental impacts, etc. Direct price comparison of transportation fuels, such as gasoline, diesel,

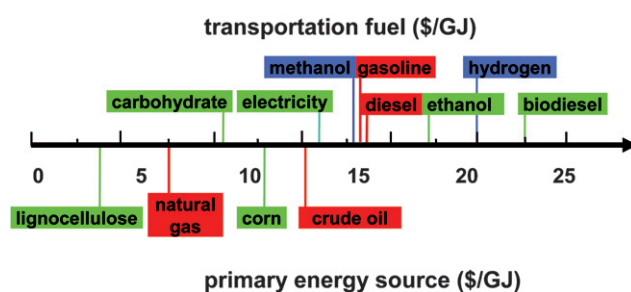


Fig. 1 Cost comparison of primary energy resources and potential transportation fuels. The prices of energy resources and fuels vary in a relatively large range and the values only represent likely recent prices.

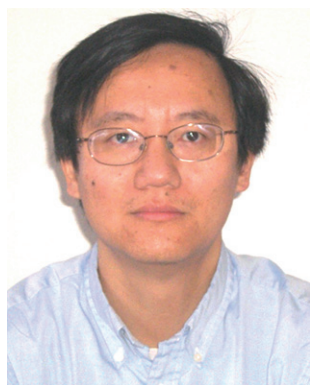
ethanol, biodiesel, methanol, hydrogen, or even electricity, is relatively straightforward for end-users because their prices include costs associated with feedstock, processing, capital depreciation, distribution, profits, and taxes. Fig. 1 shows the energy contents of potential fuels in an increasing order from carbohydrate (\$10.6 per GJ, \$0.18 per kg), electricity (\$16.7 per GJ, \$0.04 per kWh), methanol (\$17.8 per GJ, \$0.35 per kg), gasoline (\$17.6 per GJ, \$2.5 per gallon), diesel (\$19.5 per GJ, \$2.7 per gallon), ethanol (\$22.1 per GJ, \$2 per gallon), hydrogen (\$25.0 per GJ, \$3 per kg), to biodiesel (\$27.4 per GJ, \$3.5 per gallon). Carbohydrates isolated from corn kernels, sugarcane or cellulosic materials will be the least costly. Further conversion of carbohydrates to other fuels, such as ethanol, hydrogen or even synthetic bio-oil, will lead to higher prices. Electricity, a universal energy currency, can be generated from a number of resources – coal, natural gas, wind energy, nuclear energy, hydroelectric energy, and so on. Regardless of its generation means, electricity prices vary in a relatively narrow range after numerous conversions and grid distribution.

In this perspective, we briefly review the challenges for the hydrogen economy, propose an out-of-the-box solution that could systematically solve several of these challenges, discuss its technical feasibility, and emphasize future research directions.

2. The hydrogen economy

The hydrogen economy will be a linked network of processes that produces hydrogen, stores hydrogen chemically or physically, and converts the stored hydrogen to electrical energy at the point of use.^{3,6–8} Hydrogen is advantageous over electricity stored in rechargeable batteries for the transportation sector because stored hydrogen has a ~20-fold to >100-fold higher energy storage density than electricity stored in rechargeable batteries in terms of GJ per kg.^{9,10} Battery-only electric vehicles have a much shorter driving distance per recharging than hydrogen fuel cell systems.

Hydrogen can be produced from water and other hydrogen-containing compounds such as CH₄ and carbohydrates by a number of chemical, biological, electrical, photochemical, and photobiological approaches. Most hydrogen is currently produced from natural gas by a combination of steam reforming and water shift reactions, accompanied with a net release of CO₂ to the atmosphere. Because of soaring prices of fossil fuels, hydrogen production costs were more than \$2.70 per kg of hydrogen in 2005;¹¹ a situation that has clearly deteriorated since then.



Yi-Heng Percival Zhang was born in Wuhan, China. He received his BE and MS degrees from East China University of Science and Technology (Shanghai, China), and then obtained his Ph.D. of chemical engineering from Dartmouth College (USA) under supervision by biofuels pioneer Prof. Lee R. Lynd in 2002. He is an assistant professor at Virginia Polytechnic Institute and State University. His current research is focused on efficient cellulose

solvent-based lignocelluloses fractionation followed by saccharification by engineered cellulases as well as sugar-to-biofuels (e.g., hydrogen, electricity) generation through an in vitro synthetic biology approach – synthetic enzymatic pathway engineering.

Gaseous hydrogen storage is still the largest challenge. It can be stored (1) in high-pressure gas cylinders; (2) as liquid hydrogen in cryogenic tanks (at 21 K); and (3) in solid forms (e.g., adsorption on large specific surface area solid materials or hydrides (e.g., LiAlH_4 , NaAlH_4 , NaBH_4) or by the reaction of light metals and water.^{4,12} As for approaches 1 and 2, considerable energy is lost in hydrogen compression ($\sim 10\text{--}15\%$) or hydrogen liquefaction ($\sim 33\%$). Both also have low hydrogen storage densities, for example, liquid hydrogen has a hydrogen density of only 70.8 kg/m^3 (i.e., less than 7 mass $\text{H}_2\%$). Generally speaking, large scale high-pressure and cryogenic hydrogen storage systems are impractical for vehicular application due to safety concerns and volumetric constraints.¹³ Solid hydrogen storage technologies require high-gravimetric hydrogen density, adequate hydrogen-dissociation energetics, or stable and low-cost hydrogen carriers.^{12,13} Therefore, the US Department of Energy (DOE) set hydrogen storage goals at 6 mass% and 9 mass% for 2010 and 2015, respectively.⁵ Recently, possible hydrogen-storage materials meeting FreedomCar requirements (e.g., density, refilling rate, refilling time, and reuse cycle time), such as metal-organic frameworks with potential densities of 10 H_2 mass%, have been proposed in the DOE 2008 annual merit review and peer evaluation.¹⁴

Hydrogen, a small and energetic molecule, can diffuse through container materials or react with materials. For example, hydrogen cannot be simply delivered by today's natural gas pipeline systems because of steel embrittlement, accompanied with increased maintenance costs, leakage rates, and material replacement costs. Hydrogen pipelines will be much more expensive than electric transmission lines and natural gas pipelines. Proponents of the hydrogen economy propose local hydrogen stations based on local sources.^{15,16} Unfortunately developing these stations in high demand urban areas will have many challenges, including NIMBY (not in my backyard) backlash. Finally, a huge investment in the infrastructure is required for storing and distributing hydrogen, costing at least one trillion of dollars in the USA alone.^{15,17}

In order to solve the challenges associated with gaseous hydrogen storage and costly infrastructure, high-energy-density liquid fuels – such as methanol, ethanol, liquefied petroleum gas, gasoline, or biodiesel – have been proposed as hydrogen carriers. The vehicles must have an onboard chemical converter to reform them to hydrogen. Methanol, a liquid fuel, can be converted to hydrogen very easily *via* reforming or can be converted to electricity through direct methanol fuel cells (DMFC). The challenges faced by the DMFC technology include methanol crossover, high catalyst costs, low power density, poor efficiency, and short operation life.^{18–20} Ethanol and hydrocarbons can be converted to hydrogen and CO_2 plus some CO *via* catalytic steam reforming, partial oxidation, or auto-thermal reforming.^{21,22} Since a small amount of CO as a side-product of chemical catalysis can poison the catalysts of proton exchange membrane (PEM) fuel cells,²² extra purification steps are required to remove CO before entering PEM fuel cells. Carbon monoxide clean-up can be done in several ways – water gas shifting, selective CO removal, methanation, and Pd alloy membranes.²¹ These reformers have been shown to be highly complicated, difficult to operate, bulky, and expensive.²³ In order to avoid CO poisoning, ammonia, an easily-liquefied carbon-free gas, has been proposed

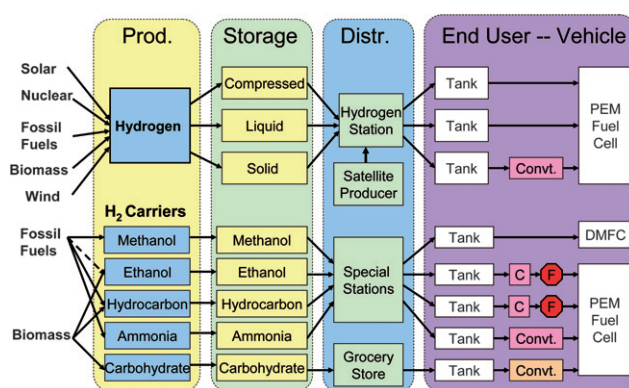


Fig. 2 Comparison of the different scenarios of the hydrogen economy.

as a hydrogen carrier. Production of NH_3 from pure hydrogen and the consequential conversion of ammonia to hydrogen is not energy- and cost-efficient. Obviously, any current high-temperature on-board reformers result in system complexity and some energy loss during such conversions, implying their infeasibility for vehicular applications.

Low-temperature PEM fuel cells are used primarily for transportation applications due to their fast startup time, low sensitivity to orientation, high energy conversion efficiency, low-operating temperature (below 100°C), and favorable power-to-weight ratio (lightweight and compact). In contrast, high-temperature fuel cells are not amenable to transportation propulsion.²⁴ Therefore, nearly all the major automakers have fuel cell projects based on PEM technology with an electric motor, but the challenge of gaseous hydrogen storage results in a shorter driving range compared to gasoline-powered vehicles (300–400 miles driving distance per tank). In contrast, the Nobel Prize winner George A. Olah advocates the methanol economy,²⁵ but DMFC may be good only for low power applications, such as portable electronics.¹⁹

Fig. 2 presents different possible scenarios of the future hydrogen economy for the transportation sector, including hydrogen production, storage, distribution, fuel cell, and end users – vehicles. Hydrogen can be produced from diverse primary energy sources, such as solar energy, biomass, fossil fuels, tidal energy, geothermal energy, and so on. Once gaseous hydrogen is produced, its storage and distribution will lead to big challenges, as described above. The use of hydrogen carriers, such as methanol, hydrocarbons, or even ammonia, may be more promising in principle than direct use of gaseous hydrogen. But the system complexity of CO removal from the thermal reformers is a show stopper for the carbon-containing hydrogen carriers through on-board reforming. Therefore, the demonstration vehicle systems based on liquid hydrocarbon on-board reforming systems followed by PEM fuel cells have been abandoned. We propose a new solution – the on-board carbohydrate-to-hydrogen-PEM fuel cell system (Fig. 2).

3. An out-of-the-box solution for the hydrogen economy

We propose solid polymeric carbohydrates ($\text{C}_6\text{H}_{10}\text{O}_5$, 14.8 H_2 mass%) as a hydrogen carrier, based on the new *in vitro* synthetic

biology approach.²⁶ The use of low-cost, sustainable biomass as the primary energy source for producing transportation fuels (e.g., cellulosic ethanol and hydrogen) provides benefits to the environment, economy, and national security.^{1,6,27–38} Biomass is an enriched chemical energy source that can solve the scale-up and storage challenges associated with low-power density solar radiation.³⁹ A number of biomass-to-hydrogen production approaches have been investigated previously:

1. gasification,^{40,41} (fast or flash) pyrolysis,^{42–46} or aqueous phase reforming;^{47–51}

2. anaerobic hydrogen fermentation^{8,31,52–57} and/or a bio-electrochemically assisted microbial fuel cell reactor that can convert acetate to hydrogen with the help of a little electricity;^{58,59}

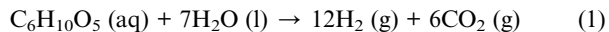
3. cell-free synthetic enzymatic pathways;^{26,60} and

4. combinatorial biological and chemical catalysis: polysaccharide hydrolysis^{31,38,61,62} and glucose–ethanol fermentation or consolidated bioprocessing^{31,63–65} followed by chemical catalysis – ethanol partial oxidation reforming.^{22,66}

The carbohydrate-to-hydrogen conversion by the cell-free synthetic enzymatic pathways (a new *in vitro* synthetic biology approach) features (i) mild reaction conditions, (ii) no CO side-product, (iii) complete conversion, and (iv) potentially high reaction rates. This allows us to propose an out-of-the-box solution for the hydrogen economy: the use of sugars as a hydrogen carrier. Potential applications include stationary power providers, local hydrogen stations, refillable sugar batteries, sugar-powered automobiles, air-independent-propulsion submarines, or even electric aircraft.

3.1. Novel hydrogen production

The novel synthetic enzymatic pathways have been designed to produce 12 moles of hydrogen per mole of glucose equivalent of glucans (starch and cellulose) and water.^{26,60} The idea is to utilize the energy stored in polysaccharides to split water and stepwise release all energy of carbohydrates in the form of hydrogen under mild reaction conditions ($\ll 100\text{ }^\circ\text{C}$ and $\sim 1\text{ atm}$) as below



These synthetic catabolic pathways that do not exist in nature are comprised of 13 enzymes in one pot (Fig. 3). Most of the reactions in the pathway catalyzed by the enzymes are reversible. The removal of gaseous products from the aqueous phase favors the unidirectional overall reaction. In addition, enzymatic biochemical reactions are well-known for their 100% selectivity at modest reaction conditions. Thermodynamic analysis suggests that the overall reaction is a spontaneous process (*i.e.*, $\Delta G^\circ = -49.8\text{ kJ mol}^{-1}$) and is an endothermic reaction (*i.e.*, $\Delta H^\circ = 598\text{ kJ mol}^{-1}$).⁶⁰ The negative value of Gibbs free energy at 25 °C suggests a nearly complete conversion. The Gibbs energy of this reaction decreased greatly with an increase in temperature, suggesting higher conversion at elevated temperatures. This reaction is driven by entropy gain rather than enthalpy loss. Another well-known entropy-driven reaction is acetate fermentation from glucose [$\text{C}_6\text{H}_{12}\text{O}_6(\text{aq}) + 2\text{H}_2\text{O}(\text{l}) \rightarrow 2\text{CH}_4\text{O}_2(\text{aq}) + 2\text{CO}_2(\text{g}) + 4\text{H}_2(\text{g})$]. In addition, the removal of both gaseous products from the aqueous reactants at mild reaction condition ($< 100\text{ }^\circ\text{C}$ and $\sim 1\text{ atm}$) drives the reaction forward to completion.⁶⁰ This

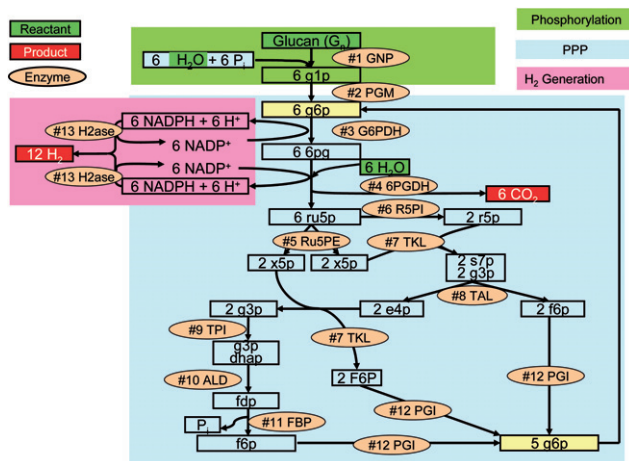


Fig. 3 The synthetic metabolic pathway for complete conversion of glucan and water to hydrogen and carbon dioxide. PPP, pentose phosphate pathway taken from ref. 26. The enzymes are: #1 GNP, glucan phosphorylase; #2 PGM, phosphoglucomutase; #3 G6PDH, G-6-P dehydrogenase; #4 6PGDH, 6-phosphogluconate dehydrogenase; #5 R5PI, phosphoribose isomerase; #6 Ru5PE, ribulose 5-phosphate epimerase; #7 TKL, transketolase; #8 TAL, transaldolase; #9 TPI, triose phosphate isomerase; #10 ALD, aldolase; #11 FBP, fructose-1,6-bisphosphatase; #12 PGI, phosphoglucose isomerase; and #13 H₂ase, hydrogenase. The metabolites and chemicals are: glp, 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 P_i, inorganic phosphate.

entropy-driven chemical reaction can generate more output chemical energy in the form of hydrogen than input chemical energy in polysaccharides by adsorbing ambient-temperature thermal energy.^{26,60}

The first proof-of-principle experiment has been conducted to validate whether or not hydrogen can be produced from starch and water.^{26,67} A number of enzymes, isolated from animal, plant, bacterial, and yeast sources, plus an archaeal hyperthermophilic hydrogenase, are put together in one pot. Although each of them has a different optimal pH, temperature, and cofactor, the compromised conditions used are 0.1 M HEPES buffer (pH 7.5) containing 5 mM thiamine pyrophosphate, 4 mM phosphate, 2 mM NADP⁺, 10 mM MgCl₂, and 0.5 mM MnCl₂ at 30 °C. Under these conditions, each enzyme remains active but is believed to be far from its optimal activity. The first reaction mediated by substrate phosphorylases plays an important role in producing glucose-1-phosphate by shortening polysaccharides without the use of ATP.^{26,63,68,69} Utilization of substrate phosphorylase enzymes is far superior to any kinase reaction involving hexokinase and ATP because of (1) no costly ATP regeneration system; (2) no accumulation of phosphate, an inhibitor of several enzymes (*e.g.*, fructose biphosphatase);⁷⁰ (3) no Mg²⁺ precipitation,⁷⁰ since Mg²⁺ is a key co-factor of several enzymes; and (4) a more homostatic pH.

