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SPECIAL ISSUE PAPER

A new high-energy density hydrogen carrier—carbohydrate—might be better than methanol

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SUMMARY

High-density hydrogen storage in the form of renewable carbohydrate becomes possible because cell-free synthetic enzymatic pathway biotransformation (SyPaB) can 100% selectively convert carbohydrate and water to high-purity hydrogen and carbon dioxide under modest reaction conditions (below water boiling temperature and atmospheric pressure). Gravimetric density of carbohydrate (polysaccharide) is 14.8% H_2 mass, where water can be recycled from polymer electrolyte membrane fuel cells or 8.33% H_2 mass based on the water/carbohydrate slurry; volumetric density of carbohydrate is >100 kg of H_2/m^3. Renewable carbohydrate would be more advantageous over methanol according to numerous criteria: substrate cost based on energy content (cost per gigajoule), energy conversion efficiency, catalyst cost and availability, sustainability, safety, toxicity, and applications. Huge potential markets of SyPaB from high-end applications (e.g., biohydrogenation for synthesis of chiral compounds and sugar batteries) to low-end applications (e.g., local satellite hydrogen generation stations, distributed electricity generators, and sugar fuel cell vehicles) would be motivation to solve the remaining obstacles soon. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS

biomass; carbohydrate; cell-free synthetic pathway biotransformation (SyPaB); hydrogen carrier; hydrogen production; hydrogen storage; sugar fuel cell vehicle

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1. INTRODUCTION

Mobility usually represents civilization level [1–3]. The utilization of liquid fuels along with internal combustion engines (ICEs) has greatly enhanced the mobility of human beings because liquid fuels have high energy storage densities, they can be easily transported and conveniently stored, and ICEs have high power-to-weight ratio (e.g., watt output/gram engine) and low production costs based on cost-per-watt output [3,4]. But concerns of depleting crude oil, soaring prices of crude oil, climate change associated with net carbon dioxide emissions, uneven resource distribution, wealth transfer, national energy security, and air pollution are driving to seek for clean and sustainable alternative transportation fuels [5,6].

The next transportation revolution would mainly occur as a transition from ICEs to the hydrogen/electricity systems [6–8]. Because electricity storage densities in the batteries (e.g., ~0.14 MJ/kg of lead acid battery, ~0.46 MJ/kg of lithium battery) [9,10] are far less than those of available hydrogen means (e.g., 5.7 MJ/kg of 4% H_2 storage container), a majority of future transportation vehicles would be based on the hydrogen/fuel cell/motor systems [8,11,12]. In addition to energy storage density, the underlying premise of the hydrogen economy is that hydrogen fuel cells have much higher energy efficiencies (~50–70%) than internal combustion engines (~20–40%) that are restricted by the second law of thermodynamics. Thus, this transition from heat engines to fuel cells would decrease consumption of primary

energy by approximately twofold and reduce air pollution generated from ICEs.

The largest obstacle to the hydrogen economy is the safe, efficient, and viable storage of hydrogen [8,13]. In general, hydrogen can be stored (i) in high-pressure gas cylinders; (ii) as liquid hydrogen in cryogenic tanks at 21K; and (iii) in solid forms, including adsorption on large specific surface area solid materials (e.g., nanomaterials, metal organic frameworks), chemical or light metal hydrides, or by the reaction of light metals or hydrides and water (e.g., $NaAlH_4$, $LiAlH_4$, AlH_3, $LiBH_4$, $Mg(BH_4)_2$, destabilized borohydrides, and amide/imide systems) [13–15]. As shown in Figure 1, most of them are far from meeting the DOE's ultimate hydrogen storage goals—8% H_2 mass and 75 kg H_2/m^3 container—which have been decreased from the original goals (9% H_2 mass in the year 2015) [16]. Some light metals and some chemical hydrides may have high gravimetric and volumetric capacities meeting the DOE's new targets. For example, ammonia borane (H_3NBH_3) can release ~18 wt% H_2 by the use of a Ni catalyst at 60°C [17]. But its synthesis requires input of ammonia [18,19], which is at least 20% more costly than hydrogen based on energy content (cost per gigajoule) because ammonia is synthesized from nitrogen and hydrogen [8]. Consequently, ammonia borane is a relatively costly hydrogen carrier. Similarly, some light metals (such as alumina) can release hydrogen when they react with water in the presence of some catalysts, but these metals have to be regenerated by electrolysis. Regeneration of light metals is energy intensive [20]. As a result, light metals or chemical hydrides may not be good as hydrogen carriers for passenger vehicles due to high energy costs for their production or regeneration, as compared with methanol or carbohydrate [8].

In order to dramatize and incentivize hydrogen research, the H-Prize has been established to competitively award cash prizes that will advance the commercial application of hydrogen energy technologies [8,21]. The 2009–2011 H-Prize will be awarded in the area of storage materials in mobile systems for light-duty vehicles. The basic requirements are (i) 7.5% reversible H_2 mass at conditions between

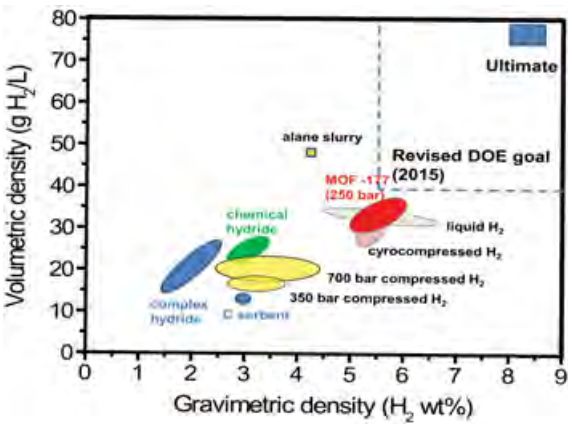

Figure 1. The available hydrogen storage means and the DOE's updated ultimate goal of hydrogen storage.

−40°C and +85°C and between 1.5 and 150 bars of H_2 pressure; (ii) 70 kg of total releasable H_2/m^3; (iii) charging kinetics—greater than or equal to 0.0004 g of hydrogen per gram of material per second at conditions between −40°C and +85°C and between 1.5 and 150 bars of H_2 pressure; (iv) discharge kinetics—greater than or equal to 0.00002 g of hydrogen per gram of material per second at conditions between −40°C and +80°C and an outlet hydrogen pressure of ⩾1.5 bar; and (v) a cycle life of 100 without a significant loss of capacity [21].

An alternative is use of hydrogen carriers, such as hydrocarbons, biodiesel, methanol, ethanol, ammonia, and carbohydrate [8]. But end users must have an on-board converter that can convert the hydrogen carrier to high-purity hydrogen before entering polymer electrolyte membrane (PEM) fuel cells. By considering the complexity, size, and control of the hydrogen generation system and hydrogen purification system, hydrocarbons, biodiesel, and ammonia are not suitable in a small room of a passenger vehicle equipped with PEM fuel cells [6]. Methanol has been proposed as a future hydrogen carrier by Nobel Prize Winner George Olah [22], whereas carbohydrate was suggested to be a future hydrogen carrier by us in 2007 [23] because of 100% chemical selectivity mediated by cascade enzymes that can implement the stoichiometric reaction as $C_6H_{10}O_5(aq) + 7\,H_2O(l) \rightarrow 12\,H_2(g) + 6\,CO_2(g)$ [23,24]. Therefore, gravimetric density of carbohydrate (starch or cellulosic materials) is 14.8% H_2 mass where water can be