Fig. 4 shows that hydrogen is produced as expected, a little later than CO₂ evolution, consistent with the designed mechanism in Fig. 3. A lag phase of hydrogen production is attributed

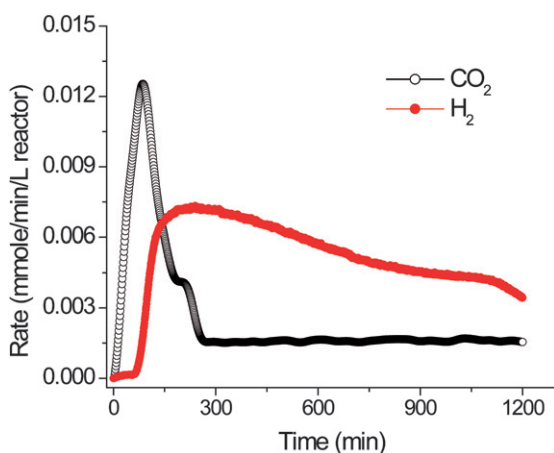


Fig. 4 Hydrogen production from starch and water at 30 °C and 1 atm modified from the ref. 26.

to the initial addition of NADP⁺ as a cofactor. When NADPH is used, there is no lag phase for hydrogen generation. This proof-of-principle experiment has been conducted by using off-the-shelf enzymes without any optimization so that the reaction rates are very low, far from the demands of practical applications.²⁶ Recently, the hydrogen production rate has been increased by 8.2 fold starting from cellulosic materials as compared to the previous results by (i) increasing the rate-limiting hydrogenase concentration, (ii) increasing the substrate concentration, and (iii) elevating the reaction temperature slightly from 30 to 32 °C (Table 1). Under the current system parameters, the measured production rate of H₂ is higher than those for photobiological systems and comparable to those reported for dark fermentations.⁵⁴ Further enhancement in hydrogen production rates will be discussed in Section 4.

3.2. Special features

The complete conversion of sugars and water to hydrogen and carbon dioxide mediated by these synthetic enzymatic pathways^{26,60} provides a number of special features suitable for mobile PEM fuel cells.

1. Highest energy efficiency. Enzymatic hydrogen production is the only one that can produce nearly 12 moles of hydrogen per mole of glucose equivalent. In addition to extracting all the chemical energy stored in the substrate sugars, the overall reaction is endothermic, *i.e.*, some of low-temperature thermal energy is absorbed and converted to chemical energy in the form

of hydrogen (22% combustion energy gain during this bioreforming).

2. High hydrogen storage density. Polysaccharides have a chemical formula C₆H₁₀O₅ with a reaction of C₆H₁₀O₅ (aq) + 7 H₂O (l) → 12 H₂ (g) + 6 CO₂ (g). As a result, hydrogen storage density in polysaccharides is 24/162 = 14.8 H₂ mass%, where water can be recycled from PEM fuel cells.

3. Mild reaction conditions (<<100 °C and ~1 atm), which do not require bulky, costly pressure reactors. The reactor temperatures are at the same range of those of PEM fuel cells, good for coupling these endothermic and exothermic reactions.

4. Nearly no costs for product separation (gas/liquid). This reaction only produces two gaseous products – CO₂ and hydrogen. Under mild reaction conditions, the reactants (sugar and water) plus the enzymes and the cofactor remain in the aqueous phase. Separation of the gaseous products and aqueous reaction is easy and nearly cost-free. Critically, the removal of the reaction products also drives the reactions forward and avoids product inhibition.

5. Clean products for PEM fuel cells along with easy power system configuration.

6. Simple and safe distribution and storage of solid sugars. Therefore, investment for upgrading infrastructure and distribution of solid sugars would be minimal.

3.3. Future applications

These enzymatic sugar-to-hydrogen reactions have several potential applications from local hydrogen generation stations to low-cost electricity generators, to high energy-density batteries, as well as sugar-powered vehicles, all of which require faster hydrogen production rates as this nascent technology is improved and optimized.

3.3.1. Local hydrogen generation station. Gaseous hydrogen distribution infrastructure is not currently available and would be very costly. Local production of hydrogen based on local renewable resources is believed to be a valuable alternative for supplying hydrogen to local end users – hydrogen fuel cell vehicles. Local satellite hydrogen generation stations could produce hydrogen based on this sugar-to-hydrogen approach, store the hydrogen, and refill hydrogen-fuel cell vehicles. The solid sugar powders produced locally will be easily collected and distributed based on current solid goods delivery systems. It is estimated that a several-fold increase in current hydrogen production rates would be sufficient for this application.

Table 1 Summary of enzymatic hydrogen production rates

Substrate	Concentration ^a /mM	Temperature/°C	V _{max,H2} /mmole h ⁻¹ L ⁻¹	References
G-6-P	2	30	0.21	140
G-6-P	2	30	0.73	26
Starch	1	30	0.48	26
Cellobiose	2	32	0.48	60
Cellopentaose	8	32	3.92	60

^a potential glucose equivalent for hydrogen production.

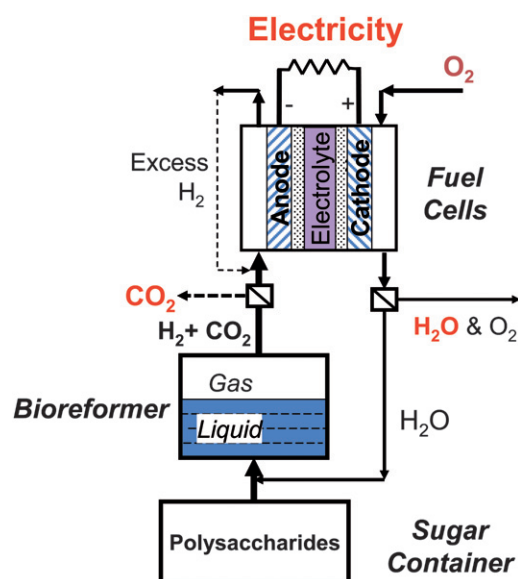


Fig. 5 Conceptual sugar-to-electricity system.

3.3.2. Low-cost (remote) electricity generator. Integration of this sugar-to-hydrogen system with fuel cells (Fig. 5) could produce low-cost electricity from low-cost sugars (\$0.18 per kg, Fig. 1), especially ideal for remote areas without electrical transmission lines and grids. The products (hydrogen and carbon dioxide) will bubble up from the aqueous reactants; pure hydrogen could be separated from CO₂ by using alkali adsorption for CO₂ sequestration, pressure swing adsorption or membrane separation; electricity will be generated by fuel cell stacks by using hydrogen and oxygen in the air. The reaction product water of fuel cells will be partially recycled for sugar dissolution. The whole system will have very high electricity conversion efficiencies since the conversion of carbohydrate to hydrogen is endothermic, *i.e.*, 22% of the combustion enthalpy of hydrogen comes from ambient thermal energy or waste heat from fuel cells. If phosphoric acid fuel cells are chosen, hot water will be co-generated. The whole energy (electricity and heat) conversion efficiency may be very close to 100%. It is estimated that a 1 kW electricity generator would have a 60 L bioreformer if a 10-fold increase in hydrogen rate is achieved. With technology improvements, the proposed enzymatic hydrogen production systems will even compete with diesel-to-electricity generators, while avoiding the use of fossil fuels and emitting no net greenhouse gases.

3.3.3. Sugar-powered vehicle. Fig. 6 shows a conceptual sugar-powered vehicles based on a hybrid of PEM fuel cells and rechargeable batteries. This combination will have both high energy storage density and power density. Solid sugar powders will be refilled into the sugar container in the car at local sugar stations; the on-board bioreformer will convert the sugar solution to hydrogen and carbon dioxide by the stabilized enzyme cocktail; a small-size buffer hydrogen storage container will balance hydrogen production/consumption; feeding of a mixture of CO₂/H₂ or pure hydrogen in the PEM fuel cells will dramatically decrease system complexity and greatly increase the system operation performances; approximately a half of water generated

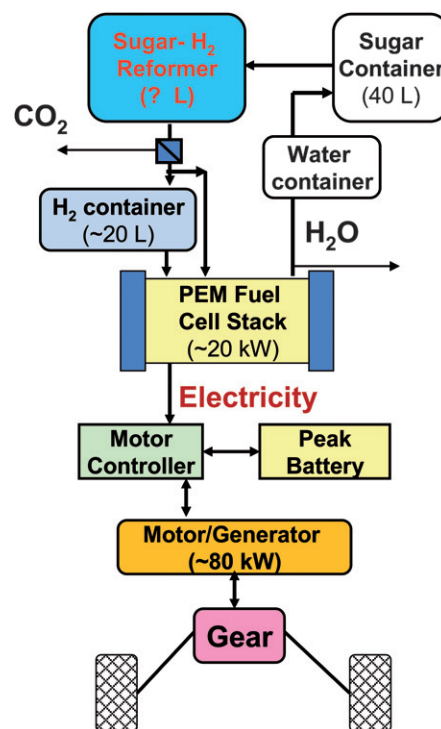


Fig. 6 Conceptual hybrid power train system including on-board sugar-to-hydrogen converter, PEM fuel cell and rechargeable battery.

from the fuel cells is used for dissolving solid sugars. Similarly, the heat output from PEM fuel cells will be coupled to the heat input needed by the bioreformer. The electrical energy from the fuel cells will be sent to the motor controller/motor/gear to generate kinetic energy. When extra energy is needed for acceleration or start-up, electrical energy stored in the rechargeable peak battery will be released. Also, similar to the gasoline-electric hybrid system, *e.g.*, the Toyota Prius, the kinetic energy on braking will be converted to electricity and stored in the battery.

Small-size hydrogen fuel cell vehicles need hydrogen production rates of ~1–2 kg per hour. Producing sufficient hydrogen at rapid rates from a small bioreformer is the number one technological challenge. Producing one kg of hydrogen per hour will need a reaction volume of 130 m³ based on the current reaction rate of 3.92 mmole of hydrogen per hour per litre, implying that this application is technically impractical. But we expect to be able to increase the hydrogen production rate by several orders of magnitude through a combination of known technologies (see Section 4). To our knowledge, the highest biohydrogen production rate is 21.8 moles of hydrogen per litre per hour,⁷¹ ~5600 times higher than the enzymatic hydrogen process.⁶⁰ If we can increase the rate by 2000-fold, the volume of the bioreformer will be as small as 65 litres, which will be small enough to replace small-size internal combustion engines. If 4–10 kg of hydrogen is needed for driving more than 300 miles before refilling, that means that 27–67.6 kg of sugar will be stored in the vehicles, occupying a volume of 38.6–96.6 litres or 10.2–25.5 gallons.

The proposed power train systems would have a very high energy conversion efficiency (overall, 55%; carbohydrate–hydrogen, 122%; hydrogen–PEM fuel cell, 50%; electricity–motor, 90%), ~3.0 times higher than that of ethanol-internal

combustion engines (overall, 18.2%; carbohydrate–ethanol, 90%; internal combustion engine, 25%; transmission, 85%). This proposed energy efficiency would be the highest among all power-train systems, including internal combustion engines, standard hydrogen–fuel cell systems, gas turbines, *etc.* If the USA's biomass resource through bioethanol–internal combustion engines replaced 30% of transportation fuels in 2030,⁷² the same amount of biomass through hydrogen–PEM fuel cell systems would achieve at least 90% transportation fuel independence through this new technology without reliance on any other energy sources.

Seemingly competitive technology –aqueous phase reforming^{47–51} – is not suitable for on-board PEM fuel cell systems because it has poor hydrogen selectivity, low yield, and dirty products (*e.g.*, CO), and requires high temperature (~ 250 °C) and pressure (*e.g.*, ~ 50 atm) reactors. Therefore, on-board reformation though aqueous phase reforming appears not to be technically feasible. Similar situations occur with on-board hydrocarbon-to-hydrogen reforming.

3.3.4. Super-high energy density sugar battery. The system integrating the sugar container, sugar-to-hydrogen reformer with PEM fuel cells can be regarded as a new biodegradable primary battery or refillable (rechargeable) secondary battery after system miniaturization. 14.8 mass% hydrogen equals an output of 2.94 kWh per kg sugar assuming an efficiency of PEM fuel cell of 50%, much higher than any current batteries (lead acid, ~ 0.030 kWh per kg; Ni–Cd, ~ 0.050 kWh per kg; Ni–MH, ~ 0.090 kWh per kg; Li ion, ~ 0.150 kWh per kg; and PL ion, ~ 0.150 kWh per kg).^{9,10} High-energy density sugar can store more energy than batteries for transportation applications before refilling or recharging.² The real energy storage density of the sugar-battery will be lower than the theoretical value of 2.94 kWh per kg of sugar because of the volume and weight of the bioreformer, whose size will be decreased as technology improvements occur in the enzyme performance and PEM fuel cell configuration. The energy storage density will also depend on the weight ratio of fuel to the other parts. A critical advantage is that sugar fuels are supplied to the cell rather than being embedded with it.⁷³ For some special applications, such as air-independent-propulsion (AIP) submarines, the energy density of the sugar battery may be very close to its theoretical value (2.94 kWh per kg of fuel + fuel cell) because of the high ratio of fuel weight to the other components. The hypothetical super-high energy density sugar will be a very promising alternative compared to other developing batteries.¹⁰

As compared to current developing enzymatic biofuel cells,^{10,73–75} the hypothetic sugar–hydrogen–PEM fuel cell systems have several advantages: (1) much higher energy extracting efficiency (122% *vs.* 15–20%), (2) several orders of magnitude higher energy output density (W m^{-2}), and (3) minimal product inhibition. Many attempts at enzymatic biofuel cells have been made recently to extract all the chemical energy in biofuels and convert it to electricity.^{76,77} All sugar batteries must overcome the challenges, such as enzyme costs and enzyme stability.^{74,78} For example, one kg of industrial immobilized thermostable glucose isomerase can convert at least 1 500 000 kg of glucose to fructose or have a turn-over number of ~ 800 000 000.^{79,80} A startup company, Akermin, has claimed

enzyme stabilization technology for three years by encasing enzymes in a proprietary, protective polymer structure. Another example is the more than one year shelf-life of glucose dehydrogenase at room temperature used in the blood sugar strips for diabetes patients. Obviously, the collaborations for enzyme and cofactor stabilization among groups of enzymatic biofuel cells, biosensors, and the hypothesized sugar-to-hydrogen–PEM fuel cell systems are expected.

4. Research and design perspectives

Before the above-mentioned applications are implemented, two major technical challenges must be overcome – (i) slow hydrogen production rate and (ii) high production cost.

Increasing the hydrogen production rate is the number one technological challenge because it is a requirement for all future applications. The proof-of-principle biohydrogen production experiment by the synthetic enzymatic pathway conducted by using off-the-shelf enzymes with some optimization has a reaction rate of 3.92 mmole of hydrogen per litre of reaction volume per hour.⁶⁰ The first significant improvement in reaction rates can be made by optimizing the enzyme ratio. We have estimated a potential improvement of at least ~ 20 -fold by optimization of the rate-limiting step enzyme ratios and increasing substrate levels.⁸¹ Second, another significant improvement will be implemented by increasing the reaction temperature. Currently, we are lacking thermostable enzymes. The rule of thumb suggests that most enzymatic reaction rates usually are doubled with every 10 °C increase (*i.e.*, Q_{10} effect). Therefore, an increase in the reaction temperature from 30 °C to 80 °C could result in another ~ 32 fold improvement. For example, the hyperthermophilic *P. furious* hydrogenase exhibits $< 1\%$ of its potential activity in the proof-of-principle experiment (32 °C). Increasing reaction temperature will decrease hydrogenase use and increase the overall reaction rate. Third, a 100-fold increase in enzyme concentration could lead another potential rate enhancement by 20–100 fold. Fourth, when the overall enzyme concentration is high, macromolecular crowding effects could lead to metabolite or substrate channeling between the cascade enzymes, which could contribute to another reaction rate enhancement by ~ 2 –100 fold, which is observed sometimes, especially in macromolecular crowding conditions.^{82–84} Finally, there will be a great enhancement potential in the turnover numbers for each enzyme by several orders of magnitude, because their catalytic efficiencies are still much lower than those catalytically perfect enzymes with a $k_{\text{cat}}/K_{\text{m}}$ of 10^8 – 10^9 per M per s.^{85,86} Based on the above analysis, an increase in hydrogen production rate by at least 3 orders of magnitude from the current levels will be reachable after intensive R&D efforts within several years. Comparatively, the power density of microbial fuel cells has been improved by greater than 10^4 – 10^6 fold during the past 10 years.^{58,87}

To our knowledge, the highest biological hydrogen production rate is 11.8 moles of hydrogen per litre of reactor volume per hour, which is mainly implemented by using two combinatorial technologies: high enzyme loading and high substrate concentration.⁷¹ This rate is high enough for some high power applications, for example, hydrogen–PEM fuel cell devices. Given the same reaction rate, a high-power vehicle equipped with a 100 kW (134 hp) PEM fuel cell stack would need an on-board

bioreformer having a reasonable volume of 210 litres, plus a peak battery with a several hundred kW electric motor.

High hydrogen production costs are associated with three key components – costly and unstable enzymes, the coenzyme (NADP⁺), and the substrates. Decreasing the enzyme costs can be carried out by two main approaches – decreasing enzyme production costs and extending enzyme lifetime. The former can be mainly implemented by (a) producing recombinant enzymes rather than purifying them from natural biological entities,⁸⁸ (b) over-expressing the target enzymes,^{88,89} (c) implementing high-cell density fermentation by using low-cost nutrients,³⁸ and (d) decreasing enzyme purification costs.^{90–92} The latter (*i.e.*, stabilization of the enzymes) can be implemented by (a) immobilization on traditional materials or nano-materials,^{93–99} (b) thermostable enzyme replacement,^{100–103} (c) enzyme formulation,^{104–106} and (d) enzyme engineering by directed evolution or rational design.^{107–113} Recently, a hyperthermostable 6-phosphogluconate dehydrogenase (#4 enzyme) from the hyperthermophilic bacterium *Thermotoga maritima* has been over-expressed in *E. coli* with a yield of more than 200 mg per litre of culture. It is found to retain >90% of its activity at 80 °C for more than 48 hours (manuscript under preparation). Stabilization of one enzyme or multiple enzymes on solid supporters is a widely-known technology.^{74,114} With the rapid development in nano-materials with much larger surface areas (*i.e.*, more enzymes can be immobilized), examples of ultra-stable immobilized enzymes have been reported to be active for one to several months.^{93,96,98,115,116} It is expected that these combinatorial technologies will stabilize the enzymes for several months or even longer at ambient temperatures and at the evaluated temperature for more than 200 hours in the near future.

NAD(P) is not a stable under certain circumstances^{117,118} but its stability can be enhanced greatly by chemical modifications or immobilization.^{114,119} Asymmetric synthesis mediated by enzymes involving NAD(P)H regeneration is becoming more and more competitive in the pharmaceutical industry.^{120,121} The reported total turnover number for cofactors is as high as 600 000¹²² or even more than 1 million,¹²³ suggesting the economical feasibility of recycling NAD(P)H for hydrogen production.

Starch is food and animal feed, and its supply is becoming more restricted again. Cellulosic material is the most abundant renewable resource; the yearly energy production is ~6 fold of all human energy consumption.^{124,125} If a small fraction of yearly cellulosic material (*e.g.*, 10%) is used for transportation, transportation fuel independence will be reached. Cellulose has the same chemical formula as starch except with different glucosidic bond linkage between anhydroglucose units.⁶¹ Producing hydrogen from cellulosic materials must overcome two obstacles: (1) increasing cellulose reactivity for fast reaction rates and (2) discovery or development of cellulose phosphorylases that can phosphorylate β -1,4-glucosidic bonds. With regard to obstacle 1, the crystalline cellulose structure can be completely broken by using cellulose solvents, such as concentrated phosphoric acid,^{126–128} ionic liquids^{129–131} and so on. The presence of lignin and hemicellulose in natural lignocellulose negatively influences cellulose hydrolysis rates and digestibility. The best lignocellulose pretreatment will be implemented if (1) hemicellulose and lignin can be removed efficiently, (2) crystalline cellulose can be

converted to amorphous cellulose, (3) low processing costs are attained, and (4) low capital investment is used. Recently, a new cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) technology that combines a cellulose solvent (concentrated phosphoric acid) and a organic solvent featuring modest reaction conditions (*e.g.*, 50 °C and atmospheric pressure) aims at lignin, hemicellulose, and cellulose at the same time.^{128,132} Very high cellulose digestibilities (~97%) by cellulase are obtained for a number of feedstocks (*e.g.*, corn stover, switchgrass and hybrid poplar) within a short hydrolysis time of 24 hours. With regard to obstacle 2, cellobiose and cellodextrin phosphorylases^{63,69,133–135} may be the starting enzymes for creating unnatural or undiscovered cellulose phosphorylase.

Costs of hydrogen production from carbohydrates (*e.g.*, \$0.18 per kg of carbohydrate) would be as low as ~\$2 per kg of H₂, assuming that feedstock costs account for 60% of overall costs and enzymes and co-enzymes account for 40%. In general, approximately 40–75% of commodity prices, such as gasoline from crude oil, hydrogen from natural gas, and ethanol from corn kernels, come from feedstock costs.¹³⁶ If the enzymes were produced as cheaply as industrial enzymes (*e.g.*, cellulase, amylase, protease), and their stability was enhanced to the same level of immobilized glucose isomerase,⁸⁰ the estimated hydrogen production costs through this enzymatic biocatalysis would be far lower than \$2 per kg of hydrogen.

An alternative way to decrease the costs of enzymes and coenzyme for hydrogen production is to put the synthetic enzymatic pathway containing 13 over-expressed enzymes into a minimal bacterium¹³⁷ or create a new super hydrogen production microorganism by total synthesis of the whole genomic sequence.¹³⁸ But the implementation of the hypothesized new bacteria will take a long time, the hydrogen yields must be a little lower than 12 H₂ per glucose unit due to cellular biomass synthesis, and the hydrogen production rates could be very slow for some applications due to membrane blockage.^{67,139}

To implement sugar-powered cars, a number of process engineering challenges have to be overcome, for example, warm-up of the bioreformer, shut-down of the bioreformer, temperature controlling for the coupled bioreformer and fuel cells, mixing and gas release control for the bioreformer, and re-generation of used enzymes and co-enzymes in the bioreformer, to name a few. But such technical challenges can be solved if the great potential is widely realized.

5. Conclusion

Hydrogen production by synthetic enzymatic pathways is the most efficient way to convert the energy stored in renewable sugars to hydrogen energy.^{26,60} In addition, an endothermic reaction at ambient temperature means absorption of some low-temperature heat energy and conversion to a high-quality chemical energy carrier – hydrogen.^{26,60,67} Hydrogen production from the enriched chemical energy source – sugars produced from photosynthesis – suggests minimal challenges for scale-up and storage of feedstocks. We now need to address both increasing the hydrogen production rates and decreasing the hydrogen production costs. With technological improvements, this carbohydrate-to-hydrogen technology will address the

challenges associated with hydrogen production, storage, safety, distribution, and infrastructure in the hydrogen economy.²⁶

We envision that we will drive sugar-powered vehicles having a driving distance of >300 miles per refill. Solid sugar (~27–68 kg of sugars or 4–10 kg of hydrogen per refilling) will be added at local outlets such as grocery stores and the like. The on-board bioreformer with a volume of several tens or hundreds of litres containing a number of stabilized enzyme cocktails will convert sugar syrup to hydrogen, which will be converted to electricity quickly with very high energy efficiency and high power density via the PEM fuel cell. As a result, driving tomorrow with renewable sugars will no longer be viewed as science fiction! These systems will be the most energy efficient and greenest power-train with high power density and high energy storage density. This ambitious project of the sugar-powered vehicle will become a hen that will lay golden eggs for various sub-directions – enzyme engineering, enzyme immobilization, synthetic biology, fuel cells, battery, powertrain system integration, and so on.

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Energy Efficiency Analysis: Biomass-to-Wheel Efficiency Related with Biofuels Production, Fuel Distribution, and Powertrain Systems

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Abstract

Background: Energy efficiency analysis for different biomass-utilization scenarios would help make more informed decisions for developing future biomass-based transportation systems. Diverse biofuels produced from biomass include cellulosic ethanol, butanol, fatty acid ethyl esters, methane, hydrogen, methanol, dimethylether, Fischer-Tropsch diesel, and bioelectricity; the respective powertrain systems include internal combustion engine (ICE) vehicles, hybrid electric vehicles based on gasoline or diesel ICEs, hydrogen fuel cell vehicles, sugar fuel cell vehicles (SFCV), and battery electric vehicles (BEV).

Methodology/Principal Findings: We conducted a simple, straightforward, and transparent biomass-to-wheel (BTW) analysis including three separate conversion elements -- biomass-to-fuel conversion, fuel transport and distribution, and respective powertrain systems. BTW efficiency is a ratio of the kinetic energy of an automobile's wheels to the chemical energy of delivered biomass just before entering biorefineries. Up to 13 scenarios were analyzed and compared to a base line case – corn ethanol/ICE. This analysis suggests that BEV, whose electricity is generated from stationary fuel cells, and SFCV, based on a hydrogen fuel cell vehicle with an on-board sugar-to-hydrogen bioreformer, would have the highest BTW efficiencies, nearly four times that of ethanol-ICE.

Significance: In the long term, a small fraction of the annual US biomass (e.g., 7.1%, or 700 million tons of biomass) would be sufficient to meet 100% of light-duty passenger vehicle fuel needs (i.e., 150 billion gallons of gasoline/ethanol per year), through up to four-fold enhanced BTW efficiencies by using SFCV or BEV. SFCV would have several advantages over BEV: much higher energy storage densities, faster refilling rates, better safety, and less environmental burdens.

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Introduction

The sustainability revolution from non-renewable sources to renewable sources is the defining challenge of our time [1,2,3]. Mobility usually represents the level of a civilization [4,5]. Light-duty passenger vehicles, which constitute the largest type of transportation energy consumption among different transportation modes, have some special requirements, such as high energy storage capacity in a small container (e.g., ~50 liters), high power output (e.g., ~20–100 kW per vehicle), affordable fuel (e.g., \$~20–30/GJ), affordable vehicle, low costs for rebuilding the relevant infrastructure, fast charging or refilling of the fuel (e.g. several min per time), and safety concerns [5,6,7]. Such strict requirements result in limited choices for fuels and respective powertrain systems. Here powertrain refers to the group of components that generate power from stored energy and deliver it to wheels of vehicles running on the road surface, including the engine, transmission, drive shaft,

differentials, and wheels [8,9]. Therefore, current light-duty passenger vehicles mainly rely on non-renewable liquid fuels and internal combustion engines (ICE). But the depletion of crude oil, accumulation of greenhouse gases, concerns of national energy security, and creation of manufacturing jobs are motivating the development of sustainable transportation biofuels based on local renewable biomass [1,3,9,10].

Most ethanol is made from corn kernels and sugarcane, but this practice raises heated debate due to competition with food supplies; furthermore, its contribution to the transport sector is minimal or modest [1,11]. Lignocellulosic biomass is presently believed to be the only major renewable bioresource that can produce a significant fraction of liquid transportation fuels and renewable materials in the future [2,9,11,12] because the overall energy stored in phytobiomass each year is approximately 30-fold of the energy consumed for transportation [9,13]. But the future role of biomass in the transport sector remains in debate [1,14,15].

A great variety of biofuels can be produced from lignocellulose biomass, including cellulosic ethanol [10,16], butanol and/or long chain alcohols [17,18], electricity [19,20], bioalkanes [21], fatty acid esters [6,22,23], hydrogen [24,25,26,27], hydrocarbons [28, 29], and waxes [22]. The biofuels that will become short-, middle- and long-term transportation fuels is a matter of vigorous debate. Among them, some biofuels may have a particular niche market. For example, jet planes require high-density liquid fuels [6,17, 21,22]. First, the analysis presented here is restricted to the largest transportation fuel market – fuels for light-duty passenger vehicles. Second, this analysis starts from less costly lignocellulosic biomass that can be collected and delivered at reasonable costs (e.g., ~\$60–100 dollars per ton) [9,11]. Third, algal biofuel production or other renewable electricity generation (e.g., solar and wind electricity) is not covered in this paper.

Several types of powertrain systems have been developed to convert stored energy to kinetic energy, including internal combustion engines (e.g., gas ICE, diesel ICE, jet turbine, and rocket turbine), external combustion engines (e.g., steam engine and steam turbine), and electric motors. Because of special requirements of passenger vehicles, such as weight-to-power ratio (e.g., one to several g/W), engine costs (e.g., tens dollars/kW), and engine lifetime (e.g., ~5,000 h), only three engines are acceptable for passenger vehicles: gas ICE, diesel ICE, and electric motor. Considering electricity stored in batteries and possible on-board electricity generation systems (e.g., hydrogen proton exchange membrane (PEM) fuel cell) plus their hybrids, this analysis attempted to compare six current and future powertrain systems: gas-based ICE vehicles (ICE-gas) [7,8], hybrid electric vehicles based on gasoline ICE (HEV-gas) [30], hybrid electric vehicles based on diesel (HEV-diesel) [30], fuel cell vehicles based on compressed H₂ (FCV) [31,32,33,34], battery electric vehicles (BEV) [20,32], and sugar (hydrogen) fuel cell vehicles (SFCV) [3,5,9].

Numerous life cycle analyses (LCA) have been conducted to investigate the potential impacts of biomass/biofuels on energy applications, greenhouse gas emissions, and even water footprint [10,14,15,35,36,37,38,39,40,41,42,43,44]. But such analyses rely heavily on numerous assumptions, uncertain inputs (e.g., fertilizers, pesticides, farm machinery), energy conversion coefficients among different energy forms and sources, system boundaries, and so on. For example, conflicting conclusions have been made even for well-known corn ethanol biorefineries [10,36,37].

Here we suggest developing an energy efficiency analysis for biomass-to-wheel (BTW), a ratio of kinetic energy of the wheels of an automobile to the chemical energy of delivered biomass (Fig. 1). Conducting this BTW analysis is simple and straightforward because it not only avoids uncertainties or debates for (i) biomass production-related issues, (ii) feedstock collection and transport, and (iii) land use change, but also excludes water consumption issues and greenhouse gas emissions in the whole biosystem. Therefore, energy efficiency analysis (but not life cycle analysis) may not only be helpful in narrowing down numerous choices before more complicated LCA and techno-economic analyses are conducted, but may also increase the transparency of such analyses.

In this article, we present a simple biomass-to-wheel (BTW) efficiency (η_{BTW}) analysis methodology involving three elements -- biomass-to-fuel (BTF), fuel distribution, and fuel-to-wheel (FTW) (Fig. 2). Using this method, 13 combinations of different biomass-to-biofuel approaches and their respective powertrain systems were analyzed as compared to a baseline – corn-ethanol-ICE. The identification of high BTW efficiency scenarios would help make a more informed decision for how to utilize (limited) biomass

resource more efficiently. Following this, a more detailed LCA should be conducted for evaluating potential impacts associated with identified inputs and releases and for compiling an inventory of more relevant energy and material inputs as well as environmental effects.

Methods

The biomass-to-wheel efficiency (η_{BTW}), an energy conversion ratio of an automobile’s kinetic energy to the harvested and delivered biomass in the front of the door of biorefineries, involves three sequential elements – biomass-to-fuel production, fuel transport and distribution, and the powertrain system responsible for the fuel-to-wheel conversion (Fig. 2). The BTW efficiency is the lumped efficiency from chemical energy in biomass to kinetic energy for vehicle driving. The η_{BTW} value can be calculated as below

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

where

W is the kinetic energy transferred to wheels;

E_B is the chemical combustion energy of the biomass, where dry corn stover as a typical biomass contains ~65% carbohydrates (cellulose and hemicellulose, mainly), ~18% lignin, ~5% ash, ~12% other organic molecules [45,46]; and the E_B value is 16.5 MJ of low heating value/kg of corn stover [47];

η_{BTF} is the biomass-to-fuel (BTF) efficiency through biorefineries or power stations without significant inputs or outputs of other energy;

η_{TDL} is the fuel loss efficiency during its transport and distribution; and

η_{FTW} is the fuel-to-wheel (FTW) efficiency from the fuel to kinetic energy through powertrain.

The η_{BTF} value can be calculated as below

$$\eta_{BTF} = E_F / E_B \quad (2)$$

where E_F is the fuel produced in biorefineries or power stations. The η_{BTF} values of current corn ethanol as a reference range from 46% to 50% [48], and the value of 49% is chosen as a baseline [10]. Through the biomass sugars platform, potential biofuels include cellulosic ethanol, butanol, fatty acid esters (ester-diesel), hydrogen, and methane. Through syngas made by a thermochemical pathway, potential biofuels are ethanol, hydrogen, methanol, dimethyl ether (DME), FT-diesel, and electricity [49,50,51]. Also, electricity can be produced through direct combustion for the generation of steam followed by a steam turbine/generator, or biomass integrated gasification combined cycle (BIGCC) to fuel cells (Table 1).

Different powertrains are required to convert different biofuels to the kinetic energy of the wheels. The η_{FTW} value can be calculated as a ratio between the kinetic energy on wheels (W) and fuel energy in the tank (E_T):

$$\eta_{BTW} = W / E_T \quad (3)$$

For liquid biofuels, powertrain systems are gasoline ICE, HEV-gas, and HEV-diesel. Fuel cell vehicles run on stored compressed hydrogen, through a PEM fuel cell stack and an electric motor. The sugar fuel cell vehicle (SFCV) is a hypothetical powertrain system, where sugar is a hydrogen carrier, an on-board biore-

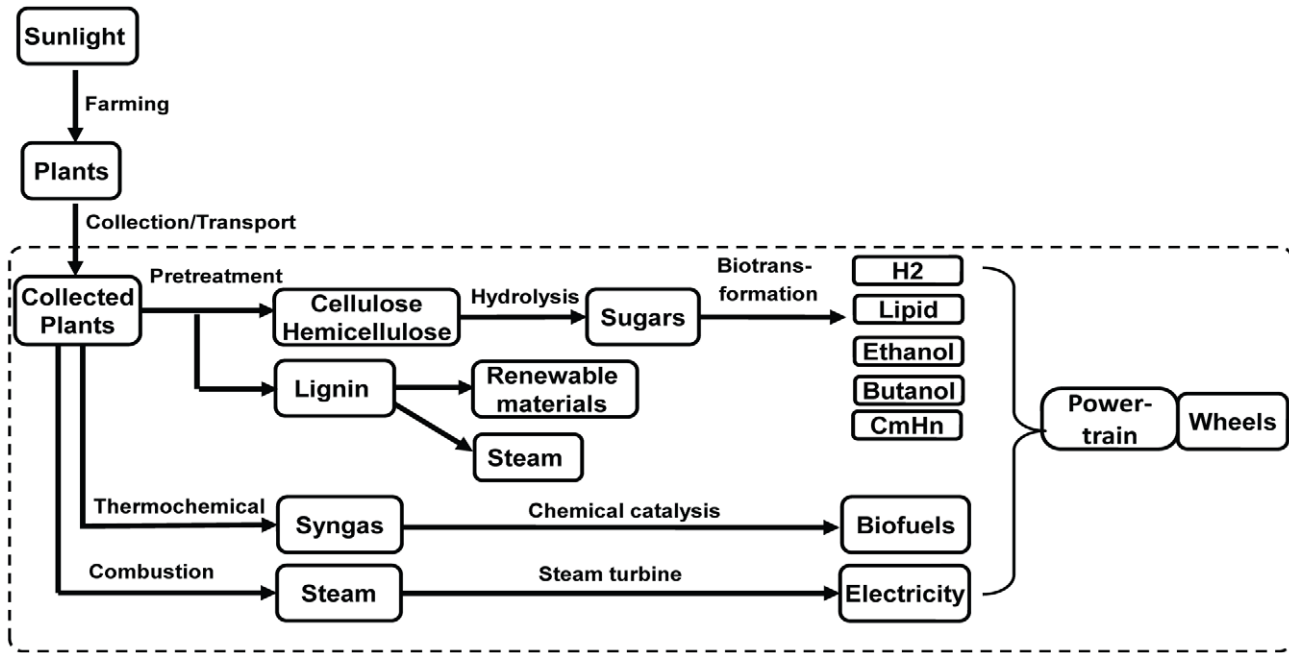


Figure 1. Different pathways for biofuels production from lignocellulosic biomass. The current energy efficiency analysis focuses on the delivered biomass-to-wheel efficiency related with conversion, transportation and power train systems.
doi:10.1371/journal.pone.0022113.g001

former generates high-purity hydrogen for PEM fuel cell stacks, and the remaining powertrain parts are the same as FCV [5,9]. The battery electricity vehicle (BEV) is a battery/motor system based on rechargeable batteries that can store electricity.

The η_{TDL} value can be calculated as fuel consumed for its transport and distribution from biorefineries to end-users (vehicles)

$$\eta_{TDL} = E_C / (E_C + E_T) \tag{4}$$

where E_C is the energy consumed in the process of fuel transport and distribution, E_T is the fuel energy delivered to end users (i.e., powertrains), and $E_F = E_C + E_T$.

Fuel losses during transport and distribution were obtained from the Argonne National Laboratory’s model Greet 1.8c [52]. Detailed data sources and efficiency calculations are available in Table 2.

Results

Different scenarios of fuel production through sugar, syngas, and steam platforms as well as six different powertrains viz.

internal combustion engine vehicle (ICE), hybrid electric vehicle-gas (HEV-gas), hybrid electric vehicle-diesel (HEV-diesel), (hydrogen) fuel cell vehicle (FCV), battery electric vehicle (BEV), and sugar fuel cell vehicle (SFCV) are shown in Figure 3.

Biomass-to-fuel efficiency (η_{BTF})

All biomass-to-fuel efficiency data plus their original data and units for different biomass pathways are listed in Table 1, and their representative η_{BTF} values are presented in Fig. 4.

In this study, we use corn stover as a representative biomass, in which total carbohydrates (including cellulose and hemicellulose) account for approximately 60–65% of combustion energy in biomass. Through the biochemical (sugar) pathway, the remaining chemical energy in biomass, mainly lignin, is consumed for running pretreatment as well as sugar isolation and product separation [45]. In general, ~35–40% of the chemical energy of biomass is enough to run biorefineries without external energy input [45,53]. The η_{BTF} values for sugar-to-biofuels mainly depend on sugar isolation yields and sugar-to-fuel yields during microbial fermentation or enzymatic biotransformation. In this study, the η_{BTF} value is 57%, i.e., ~88–95% of sugar release from

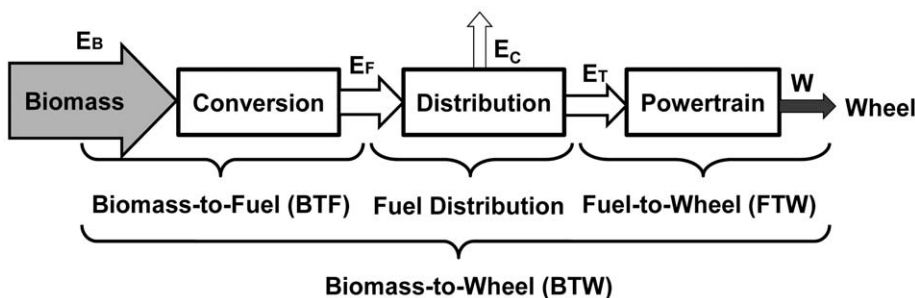


Figure 2. The scheme of energy efficiency analysis for biomass-to-wheel efficiency calculation -- $\eta_{BTW} = \frac{W}{E_B} = \eta_{BTF} * (1 - \eta_{DL}) * \eta_{FTW}$.
doi:10.1371/journal.pone.0022113.g002

Table 1. Biomass-to-fuel (BTF) efficiency through different biomass utilization pathways.

Biofuel	Technology	Feedstock	Efficiency	Original Data	Original Data unit	Reference
corn ethanol	fermentation	corn	46.4%	0.372	L/kg dry	[95]
	fermentation	corn	49.4%	0.396	L/kg dry	[10]
	fermentation	corn	50.1%	0.402	L/kg dry	[48]
cellulosic ethanol	fermentation	corn stover	48.4%	0.298	kg/kg	[45]
	fermentation	corn stover	55.6%	0.342	kg/kg	[53]
sugar	hydrolysis	corn stover	55.8%	0.652	kg/kg	[53]
	hydrolysis	corn stover	61.1%	0.714	kg/kg	[58]
hydrogen	gasification	wood	55.0%	55.00	%LHV	[57]
	gasification	almond shells	70.8%	74%	HHV	[58]
methanol	gasification	wood	50.9%	0.477	kg/kg	[59]
	gasification	lignocellulose	54.9%	59.0	%HHV	[58]
DME	gasification	energy crop	39.0%	39–56.8%	LHV	[60]
FT-diesel	gasification	lignocellulose	41.4%	42.0	%HHV	[31]
	gasification	lignocellulose	52.0%	52.0	%LHV	[61]
ester micro-diesel	fermentation	glucose	7.2%	14.0	% theoretical efficiency	[22]
	fermentation	glucose	36.5%	64	%LHV	[6]
butanol	fermentation	glucose	46.7%	0.350	g/g glucose	[17]
	fermentation	glucose	52.8%	92.6%	LHV	[6]
methane	fermentation	ley crops	62.2%	10.6	GJ/dry ton	[54]
	fermentation	energy maize	81.3%	0.374	m ³ /kg dry maize	[55]
electricity	boiler	lignocellulose	25–43%	25–43%	LHV	[62]
electricity	BIGCC	lignocellulose	45.0%	45.0%	LHV	[63]
		lignocellulose	32–40%	32–40%	LHV	[62]
electricity	molten carbonate FC	lignocellulose	40.2%	40.2%	LHV	[64]
electricity	FC	lignocellulose	51.0%	51.0%	LHV	[65]

doi:10.1371/journal.pone.0022113.t001

biomass, in agreement with data elsewhere [45]. Given sugar yields of 88–99% for cellulose and hemicellulose and sugar-to-ethanol yields of 92–95%, the η_{BTF} value of cellulosic ethanol would be 50%, with a range of 48–56% [10,53]. Given the sugar-to-butanol yields from 82% (now) [17] to 93% (future) [6], the

η_{BTF} value for butanol fermentation would be about 48% with a range of 47–53%. Methane can be produced by anaerobic fermentation mediated by a microbial consortium, where microorganisms convert all organic components except non-hydrolytic lignin to methane. Therefore, η_{BTF} values range from 62 to 81% [54,55]. The practical η_{BTF} value of methane may be approximately 65%, higher than 50% (ethanol) and 48% (butanol). In contrast to anaerobic biofuels fermentations, long chain fatty acid esters (microdiesel) must be produced from sugars through semi-aerobic fermentation due to an imbalance of NAD(P)H [6,22,23]. Because semi-aerobic fermentation consumes a significant amount of sugar for the synthesis of cell mass than anaerobic fermentation, less carbohydrate would be allocated to the production of microdiesel [6,56]. The η_{BTF} values of the ester-diesel fermentation would be about 35%, in the range of 7 to 37% depending on the fuel yields, from 13% [22] to 64% (future) [6].

Syngas can be produced from biomass through gasification – partial combustion at temperatures above 1000 K and in the presence of oxygen and/or water. Gasification is a relatively mature technology, so a significant fraction of biomass must be consumed for partial combustion, resulting in relatively low energy efficiencies, even though all organic components can be utilized [49,50,51]. The η_{BTF} values for hydrogen generation from biomass range from 55% [57] to 71% [58] with a mean value of ~60%. The η_{BTF} values for methanol, DME and FT-diesel vary from 51% [59] to 55% [31], from 39% to 57% [60], and from 41% [31] to 52% [61], respectively. Preferred η_{BTF} values

Table 2. Distribution energy efficiency loss*.

Distribution energy efficiency loss		Input data (Greet 1.8c *)	
Biofuel	Efficiency loss %	Energy input	Unit
Electricity	8.00	8.00	%
FT-diesel	1.53	15,557	btu/mmbtu
Dimethylester	3.10	31,980	btu/mmbtu
Methanol	3.29	34,021	btu/mmbtu
Hydrogen	17.5	211,654	btu/mmbtu
Methane	7.54	81,550	btu/mmbtu
Sugar	1.47	5,979	btu/bushel
ester-diesel	0.75	7,541	btu/mmbtu
Butanol	1.35	13,636	btu/mmbtu
Ethanol	1.71	17,387	btu/mmbtu

*http://www.transportation.anl.gov/modeling_simulation/GREET/index.html. doi:10.1371/journal.pone.0022113.t002

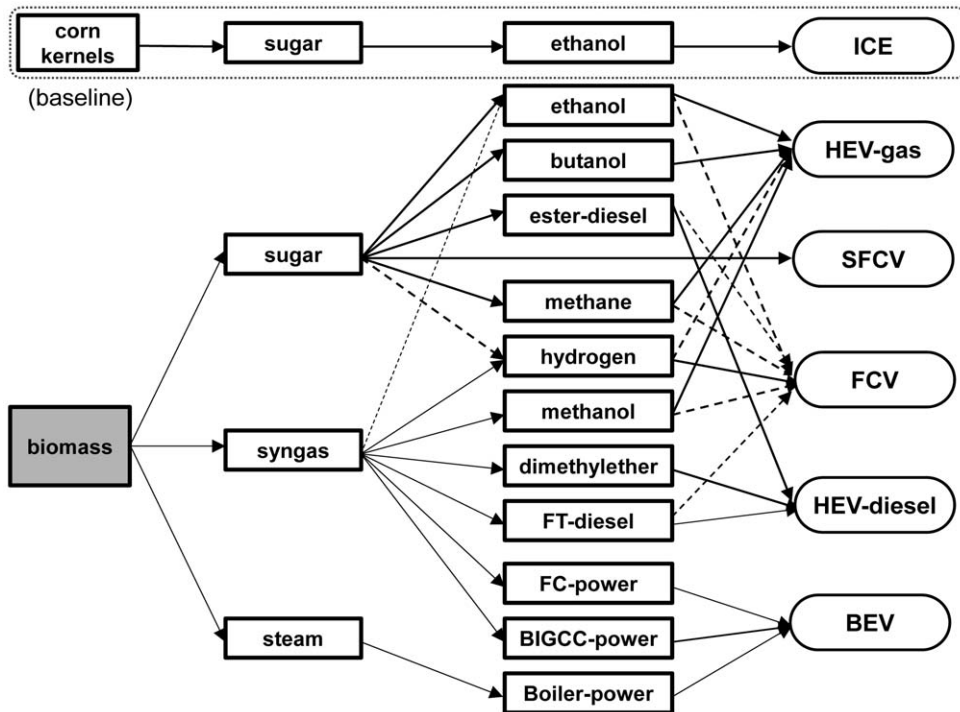


Figure 3. Scenarios of the production of fuels from biomass and their respective fuel power train systems. Solid lines represent the scenarios that we analyzed; the dotted lines represent possible scenarios that we did not analyze. doi:10.1371/journal.pone.0022113.g003

are 54% (methanol), 52% (DME), and 51% (FT-diesel), respectively. Clearly, the η_{BTF} values for liquid biofuels (methanol, DME and FT-diesel) are lower than those of hydrogen because of more catalysis steps and their accompanied energy losses.

Bioelectricity can be produced simply through boiler/steam turbine technology, with η_{BTF} values ranging from 25% (now) to 43% (future) [62]. The assumed η_{BTF} value is approximately 32%. Biomass integrated gasification, combining gas and steam turbine for electricity production (BIGCC), would have improved overall efficiencies, ranging from 32 to 45% [62,63]. In order to increase electricity generation efficiency without restriction of the second law of thermodynamics for turbines, the integrated biomass

gasification and fuel cells would have η_{BTF} values of 40 to 51% [64,65].

Transport and distribution loss efficiency (η_{TDL})

Fuel distribution processes consume a fraction of fuel produced from biorefineries or power stations (Fig. 5). Original data and units were obtained from the GREET1.8c software (Table 2). Typical η_{TDL} values for different fuels after normalization are shown in Figure 5. In general, liquid biofuels have similar efficiency losses (e.g., 0.8–3.3%). Gaseous fuels, such as hydrogen and methane, have more energy consumption for their compression, transport, refilling, and so on. The η_{TDL} values are 17% for compressed

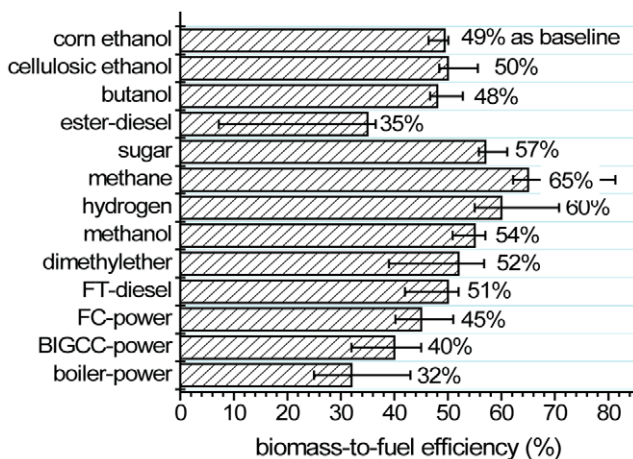


Figure 4. Comparison of biomass-to-fuel (BTF) efficiency in the biorefineries or power stations. doi:10.1371/journal.pone.0022113.g004

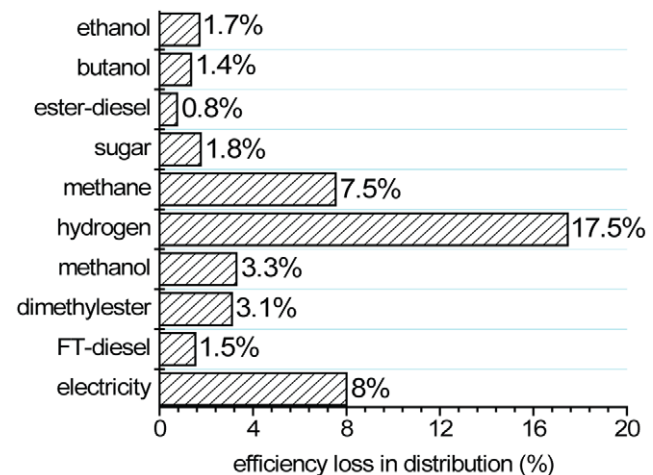


Figure 5. Comparison of transport and distribution loss efficiency for different fuels. doi:10.1371/journal.pone.0022113.g005

hydrogen and 8% for compressed methane (Greet1.8c). The well-documented distribution efficiency of electricity is 92%, i.e., 8% of electricity is lost during its distribution (Greet1.8c).

Fuel-to-wheel efficiency (η_{FTW})

Two major internal combustion engines for passenger vehicles are gasoline Otto (spark plug firing) ICE and diesel (compression ignition) ICE. Gasoline ICEs have a low weight-to-power ratio (e.g., ~1 g engine per W output) but their maximum efficiencies are relatively low, approximately 32%, due to low compression ratios [66]. In contrast, diesel ICEs have a higher weight-to-power ratio (e.g., ~3–4 g engine per W output) and a much higher energy conversion efficiency, more than 40% [66]. It is reasonable that diesel ICEs are widely used in heavy-duty trucks, tanks, and tractors. In Europe, diesel ICE passenger vehicles are more popular mainly due to higher fuel costs and more climate change concerns. Audi A3 vehicles based on ICE-diesel have 35.4 miles per gallon of diesel, higher than ICE-gasoline (24.7 miles per gallon of gasoline) [67], suggesting a ~26% enhancement in η_{FTW} efficiency. (Note: the volumetric energy density of diesel is ~13–14% higher than that of gasoline) [7].

Practical η_{FTW} values of ICEs are much lower than their maximum efficiency because of (i) the engines operate at ~70% of their maximum efficiency during most driving conditions, (ii) ~17% loss for engine idling, (iii) ~2% consumption for accessories (e.g., air conditioning, lighting), and (iv) ~25% loss in transmission [30,66,68]. Therefore, the η_{FTW} for ethanol-ICE is approximately 14% as a baseline [69], and this value would be improved through higher compression rate ethanol engine and better transmission [70,71,72]. Advanced diesel vehicles are expected to have η_{FTW} values of 20–24% [71]; the η_{FTW} value of 23% is used in this study.

Hybrid electric vehicles (HEV) can eliminate idling losses, allow a small engine to work at nearly optimal conditions, and utilize braking energy with regenerative braking [30,73]. Therefore, advanced HEV-gas is estimated to have η_{FTW} values of 29–34% [30,74]. Similarly, the η_{FTW} values of HEV-diesel can be increased to 32–38%, with a preferred value of 37%.

The hydrogen fuel cell vehicle (FCV) is a complicated powertrain system involving compressed hydrogen, FEM fuel cells, an electric motor, and a rechargeable battery [32,75]. FCVs feature zero tailpipe pollution and high energy conversion efficiencies due to PEM fuel cells, whose theoretical energy efficiency from hydrogen to electricity is up to 83%. As a result, many companies have attempted big research FCV projects, and some of them produced prototype FCVs, such as the GM Sequel, the BMW Hydrogen 7, the Ford Focus FCV-Fuel Cell, the Toyota Fine X, and the Honda FCX Clarity. The η_{FTW} values of FCVs range from 41 to 54% [32,75], with a mean value of 45%. SFCVs based on FCVs would have an on-board bioreformer that can convert the sugar slurry to high-purity hydrogen and absorb waste heat from PEM fuel cells. Because the efficiency of sugar-to-hydrogen is 107% based on low heating value [9,24,25], the η_{FTW} value for SFCV is estimated to be 48% with a range of 44–57%.

Battery electric vehicles (BEV) have the highest η_{FTW} values, although they still have some energy losses in battery recharging and release, storage loss, motor, and so on [32,76]. BEVs have predicted η_{FTW} values from 64 to 86% [32,76,77], with a mean value of 68%. All fuel-to-wheel efficiencies of different vehicles are summed up in Table 3 and Fig. 6.

Biomass-to-Wheel (BTW) efficiency (η_{BTW})

A combination of 12 kinds of biofuel production approaches and 6 kinds of advanced powertrains for passenger vehicles results

Table 3. Fuel-to-wheel (FTW) efficiency for different powertrains.

Powertrain	Efficiency	Reference
ICE-gas	11.3–15.2%	[30,69,70,71]
ICE-diesel	20–24%	[71]
HEV-gas	28.8–31.4%	[30,74]
HEV-diesel	34.6–37.6%	based on HEV-gas [30,74] and ICE-diesel [71]
FCV	41.0–53.8%	[32,75]
SFCV	43.7–57.3%	based on FCV plus sugar to H ₂ biotransforming efficiency [6,24,25]
BEV	64.4–86%	[32,76,77]

doi:10.1371/journal.pone.0022113.t003

in more than 20 scenarios (Fig. 3). In this analysis, 14 scenarios were calculated (Fig. 7). The current corn ethanol/ICE scenario has η_{BTW} value of ~7%, i.e., only 7% of the chemical energy in corn kernels is converted to the kinetic energy on wheels, implying a great potential in increasing biomass utilization efficiency. An ethanol HEV-gas system would double η_{BTW} values to 14–18%, suggesting the importance of developing hybrid electric vehicles based on available liquid fuel distribution system. There is no significant difference in η_{BTW} between butanol and ethanol, but butanol may have other important future applications, such as powering jet planes. The η_{BTW} values of methane/HEV-gas and methanol/HEV-gas are 19% and 17%, respectively, higher than those of ethanol and butanol, mainly due to higher product yields. Since ICE-diesel has higher η_{FTW} efficiencies than ICE-gas, the scenarios based on HEV-diesel through DME and FT-diesel (except ester-diesel) would have higher η_{BTW} values than HEV-gas scenarios. For ester-diesel, a significant amount of energy is lost during aerobic fermentation due to thermodynamic and bioenergetic limits [6], resulting in low η_{BTW} values. Even for the niche jet fuels market, the production of ester-diesel through semi-aerobic microbial fermentation might not be competitive with anaerobic butanol fermentation [78] and a high-energy-retaining efficiency hybrid of biocatalysis and chemical catalysis [28].

Although (hydrogen) fuel cell vehicles (FCVs) have higher η_{FTW} efficiencies than ICE-gas and ICE-diesel, the H₂/FCV scenario

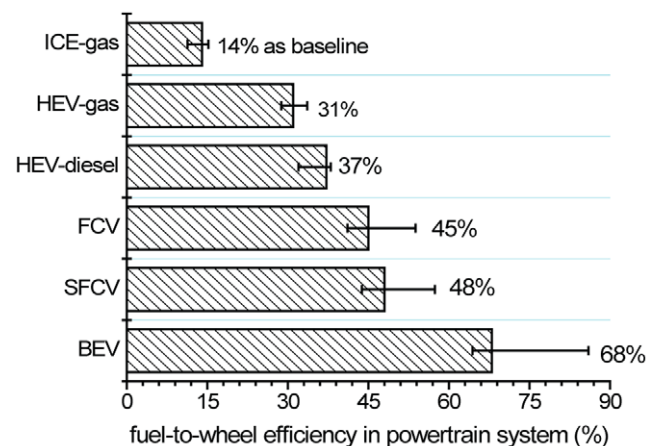


Figure 6. Comparison of fuel-to-wheel (FTW) efficiency for different powertrain systems.

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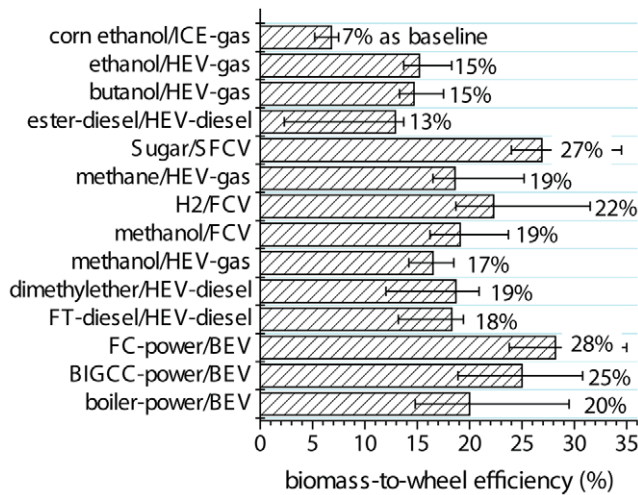


Figure 7. Comparison of biomass-to-wheel (BTW) efficiency for different biomass utilization scenarios.
doi:10.1371/journal.pone.0022113.g007

shows ~46% and ~15% η_{BTW} enhancements over ethanol HEV-gas and DME HEV-diesel, respectively, because significant energy loss in hydrogen distribution discounts FCV's advantages over HEV-diesel. The sugar/SFCV scenario would have very high η_{BTW} values of approximately 27% due to lower energy consumption in fuel transport and heat recapture in the sugar-to-hydrogen biotransformation, compared to the H₂/FCV scenario.

BEV scenarios are among the highest η_{BTW} values, from 20% to 28%, with increasing electricity generation efficiencies from direct combustion, BIGCC, to FC-power.

Discussion

Conducting energy efficiency analysis is simpler, faster, and less controversial than conducting life cycle analysis because the latter heavily depends on so many different assumptions and uncertain inputs. Here we present a straightforward energy efficiency analysis from biomass to wheels for different options, which contains three elements. Each element can be analyzed separately and adjusted individually; most of which have data well-documented in literature (Tables 1–3). Because of the same input and output in all cases, an increase in energy conversion efficiency nearly equals impact reductions in carbon and water footprints on the environment. Most of the results obtained from this biomass-to-wheel analysis were in good agreement with previous, more complicated life cycle analyses, supporting the validity of this methodology. Our analysis suggested that the hydrogen fuel cell vehicle (H₂/FCV) scenario would have at least comparable efficiency with or a little higher than hybrid electric vehicle (HEV) systems, which was supported by a previous paper [76]. Another analysis suggested that the H₂/fuel cell scenario had three times higher efficiency than ethanol/internal combustion engines (ICE) [33], in good agreement with our analysis (Fig. 7). Through comparison of four biofuels (i.e., hydrogen, methanol, Fischer–Tropsch (FT)-diesel, and ethanol) and two powertrain systems (i.e., ICE and FCV), they recommended FCV due to the highest energy efficiency [31]. These data were comparable with our analysis (Fig. 7). Both the sugar/sugar fuel cell vehicle (SFCV) and fuel cell (FC)-power/battery electric vehicle (BEV) scenarios would have nearly four times that of corn ethanol/ICE-gas, implying the importance of enhancing BTW efficiency in each conversion element.

A new solution -- sugar-fuel cell vehicles (SFCV)

The concept of SFCV was proposed to address problems associated with H₂/FCV, such as high-density hydrogen storage in FCV, low-cost sustainable hydrogen production, costly hydrogen distribution infrastructure, and safety concern [9,25]. In this system, renewable sugar (carbohydrate) is suggested as a high hydrogen density carrier, with a gravimetric density of 8.33% mass H₂ and a volumetric density of more than 100 g H₂ per liter [3,5,9]. Transportation and distribution of the sugar/water slurry or sugar slurry would be easily achieved using available infrastructure. This hypothetical SFCV based on FCV would contain a sugar tank and an on-board sugar-to-hydrogen bioreformer, with a combined sugar tank and bioreformer volume that is much smaller than a compressed hydrogen tank or other hydrogen storage approaches [3,5]. The sugar/water slurry would be refilled rapidly into the sugar container in SFCVs at local sugar stations; the on-board biotransformer would convert the sugar solution to high-purity hydrogen and carbon dioxide using a stabilized enzyme cocktail; and a small-size hydrogen storage container would serve as a buffer, balancing hydrogen production and consumption. In addition, feeding a mixture of CO₂/H₂ or pure hydrogen in the proton exchange membrane (PEM) fuel cells would dramatically decrease system complexity and greatly increase system operation performance, and the waste heat release from PEM fuel cells would be coupled to the heat needed by the bioreformer. Electrical energy from PEM fuel cells would be sent to the motor controller/motor/gears to generate kinetic energy [9]. When extra kinetic energy is needed for acceleration or start-up, electrical energy stored in the rechargeable battery would be released, like in a hybrid electric vehicle [9]. The on-board bioreformer in SFCVs, mediated by the thermoenzyme cocktails under modest reaction conditions (e.g., ~80°C and ~1 atm), may be capable of providing high-purity hydrogen at a rate of ~23.5 g H₂/L/h or higher. Given a bioreformer size of 42.8 L, one kg of hydrogen per hour could then be produced to drive the PEM fuel cell stack, followed by the electric motor [5]. High-speed biohydrogen production rates have been implemented by high cell-density microbial fermentation [79]. It is widely known that enzymatic reactions usually are at least one order-of-magnitude faster than microbial fermentations because the former has no cellular membrane to slow down mass transfer and much higher biocatalyst loadings, without the dilution of other biomacromolecules (e.g., DNA, RNA, other cellular proteins) [3,56,80,81]. Current gasoline/ICE cars require maintenance every 3,000 miles (e.g., 4,800 km) or 3 months, i.e., 50–100 driving hours. Discovery of thermophilic enzymes that are stable at ~80°C for more than 100 h has been demonstrated, for example, *T. maritima* 6-phosphogluconate dehydrogenase [82]. We expect that enzyme deactivation in the biotransformer will be solved through infrequent service maintenance, similar to the oil/air filter change for gasoline/ICE vehicles. Several technical obstacles of SFCVs include poor enzyme stability, labile and costly coenzymes, low reaction rates, and complicated system configuration and control [3,9,56,80]. A huge potential market (e.g., nearly one trillion of US dollars per year) provides the motivation to solve these issues within a short time. Current progress includes the discovery of thermostable enzymes from extremophiles and low-cost production of recombinant enzymes [80,82,83,84,85,86], engineering redox enzymes that can work on small-size biomimetic cofactors [56,87,88], and accelerating hydrogen generation rates [5,9,24,89].

SFCV is better than BEV

Although the biomass-to-wheel efficiency may be the most important criterion in analyzing future transportation systems, many factors were related with future choices, including energy

storage density, system compactness, fuel costs, infrastructure, safety, operation reliability, environmental costs, resource availability, technology maturity, and improvements potential. Because the energy densities of lithium ion batteries (0.46–0.72 MJ/kg) [90,91] are much lower than those of liquid fuels (~30–40 MJ combustion energy/kg) and sugars (~11–14 MJ electricity/kg sugar) [3,5], BEVs will have a very short driving distance, making the BEV poorly suited for long-distance transportation [32]. If the energy densities of rechargeable batteries were increased by 10-fold in the future, safety concerns would likely come into play, slowing or even preventing wide deployment of such batteries in BEVs. In fact, it is impossible to increase energy densities of lithium rechargeable batteries by 10-fold due to physical limits [90]. Metal/air batteries are supposed to have the highest energy storage density of all batteries [90]. But regeneration of oxidized metals is so energy intensive that metal/air batteries may be too costly for the transport sector. SFCV would have a comparable η_{BTW} with the FC-boiler/BEV scenario but with much longer driving distances based on the same fuel weight (i.e., broader applications). Also, refilling of solid sugar or sugar/water slurry into SFCVs would be much faster and safer than recharging batteries for BEVs or refilling compressed hydrogen for FCVs. If the obstacles to ultra-fast recharging and the life-time of batteries were solved, a huge infrastructure investment would be required for upgrading electrical grids, sockets for quick recharging, power stations, etc. Since SFCV would have ~3.4 times the FTW efficiency of ethanol/ICE-gas (Fig. 6), one kg of sugar (i.e., 17 MG/kg) would release more kinetic energy than one kg of gasoline (i.e., 46.4 MJ/kg) from ICE-gas. Thus, the mass of sugar delivered in the future may be less than the mass delivered by the current liquid gasoline/diesel distribution system. Another advantage is the much shorter sugar slurry transportation distance compared to that of gasoline/diesel, due to local production and distribution. The distribution of sugar would be done based on available goods distribution systems. Since SFCVs use biodegradable enzymes as catalysts, they would greatly decrease the environmental burdens related to BEVs, such as disposing and recycling used batteries.

Beyond BTW

Assessment of any energy system is really challenging because it involves so many factors. Generally speaking, efficiency and cost are usually the two most important criteria. Since thermodynamics (energy efficiency) determine economics in the long term, SFCVs and FC-power/BEV seemed to be long-term winner candidates, but SFCVs have other important advantages. Currently and in the short term, costs mostly determine market acceptance and

dominance. But cost analysis is more complicated than energy efficiency analysis, because the former involves direct costs (e.g., fuel, vehicle, etc.), indirect costs (e.g., vehicle service, taxes, subsidies, infrastructure costs for repairing and rebuilding, resource availability, etc.), and hidden costs (e.g., safety, toxicity, waste treatment, greenhouse gas emissions, military expenditures, etc.). In the short term, cellulosic ethanol plus HEV-gas and methane-HEV-gas may be the most promising options.

Potential roles of biomass

It was important to estimate the role of US biomass resources in the future transport sector. The net primary production of biomass in the USA would be approximately 9.83 billion of dry metric tons in 2030, based on the current net primary (biomass) production with an annual growth rate of 1% [92], mainly due to higher photosynthesis yields accompanied with rising CO₂ levels [93,94]. Considering the fact that gasoline/bioethanol consumption in 2008 was approximately 140 billion gallons per year and an assumed annual growth rate of 1%, a switch from ethanol/ICE to sugar/SFCV would require net biomass energy of 11.60 EJ/year in 2030. That is, approximately 700 million metric tons of biomass in 2030, i.e., ~7.1% of calculated annual US biomass (i.e., net primary production including natural ecosystems plus agricultural systems), would be sufficient to meet 100% of transportation fuel needs for light-duty passenger vehicles.

On the prospect of meeting transportation energy needs at acceptable fuel costs, we would like to suggest that short-term or middle-term solutions would be ethanol/butanol/methane plus HEV considering available current fuel distribution infrastructure and enhanced BTW efficiencies. In the long term, SFCVs will likely win over BEVs due to advantageous energy storage densities, safety, infrastructure, and environmental impacts. The great potentials for increasing η_{BTW} values from ethanol-ICE to the future systems (HEV and SFCV) suggest that more efficient utilization of biomass would greatly decrease greenhouse gas emissions, and biomass use could result in more benefits to the environment, rural economy, and national security than originally expected [1]. Through SFCVs, about ~7% of annual US biomass resources may be sufficient to meet 100% of US light-duty transportation fuel needs in the future.

Author Contributions

Conceived and designed the experiments: YPZ. Performed the experiments: WDH. Analyzed the data: WDH YPZ. Wrote the paper: WDH YPZ.

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Simpler Is Better: High-Yield and Potential Low-Cost Biofuels Production through Cell-Free Synthetic Pathway Biotransformation (SyPaB)

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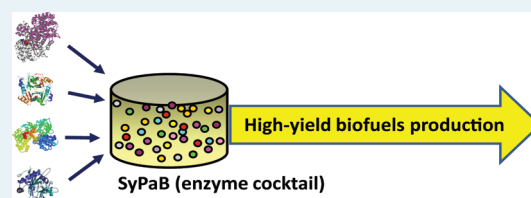
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ABSTRACT: The production of biofuels from renewable sugars isolated from plants or produced through artificial photosynthesis would provide a sustainable transportation fuel alternative for decreasing reliance on crude oil, mitigating greenhouse gas emissions, creating new manufacturing jobs, and enhancing national energy security. Since sugar costs usually account for at least 50% of biofuels' selling prices, it is vital to produce desired biofuels with high product yields and at low production costs. Here I suggest high-product yield and potentially low-cost biofuels production through cell-free synthetic enzymatic pathway biotransformation (SyPaB) by in vitro assembly of stable enzymes and (biomimetic) coenzymes. SyPaB can achieve high product yields or high energy efficiencies that living entities cannot achieve. Great potentials of SyPaB, from chiral compounds, biodegradable sugar batteries, sulfur-free jet fuel, hydrogen, sugar hydrogen fuel cell vehicles, high-density electricity storage, to synthetic starch, are motivation to solve the remaining obstacles by using available technologies, such as protein engineering, enzyme immobilization, unit operations, and technology integration. The biotransformation through in vitro assembly of numerous enhanced-performance and stable enzymes in one bioreactor that can last a very long reaction time (e.g., several months or even years) would be an out-of-the-box solution for high-yield and low-cost biofuels production.

KEYWORDS: artificial photosynthesis, biofuels, biological CO₂ fixation, hydrogen, in vitro synthetic biology, biocatalysis and biotransformation, synthetic pathway biotransformation (SyPaB)



1. INTRODUCTION

Biofuels are usually defined as transportation fuels produced from biological resources (e.g., corn kernels, sugar cane, lignocellulosic biomass, and algal biomass) and/or through biological conversions. As compared to the other energy consumption sectors (e.g., industrial, residential, and commercial), transportation fuels that account for approximately 20% of total energy consumption have some special requirements: high energy storage capacity in a small container (e.g., ~50 L), high power output (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 min per time), safety, and so on.^{1–3} Currently, approximately 95% transportation fuels are produced from crude oil. Concerns of depleting crude oil reserves, climate change, national energy security, and wealth transfer are driving the search for sustainable transportation fuel alternatives.^{1,3,4}

The production of chemicals mediated by biocatalysts usually has numerous advantages over chemical catalysis, such as higher energy efficiency, higher chemical selectivity (i.e., higher product yield), more modest reaction conditions, and lower costs of bioreactors.^{5–7} Different scenarios of biofuels production have been proposed starting from plant biomass, algal biomass, or

even CO₂ plus hydrogen or electricity, but nearly all biofuels (secondary energies) originate from the most abundant primary energy—solar energy. Since carbohydrates (e.g., cellulose, hemicellulose, and starch) are the most abundant renewable bioresource (e.g., ~100 billion tons per year), biofuels production through carbohydrates would become a dominant platform in the future. The scope of this perspective is restricted to compare two different biocatalysts, living entities and synthetic cascade enzymes, for the production of the best future biofuel, namely, hydrogen, and the production of synthetic starch from CO₂ but is not involved in bioenergy plants, cellulase engineering, other biofuels production, and algal biofuels. (Note: hydrogen is believed to be the best biofuel in the future because (i) it can be utilized through fuel cells featuring higher energy efficiencies compared to internal combustion engines, (ii) less pollutants are produced, and (iii) it can be produced from diverse energy sources.)

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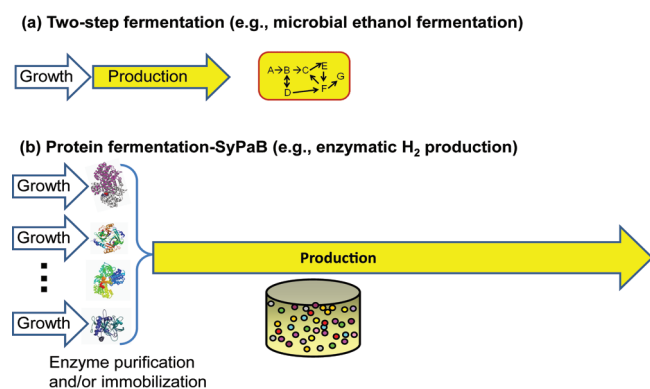


Figure 1. Comparison of microbial two-step biofuels production (a) and a hybrid of microbial fermentation for bulk enzyme production and cell-free synthetic enzymatic biotransformation (SyPaB) (b). Arrows represent microbial fermentation or biotransformation.

Biofuels production R&D is typical of goal-oriented projects with numerous constraints from economical, technological, environmental, social, scalability, competing technologies, and so on. Although so many advanced biofuels, including cellulosic ethanol, long-chain alcohols (e.g., *n*-butanol, iso-butanol), fatty acid ethyl esters, hydrogen, electricity, methane, bioalkanes, and so on, have been produced in laboratories, most of them might not be produced economically in the future. In industrial processes, three key elements—product yield, product titer, and reaction rate—mainly decide process economy. For biofuels production based on available sugars, energy conversion efficiency (or product yield) must be the No.1 cost factor because sugar costs usually account for ~50–70% of prices of mature biofuels.^{8–11} The second important factor is product titer, which is closely associated with separation costs, followed by production rate. In it, a default assumption is that reasonable biofuels production rates have been or will be accomplished, for example, ~0.2–1 g sugar consumed per liter per h.

Biofuels can be produced from sugars mediated by (i) growing microbes, (ii) resting cells that are not active in the process of cell division, and (iii) cascade enzymes. When some constituent of cells (e.g., fatty acids) is a desired product, the formation rates of such product are directly related to the rates of cell growth, called growth-associated production. At this situation, growing microbes insist on metabolizing sugars for anabolism (i.e., allocation of sugars to other cell components). Therefore, practical product yields are far below from their theoretical yields.^{8,12,13} From the point of view of synthetic biology, both cell growth and undesired product formation by living entities are a dissipation of the task that we want them to do, resulting in relative low product yields. To increase biofuels yields, it is vital to insulate basic anabolism from biofuels production (Figure 1a). In practice, industrial ethanol fermentations are usually conducted in two steps. At the first step, yeasts consume sugars to produce a large amount of cell mass with oxygen supplies; at the second step, yeasts produce high-yield ethanol from glucose in the absence of oxygen. When ethanol titer is high, it can stop yeast growth so that yeasts turn to resting cells that produce ethanol only without significant synthesis of cell mass¹⁴ (Figure 1a). Here I extended the concept of high-yield resting cell biotransformation to high-yield cell-free synthetic pathway biotransformation (SyPaB) that can implement complicated biochemical

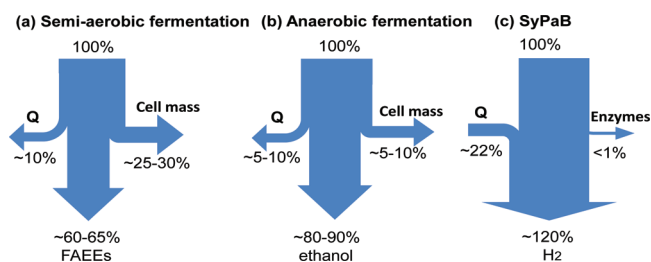


Figure 2. Energy efficiency comparison for fatty acid ethyl esters fermentation by semiaerobic fermentation (a), ethanol fermentation by anaerobic fermentation (b), and hydrogen production by SyPaB (c).

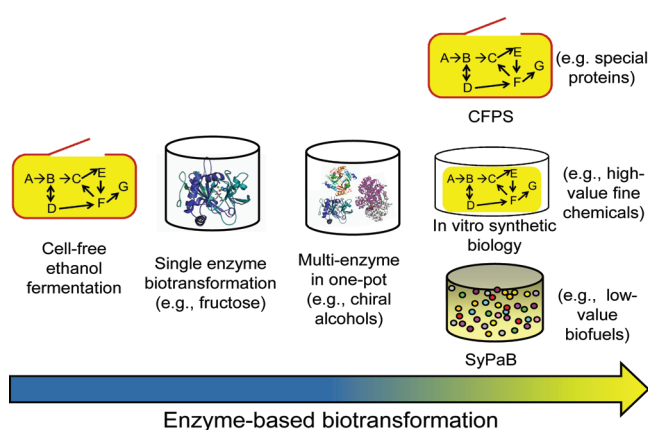


Figure 3. Evolution of enzyme-based biotransformation from cell-free ethanol fermentation (discovery of enzymes), single enzyme biotransformation, multiple-enzyme one pot to cell-free protein synthesis, in vitro synthetic biology, and cell-free synthetic pathway biotransformation in terms of time and increasing system complexity.

reactions by the in vitro assembly of numerous enzymes and coenzymes.^{9,15–17} In SyPaB, the insulation of cell growth from product formation is implemented by process operations (Figure 1b).

An analysis based on thermodynamics and bioenergetics was conducted for assessing upper limits of energy efficiency for the production of fatty acid ethyl esters (FAEEs) by semiaerobic fermentation, ethanol by anaerobic fermentation, and hydrogen by SyPaB (Figure 2).⁸ According to their biochemical pathways and thermodynamics, 100% product yields result in ~10% combustion energy loss for FAEEs, ~5% loss for ethanol, and ~22% gain for hydrogen.^{8,18,19} Since a fraction of sugar must be consumed for biocatalyst synthesis, potential yields of biofuels should be lower than their theoretical yields. An imbalance of coenzymes in microbial FAEEs production leads to a significant fraction of carbohydrate loss for the synthesis of cell mass in semiaerobic fermentation. As a result, only 60–65% of the combustion energy in sugar would be converted to FAEEs. Ethanol fermentation has much better energy-retaining efficiencies because of (i) anaerobic fermentation and (ii) uncoupling of cell growth and product formation. Therefore, ethanol is a very good liquid biofuel now. The best case would be hydrogen produced by SyPaB because of 22% of extra enthalpy gain by absorbing waste heat (i.e., the combustion energy of hydrogen is more than that of sugar) and a very small amount of sugar consumed for the synthesis of cascade enzymes when enzymes

have total turnover number (TTN) values of 10^7 – 10^8 mol product per mol enzyme.^{8,15,20}

In this perspective, I present an out-of-the-box solution for a high-yield and potentially low-cost biofuels production platform, SyPaB, featuring very high product yields and fast reaction rates that can insulate protein synthesis from biofuels production, review the brief history of enzyme-based biotransformations, argue SyPaB as a new low-cost biomanufacturing platform, and discuss challenges and opportunities of SyPaB.

2. HISTORY OF ENZYME-BASED BIOTRANSFORMATIONS

Long before people had a clue about the nature of biotransformation, certain properties of microorganisms had been long exploited for commercial processes, such as in the production of beer, wine, vinegar, soy sauce, and cheese, and the preservation of vegetables by pickling. Central to the rational use of biocatalysts has been a stream of theoretical understanding of the nature of living biocatalysts and related enzymes. The developments of enzyme-based biotransformations (Figure 3) can be divided roughly into four phases:

Phase 1 (1897). Recognition of biotransformation occurrence in the absence of living cells (cell-free ethanol fermentation by Eduard Bucher, Nobel Prize in Chemistry, 1907).⁹ Later, more studies were focused on studies of enzymes responsible for natural enzymatic pathways in basic metabolisms. For example, Otto Fritz Meyerhof won the Nobel Prize in Physiology or Medicine in 1922 for his elucidation of the glycolytic pathway.²¹ Even now, *in vitro* reconstitution of natural pathways is still an important tool to understand and discover *in vivo* complicated biochemical reactions or pathways.^{22,23}

Phase 2 (1960s). Utilization of one enzyme for simple biotransformation.^{5,24} Clearly, the use of isolated enzymes for the production of chemicals has a much shorter history than microbial fermentation. Invertase may be the first immobilized enzyme used commercially for the production of Golden Syrup by Tate & Lyle in World War II. Industrial process for *L*-amino acid production by soluble aminoacylase was developed in 1954. In 1969, Tanabe Seiyaku Co. (Japan) started the industrial production of *L*-methionine by using immobilized aminoacylase in a packed bed reactor. In 1967, the Clinton Corn Processing Company (U.S.A.) was the first to produce fructose corn syrup by glucose isomerase. Currently, immobilized glucose isomerase Sweetzyme T (Novo, Denmark) is packed into columns for conversion of glucose into fructose. The longest working lifetime of immobilized glucose isomerase is 687 days at 55 °C and pH 7.5 by Kato Kagaku (Japan). Now, annual enzymatic fructose production from glucose exceeds 9 million tons.²⁴ Enzymatic acrylamide production was initiated in 1985. Currently, more than 100,000 tons of acrylamide is produced by using immobilized nitrile hydratases per year.²⁴ Discovery and utilization of thermoenzymes, protein engineering including directed evolution, rational design and their combination, high-cell density fermentation for low-cost recombinant protein production, and enzyme immobilization have enabled the production of very stable recombinant enzyme at very low costs.^{25–28}

Phase 3 (1990s). Utilization of multienzyme one pot for relatively complicated biotransformation because most enzymes can function under similar conditions. Multienzyme one pot has numerous benefits: fewer unit operations, smaller reactor volume, higher volumetric and space-time yields, shorter cycle

times, and less waste generated. Also, by coupling steps together, unfavorable equilibria can be driven toward the formation of desired products.^{9,29,30} For cofactor-dependent enzyme reactions, it is not economically feasible to continuously provide costly cofactors in biomanufacturing. Therefore, *in situ* NAD(P)H-regenerated by another enzyme is becoming more and more accepted, especially for the synthesis of high-value chiral compounds in the pharmaceutical industry.^{17,31,32} NAD(P)H is usually generated by using a pair of a hydrogen-donor substrate and a single enzyme, including formate/formate dehydrogenase,³³ glucose/glucose dehydrogenase,³⁴ glucose-6-phosphate/glucose-6-phosphate dehydrogenase,²⁶ dihydrogen/hydrogenase,³⁵ and phosphite/phosphite dehydrogenase.³⁶ In another case, enzymatic hydrolysis of crystalline cellulose require a synergetic action of endoglucanases, cellobiohydrolases, and beta-glucosidases.^{37–39} In the organic chemistry field, the synthesis of monosaccharides, activated monosaccharides, oligosaccharides, and glycopeptides by using multienzyme one pot has been intensively investigated.^{40–46}

Phase 4 (2000s). Utilization of numerous cascade enzymes for very complicated biotransformation. It includes three representative directions: (1) cell-free protein synthesis (CFPS), which utilizes natural protein synthesis systems in cell lysates for fast synthesis of proteins for research purpose and the production of high-value antibodies or other proteins,^{47,48} (2) *in vitro* synthetic biology for the production of high-value products,^{25,49–52} and (3) synthetic pathway biotransformation (SyPaB) for low-value biofuels production.^{9,15,17} Different from CFPS and high-value product formation, SyPaB must have balanced cofactors and ATP *in vitro*.¹⁷ In addition, thermodynamics must be analyzed to ensure designed non-natural processes to take place as expected. The development cycle of SyPaB is composed of five parts: (i) pathway reconstruction, (ii) enzyme selection, (iii) enzyme engineering, (iv) enzyme production, and (v) bioprocess engineering.^{9,15,17} Whole SyPaB processes can be improved in an iterative manner, gradually leading to a low-cost industrial bioprocess.^{9,15,17} The SyPaB technology has successfully achieved some breakthroughs that neither microbes nor chemical catalysts could implement before, such as production of nearly 12 mol of hydrogen from per mol of anhydroglucose and water,^{19,53} ultrahigh-yield regeneration of NAD(P)H in microbe-toxic biomass hydrolysate,²⁰ enzymatic conversion of ethanol and CO₂ to lactate,⁵⁴ and so on.

3. BIOCATALYSTS: LIVING ENTITIES VERSUS SYPAB

Although SyPaB and living entities are responsible for transforming similar-level complicated biochemical reactions, SyPaB featuring high product yields and fast reaction rates enable it to play more important roles in biofuels production because (energy) conversion efficiencies will be important to decide their production economics in a long-term⁸ and their reaction rates will be vital to their potential applications.^{1,55} Here we present two SyPaB examples, which do much better than do natural living entities.

3.1. Hydrogen Production from Sugars. The global biosphere produces more than 250 million tons of biohydrogen per year.⁵⁶ Most hydrogen arises from anaerobic fermentation of carbohydrate previously fixed by photosynthesis, followed by its consumption along with CO₂ or organic acid reduction by methanogenic archaeobacteria. On oceanic continental shelves

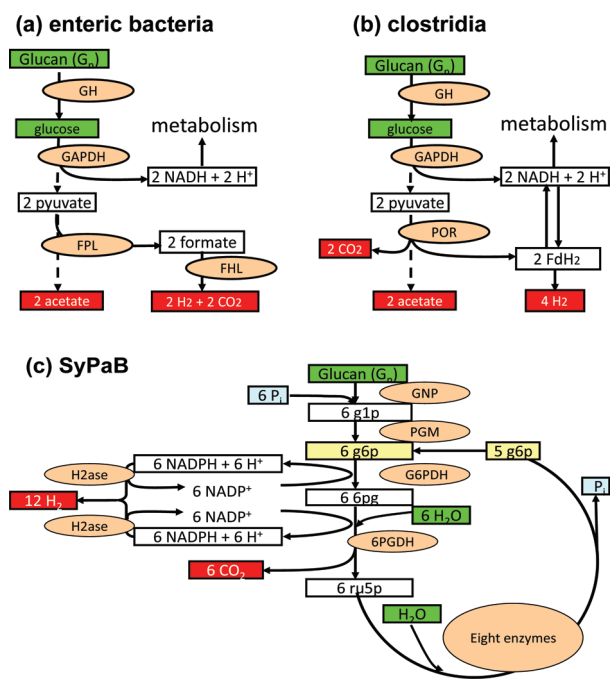
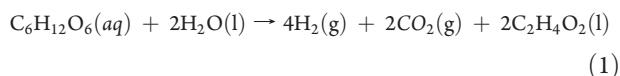


Figure 4. Scheme of microbial hydrogen production by enteric bacteria (a), clostridia (b), and by SyPaB (c).

and in permafrost regions, methane has been accumulated extensively as methane hydrate deposits, which exceed petroleum, coal, and natural gas deposits combined. In other locales, methane is released to the atmosphere, a much stronger greenhouse gas than carbon dioxide.

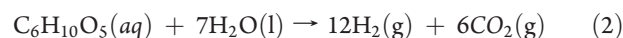
Natural microorganisms can produce hydrogen from sugars through different pathways.^{38,57–59} The upper limit of hydrogen yield for living entities is 4 mol of H₂ produced per mol of glucose equivalent consumed plus 2 acetic acids, called the Thauer limit (eq 1).^{58,59}



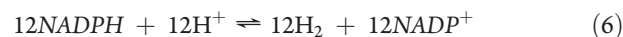
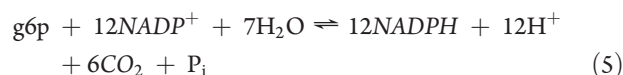
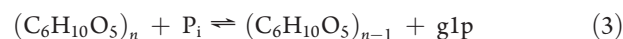
Enteric microorganisms include facultative anaerobic bacteria, such as *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Escherichia coli*. Since they can grow under aerobic conditions for high-cell mass concentrations, such high-cell masses result in extraordinarily high volumetric H₂ productivities. However, specific H₂ yields of enteric bacteria are relatively low, usually less than two mol of H₂ per mol of glucose because of their central metabolism, where hydrogen is generated from pyruvate–formate lyase and formate hydrogen lyase⁶⁰ (Figure 4a). NADH is generated from the glycolytic pathway, but this coenzyme is not a favorable electron carrier for hydrogen generation at moderate temperatures. The clostridia are obligate anaerobes capable of producing organic solvents as well as H₂ through a mixed acid pathway (Figure 4b). These microorganisms are the predominant organisms in mixed microflora capable of producing H₂ from biomass waste treatment.⁶¹ Different from enteric bacteria, H₂ production by many clostridia species is catalyzed by the combination of pyruvate:ferredoxin oxidoreductases (POR) and Fe-only hydrogenase.⁶² NADH can donate electrons to ferredoxin by NADH:ferredoxin oxidoreductase.³⁸ Several clostridial species have been evaluated for their potential as

biohydrogen producers.⁶¹ Hyperthermophiles can produce nearly 4 mol of H₂ produced per mol of glucose equivalent, albeit at lower volumetric productivities than observed for mesophilic bacteria.⁵⁷ In spite of intensive efforts in metabolic engineering and synthetic biology, none of natural or engineered microorganisms can produce hydrogen more than the Thauer limit.^{57,63–66}

To break the Thauer limit, a non-natural synthetic pathway has been designed to split water by using the chemical energy in starch.¹⁹ As a result, far more than 4 mol of hydrogen per mol of glucose unit from starch and water is produced.¹⁹



This non-natural synthetic catabolic pathway is composed of 13 enzymes together (Figure 4c). The pathway contains four biocatalytic modules: (i) a chain-shortening phosphorylation reaction for producing glucose-1-phosphate (g1p) catalyzed by glucan phosphorylase (eq 3); (ii) generation of glucose-6-phosphate (g6p) from g1p catalyzed by phosphoglucosmutase (eq 4); (iii) generation of 12 NADPH from g6p through a pentose phosphate pathway plus four enzymes in the glycolysis and gluconeogenesis pathways (eq 5); and (iv) generation of hydrogen from NADPH catalyzed by hydrogenase (eq 6).



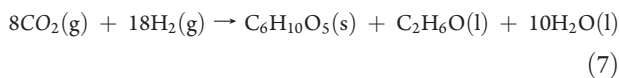
Thermodynamic analysis suggests that the overall reactions from starch or cellulosic materials and water are spontaneous and endothermic (i.e., $\Delta G^\circ = -49.8$ kJ/mol and $\Delta H^\circ = +598$ kJ/mol).^{19,53} Such reactions are driven by entropy gains rather than enthalpy losses. These entropy-driven chemical reactions can generate the chemical energy in the form of hydrogen more than the chemical energy in polysaccharides by absorbing ambient-temperature thermal energy.^{19,53} The removal of gaseous products, H₂ and CO₂, from the aqueous phase under mild reaction conditions (<100 °C and ~1 atm) favors the unidirectional reactions for the formation of hydrogen.^{19,53} Similarly, another entropy-driven bioreaction is C₂H₄O₂(aq) → CH₄(g) + CO₂(g) mediated by methanogenesis microorganisms, resulting in 1.7% combustion energy gain. Two spontaneous endothermic chemical reactions are N₂O₅(s) → 2 NO₂(g) + ¹/₂O₂(g) and Ba(OH)₂·8H₂O(s) + 2NH₄SCN(s) → Ba(SCN)₂(aq) + 2NH₃(aq) + 10H₂O(l).⁹ All of the above entropy-driven reactions involve phase changes from more orderly to less orderly.

3.2. Biological CO₂ Fixation. Carbon dioxide can be biologically fixed by plants, microorganisms, and animals. Most plants fix carbon dioxide by using chloroplasts through the reductive pentose-phosphate cycle, that is, the Calvin–Benson cycle. Microorganisms can fix CO₂ through six pathways: the reductive citric acid cycle,⁶⁷ the reductive acetyl-CoA pathway (Wood–Ljungdahl pathway),⁶⁸ the 3-hydroxypropionate pathway,⁶⁹ the 3-hydroxypropionate-4-hydroxybutyrate cycle, and dicarboxylate-4-hydroxybutyrate cycle.⁷⁰ Sometimes, animal tissues, such

as liver cells, have been found to fix CO₂ to produce cell constituents (e.g., glycogen).⁷¹ All of natural biological CO₂ fixation pathways require 12 mol of the reduced cofactor (NADPH) or its equivalents plus several mol of ATP for the generation of one mol of glucose from 6 mol of CO₂. The ATP number consumed depends on the pathways in microorganisms and plants, ranging from 2 to 30.^{70,72} Here ATP is an extra energy driving force for implementing thermodynamically-unfavorable reactions because most times CO₂ concentrations in the environments are very low. When high concentration of CO₂ is available, the number of ATP consumed per glucose unit may be decreased greatly.

Plant photosynthesis utilizes intermittent low-energy concentration solar energy (e.g., ~170 W/m²) and fixes CO₂ in the form of carbohydrate. But natural plant photosynthesis has pretty low energy efficiencies from solar energy to chemical energy of 4.6–6.0% (theoretical), ~3–4% (peak), ~1–2% (dedicated crops), and ~0.2–0.3% (global average).^{73–75} Such low efficiencies are mainly attributed to four factors: (i) narrow light absorption spectrum by chlorophylls, (ii) unmatched reaction rates between light reactions and dark reactions, (iii) relatively low efficiencies of carbohydrate synthesis, and (iv) carbohydrate losses because of respiration of living entities.^{73–76}

To surpass low-efficiency plant photosynthesis for CO₂ fixation, another potential application of SyPaB is to fix CO₂ through a non-natural ATP-neutral high-efficiency pathway⁷⁷ (Figure 4, eq 7)



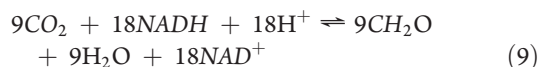
where the inputs are CO₂ and hydrogen; the outputs are water-insoluble amylose (linear starch), volatile ethanol (C₂H₆O), and water.

The hypothetical hydrogen/CO₂-to-carbohydrate process is composed of six biocatalytic modules, including

- (1) NADH is generated from hydrogen by using hydrogenase (eq 8)^{78,79}



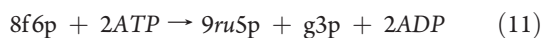
- (2) CO₂ fixation to formaldehyde (CH₂O) mediated by formate dehydrogenase and formaldehyde dehydrogenase (eq 9),^{80–82}



- (3) conversion of formaldehyde to fructose-6-phosphate (f6p) by 3-hexulose-6-phosphate synthase and hexulose phosphate isomerase from the ribulose monophosphate pathway (eq 10),^{83,84}



- (4) ribulose-5-phosphate (ru5p) regeneration by the eight enzymes from the nonoxidative pentose phosphate pathway (eq 11),^{19,72}



- (5) ethanol production from glyceraldehydes-3-phosphate (g3p) by the seven enzymes from the glycolysis and

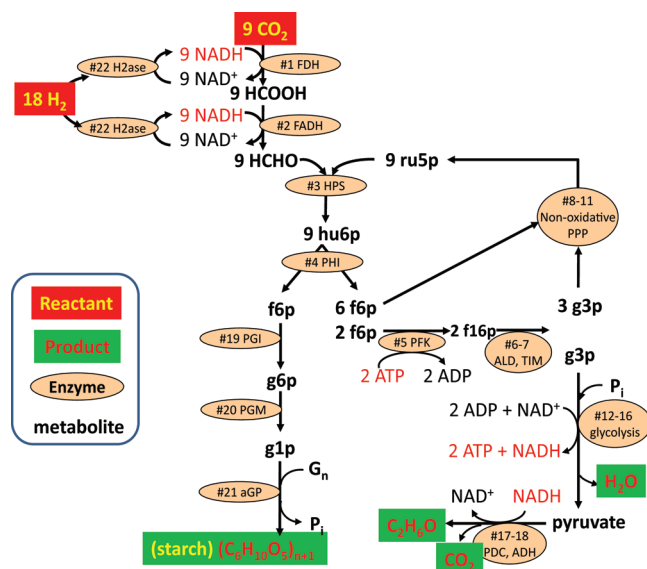
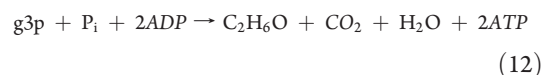
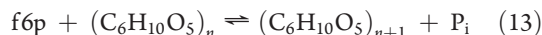


Figure 5. In vitro ATP-balanced synthetic pathway of CO₂ fixation by using hydrogen for the production of synthetic starch and ethanol.

ethanogenesis pathway (eq 12),⁷²



- (6) starch (amylose, a linear α -1,4-glucosidic bond starch) lengthening reaction mediated by starch phosphorylase along with phosphoglucose isomerase and phosphoglucose mutase (eq 13),^{19,72}



The combination of eqs 8–13 results in eq 7 with an energy conversion efficiency of 81%.⁷⁷ The standard Gibbs free energy of eq 7 is -54.5 kJ/mol, implying that the above reaction may occur spontaneously under standard conditions. The overall reaction could be operative since (i) nearly each reaction is reversible, except 6-phosphofructokinase and pyruvate kinase, both of which control the overall reaction direction, (ii) each module (eqs 8–13) involving several enzymatic steps has been implemented successfully in the literature, and (iii) the Gibbs free energy is negative. This process can drive forward the desired products through several process operations: (i) high-pressure and high concentration CO₂ from a power station or a CO₂ storage site is used for a high driving force for this artificial photosynthesis, (ii) the amylose-lengthening reaction occurs on the nonreducing ends of amylose and amylose is more insoluble in the presence of ethanol, and (iii) ethanol can be stripped from the aqueous phase. Instead of putting all of enzymes in SyPaB in one reactor, it is possible to separate several cascade reactions into several bioreactors in series, as demonstrated in the synthesis of D-ribulose-1,5-bisphosphate from 3-phospho-glycerate.⁸⁵ In the starch synthesis step (eq 13), this reaction may be run like solid-phase synthesis, where anhydroglucose units are added on the nonreducing ends of amylose one by one.

The major potential applications of such artificial photosynthesis could be the storage of low-cost renewable hydrogen or electricity in the form of starch and ethanol on a large scale

Table 1. Challenges on SyPaB Technology and Their Respective Solutions and Supportive Examples

challenge	solution	example	ref.
enzyme instability	utilization of thermoenzymes	Taq polymerase, amylase, glucose isomerase	121,122
	protein engineering (directed evolution and rational design)	subtilisin, cellulase	123–125
	enzyme immobilization	immobilized glucose isomerase, immobilized phosphoglucose isomerase	5,126
costly enzymes	TTN value $> 10^7$	CthPGI, CthPGM, Tm6PGDH, TmFBP ^a	26,28,94,95
	high-cell density microbial fermentation production	cellulase (\$5/kg), amylase (\$~10/kg), Hyperthermophilic 6PGDH	14,26,37
	recombinant overexpression by <i>E. coli</i>	<i>P. furiosus</i> hydrogenase, CthPGI, CthPGM, Tm6PGDH, TmFBP,	26,28,94,95,127
	simple scalable purification techniques (e.g., heat precipitation, $(\text{NH}_4)_2\text{SO}_4$ precipitation, adsorption/desorption)	heat precipitation (Tm6PGDH), one-step CthPGI purification and immobilization	14,26,28,96,97
costly and labile coenzymes	coenzyme immobilization and recycling	chiral alcohol synthesis in pharmaceutical industry	128,129
lack of thermoenzyme library	stable and low-cost biomimetic coenzyme replacement	P450, horse peroxidase, alcohol dehydrogenase	100,101,103,104
	meta-genomics, bioinformatics tools, robotic automation, and high throughput cloning	screening ~500 recombinant enzymes in one biocatalysis reaction	14
different optimal conditions for different enzymes	reaction conditions compromised	> 1400 <i>T. thermophilus</i> HB8 thermoenzyme library	19,20,53
	numerous enzymes obtained from one source or modify them	sugar-to-hydrogen, biohydrogenation	
scalability potential	production of 75 million tons of H ₂ replacing 450 million tons of gasoline (i.e., 150 billion gallons)	<i>T. thermophilus</i> HB8 thermoenzyme library	14
		~250,000 tons of enzyme mixtures (i.e., ~300 kg of H ₂ per kg of enzyme mixture) ^b	120

^a CthPGI, *C. thermocellum* phosphoglucose isomerase; CthPGM, *C. thermocellum* phosphoglucosmutase; Tm6PGDH, *T. maritima* 6-phosphoglucosmutase; TmFBP, *T. maritima* fructose-fructose-1,6-bisphosphatase. ^b One kg of enzyme mixture can produce 300 kg of hydrogen based on two assumptions: (i) all enzymes have TTN values of 30,000,000 mol product per mol enzyme and (ii) the average molecular weight of the enzyme mixture is 50,000.

Table 2. Analysis of Potential Hydrogen Rate Increases for Sugar-to-Hydrogen Mediated by SyPaB

technology	potential fold	ref.	predicted fold ^a
increasing reaction temperatures from 30 to 80 °C or even higher	32	Q10 effect for hyperthermophilic hydrogenase ^{b130}	4–20
increasing the use of enzymes responsible for rate-limiting reactions	10	53	2–5
increasing overall enzyme concentration	10	106	5
increasing substrate concentration by 50-fold	10	53	5
creating metabolite channeling among cascade enzymes	~2–50	95,131,132	2
increasing catalytic efficiency of enzymes	~10		
overall accelerating rates	640,000–32,000,000		500–5,000

^a Predicted folds based on each technology may change greatly. It is feasible to increase reaction rates by 3000-fold to be the same level as compared to the highest microbial hydrogen generation rates.¹⁰⁶ ^b *P. furiosus* hydrogenase responsible for the rate-limited step in the sugar-to-hydrogen production, exhibited approximately 1% of its maximum activity at ~30 °C. Increasing reaction temperature along with the use of other thermoenzymes would accelerate hydrogen generation rates greatly.

and the production of feed and food in emergency cases, such as volcanic winter. A combination of high efficiency solar cells with solar-to-electricity efficiencies of 18–42%,⁸⁶ water electrolysis with electricity-to-hydrogen efficiencies of ~85%,⁸⁷ and carbohydrate generation from H₂ and CO₂ with an efficiency of ~80% here would have overall solar energy-to-carbohydrate efficiencies from 12 to 29%, much higher than those of natural plant photosynthesis.^{73,75} The much higher efficiencies of this artificial photosynthesis are mainly attributed to (i) higher efficiency solar cells that can utilize a broader wavelength range of solar insolation, (ii) no respiration (energy) losses in cell-free biocatalysis systems, and (iii) higher-energy efficiency synthetic pathway of starch (Figure 5). Since solar/wind electricity can be easily collected by wires and be distributed by grids, it would be feasible to produce synthetic starch 24/7 at well-controlled bioreactors. More appealing, this artificial photosynthesis does not require a large amount of water for plant transpiration, resulting in potential conservation of fresh water by about 500-fold or higher.^{88,89} The pollutants generated from bioreactors can be treated more easily than those from agricultural land because they are point pollution sources.⁹⁰ Modern farming requires significantly high inputs from nutrients (e.g., nitrogen and phosphorus), herbicides, and pesticides for high crop productivities.⁹¹ Only a fraction of fertilizers (e.g., ~30–50%) are utilized by plants, resulting in severe nonpoint water pollution from agricultural land.⁹² Waste water pretreatment for bioreactors would be much easier than those from agricultural land.

Approximately 10–60 fold increases in area-specific starch productivity and ~500–1000 fold water reduction per weight of starch synthesis through this artificial photosynthesis would drastically decrease land uses for biofuels production and reduce or eliminate land/water competition with food and feed production. Also, the conversion of starch to biofuels and value-added chemicals is much more easy than that of nonfood biomass.^{38,73,93}

4. CHALLENGES AND OPPORTUNITIES

Construction of in vitro synthetic enzymatic pathways is much easier than modification of living biological entities so that in vitro reconstitution of enzymatic pathways has long been used for understanding natural pathways.^{22,23} In the future, in vitro synthetic cascade enzymes would become a low-cost biomanufacturing platform, where product yield is the most critical factor for economically viable production of biofuels. Different from living biological entities operated far

from thermodynamic equilibrium and their complicated regulation mechanisms, which are being elucidated by intensive efforts of systems biology and synthetic biology, cell-free systems can be accessed, regulated, operated, and scaled up easily. For example, it is relatively easy to get very high product yields, although all of the enzymes are obtained from different sources and their optimal conditions are not matched well.^{20,50,53}

The challenges or doubts of low-cost biomanufacturing SyPaB are attributed to a fixed paradigm of most bioengineers and scientists. The possible causes include (i) enzyme instability, (ii) costly enzymes, (iii) costly and labile coenzymes, (iv) a lack of stable enzymes, (v) different optimal conditions for different enzymes, and (vi) scalability potential.^{9,14} To address the above challenges, the respective solutions and supportive examples are listed in Table 1. For example, enzyme instability can be addressed by thermoenzymes, protein engineering through directed evolution and rational design, enzyme immobilization, and their combinations. The previous economic analyses suggest that enzyme costs would be minimal when total turnover numbers (TTN) of all enzymes are larger than 10⁷–10⁸ mol of product per mol of enzyme.^{14,15,20} In practice, it is very feasible to obtain enzymes with such high TTN values from natural thermoenzymes, for example, *Clostridium thermocellum* phosphoglucomutase,⁹⁴ *Thermotoga maritima* 6-phosphogluconate dehydrogenase,²⁶ *T. maritima* fructose-1,6-bisphosphatase,⁹⁵ and *C. thermocellum* phosphoglucose isomerase.²⁸ With respect to costly enzyme, bulk industrial enzymes can be produced and obtained at very low costs, for example, \$~5 per kg of crude protease produced by *Bacillus subtilis*, \$5–10 per kg of cellulase produced by *Trichoderma* spp., and tens of U.S. dollars per kg of recombinant proteins produced in *E. coli*.¹⁴ Several low-cost scalable protein purification approaches are available, for example, simple centrifugation for secretory enzymes, adsorption/desorption on low-cost cellulosic materials,^{96,97} heat precipitation for thermostable enzymes,^{26,98} ammonia precipitation,^{14,99} and one-step enzyme purification and immobilization.²⁸ Therefore, purification costs for bulk recombinant thermoenzymes would become minor.

Currently, the largest obstacle to SyPaB may be costly coenzymes, NADH and NADPH. The labile coenzyme issue can be addressed by the use of low-cost and stable NAD biomimetic coenzymes. But this research area is in its infancy^{100,101} because there were no large markets before. Several redox enzymes (e.g., P450 and alcohol dehydrogenase) have been engineered for better performance on biomimetic coenzymes.^{102–104} With developments in (i) engineered oxidoreductases that can use biomimetic NAD coenzymes and (ii) stable enzymes as building blocks of SyPaB, we

Table 3. Selected SyPaB-Based Applications, As Compared to Competing Technologies, Their Technology Readiness Levels (TRL) for the Y-12 National Security Complex,¹⁰⁸ Their Remaining Obstacles, and Respective Solutions

application	competing technology	market size ^a (US \$/year)	TRL	remaining obstacle	solution	ref.
biosynthesis of chiral drugs via bioreduction environmentally friendly sugar batteries (enzymatic biofuel cells)	one-enzyme NAD(P)H regeneration	~ billions	TRL 6	separation of metabolites/products with enzymes	enzyme immobilization, membrane reactor	20,36
	primary batteries, rechargeable batteries, DMFC	~ 2 billion	TRL 4	low power output, incomplete oxidation, short lifetime	system optimization, nanobiotechnology, cascade pathways, thermoenzymes, enzyme engineering and immobilization	9,110,111
					Tables 1 and 2	9,14,19,53
sugary H ₂ for local hydrogen users	made from natural gas and coal, or biomass, solar, or wind energy	~20 billion (e.g., ~8 million tons of H ₂)	TRL 4	enzyme stability, enzyme costs, labile coenzymes, slow reaction rates	Table 1, membrane reactor	9,14,20
	microbial fermentations, FT process, pyrolysis	~ 50 billion (e.g., 75 million tons of jet fuel)	TRL 3	<i>ditto</i> as bioreduction, and metabolite, and enzyme removal		
electricity generators sugar fuel cell vehicles (SFCV)	Diesel electricity generators	~ billions	TRL 2	<i>ditto</i> as sugary H ₂	Tables 1 and 2	1
	BEV ^a FCV ICE	~500 billion (e.g., 450 million tons of gasoline)	TRL 2	<i>ditto</i> as sugary H ₂ slow reaction rate	Tables 1 and 2	1,3,16
CO ₂ fixation for starch production	dedicated bioenergy plants, mass electricity storage	NA	TRL 2	<i>ditto</i> as enzymatic fuel cells	Tables 1 and 2	77

^a U.S. market only. ^a BEV, battery electric vehicle; FCV, (hydrogen) fuel cell vehicle; ICE, internal combustion engine-based vehicle.¹²⁰

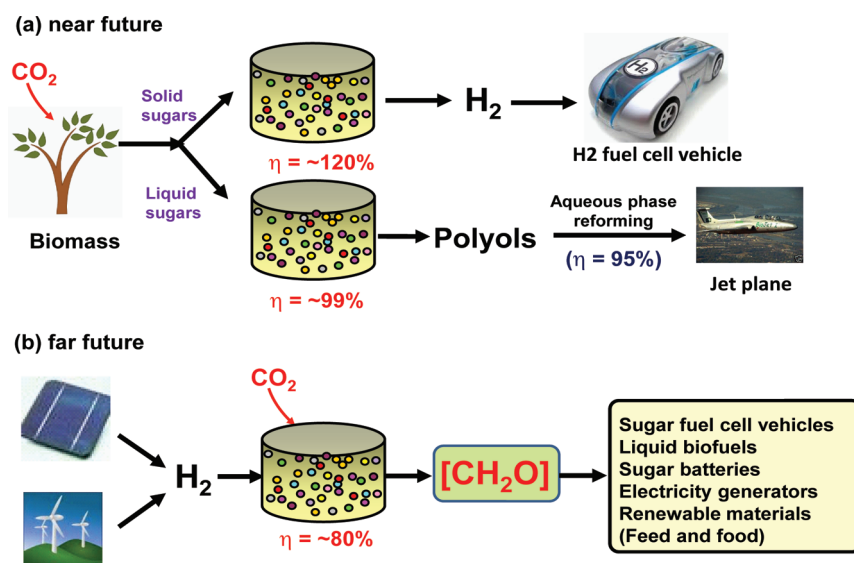


Figure 6. Different biofuels scenarios based on plant biomass through natural photosynthesis (near future) and starch produced by artificial photosynthesis (far future), where high-yield and low-cost SyPaB would have a central role for different biofuels production. The data in red represent energy efficiencies mediated by SyPaB featuring $\sim 99\%$ mass conversion.

estimate that ultimate hydrogen production costs may decrease to $\sim \$1.50$ per kg of hydrogen, where carbohydrate ($\$0.22/\text{kg}$) accounts for $\sim 95\%$ of its production costs, in part because biohydrogen has very low separation and purification costs and the other chemicals in reactors can be recycled.^{14,15}

Enzymatic reactions are usually faster than microbial fermentations^{9,105} mainly because neither the dilution of biomacromolecules (e.g., DNA, RNA, other proteins, etc.) nor the mass transfer barriers resulted from the cellular membrane.^{1,14} Current enzymatic hydrogen generation rates are comparable with those of anaerobic hydrogen fermentation and are much faster than photobiological hydrogen fermentation.⁵³ As compared to the highest microbial hydrogen production rates (i.e., $23.6 \text{ g H}_2/\text{L/h}$) in the literature,¹⁰⁶ the current enzymatic hydrogen rate⁵³ would have a potential of ~ 3000 -fold reaction rate increases. Table 2 shows potential methods for increasing reaction rates for sugary hydrogen mediated by SyPaB. They are: (i) increasing reaction temperatures, (ii) increasing the use of enzymes responsible for rate-limiting reactions, (iii) increasing substrate concentrations, (iv) increasing overall enzyme concentrations, (v) accelerating the reaction rates by metabolite (product) channeling, and (vi) increasing the catalytic efficiency of enzymes to catalytically perfect enzymes. With more collaboration among biologists, chemists, and engineers all around the world and system optimization, the reaction rates of SyPaB would be accelerated by several orders of magnitude.¹ In partial support to this prediction, power densities of microbial fuel cells have been enhanced by nearly 10,000,000 fold through intensive efforts during the past one and a half decade.¹⁰⁷

SyPaB-based applications are increasing greatly. Table 3 presents several potential applications, as compared to their competing technologies, technology readiness levels (TRL),¹⁰⁸ remaining obstacles, and respective solutions. Since each application has its unique market, it has different technology challenges (Table 3). For example, a promising application is enzymatic fuel cells (EFC) powering (low-power) portable electronics, such as cellular phones and MP3 players.^{105,109,110} Several big companies (e.g., Sony and Nokia) and small

companies (e.g., Gate Fuels and Akermin) are developing enzymatic fuel cells. To our knowledge, the highest power densities of enzymatic fuel cells based on sugar are about $5\text{--}10 \text{ mW}/\text{cm}^2$ of anode, sufficient to power a Sony Walkman.^{111,112} To increase fuel utilization efficiency, cascade enzymes are usually employed.^{110,113–115} Complete conversion of sugar energy to electricity would have 4-fold benefits: high energy utilization efficiency, high energy storage density, low product inhibition, and high power density.^{9,105,116} It is estimated that complete oxidation of a 20% sugar/water solution ($17 \text{ MJ}/\text{kg}$ sugar $\times 20\%$) would lead to energy storage densities of up to 1.7 MJ (i.e., 470 Wh) electricity per kg of the fuel solution based on $\sim 100\%$ Coulombic efficiency and $\sim 50\%$ voltage efficiency. Clearly, such high-energy density biodegradable EFCs might replace some primary batteries and secondary batteries in the future.^{55,117}

5. BIOFUELS PERSPECTIVE

Enzyme-based biotransformations are evolving from a single enzyme to multienzyme one pot to synthetic cascade enzymes. SyPaB features unique advantages: great engineering flexibility, high product yields, fast reaction rates, broad reaction conditions (e.g., high temperature and/or low pH), easy operation and control, and tolerance of microorganism-toxic compounds.^{9,15,16,20} Therefore, SyPaB would play more important roles in some yield-sensitive applications, such as biofuels production, because thermodynamics (energy efficiency) determines economics (cost) in the long term.¹¹⁸

What biofuels would be short-term (e.g., 5 years), middle-term (e.g., 10–20 years), and long-term (e.g., > 20 years) winners is under debate. But it is worth pointing out that high-yield conversion would defeat low-yield conversion eventually because of a megatrend of increasing energy utilization efficiency. In the future, transportation fuels could mainly consist of hydrogen from carbohydrates for light-duty vehicles, electricity from renewable energy sources for short-distance vehicles, and high-energy density liquid biofuels (e.g., hydrocarbons and

butanol) made from biomass for jet planes.^{1,119} On the basis of available biomass resources and pretreatment (Figure 6a), liquid hemicellulose sugars and solid cellulosic materials may be converted to jet fuel and hydrogen through high-yield SyPaB, respectively. Liquid jet fuel can be produced through a hybrid of high-yield SyPaB and aqueous phase reforming with an overall energy retaining efficiency (~95%), much higher than fatty acid ester fermentation (~60–65%) and butanol fermentation (~85%).^{8,20} Cellulosic materials can be converted to hydrogen in local stations for providing hydrogen for proton exchange membrane fuel cell vehicles.^{3,14} In the far future, synthetic starch used for electricity/hydrogen storage (e.g., > 8 mass H₂% or 11–14 MJ electricity/kg starch) may be generated through artificial photosynthesis with an hydrogen-to-starch efficiency of ~80% mediated by SyPaB. Also, starch can be converted back to hydrogen or electricity for different applications. For example, fuel cell-based sugar vehicles that would store starch as a high-density hydrogen carrier might become ultrahigh energy efficiency prime movers.^{1,3,120}

In a word, great potentials of high-yield SyPaB (Table 3) would motivate the transformation of basic research to real applications by integrating well-known technologies (Table 1). The maturation of genomics, molecular biology, techniques for enzyme engineering, low-cost enzyme production, purification, and immobilization has led to highly efficient, tunable enzymes tailored for specific large-scale industrial production. The biotransformation through in vitro assembly of numerous enhanced performance and stable enzymes in one bioreactor that can last a very long reaction time (e.g., several months or even years) would become a disruptive technology for low-cost biomanufacturing, especially for the production of biofuels where product yield is the most important cost factor.

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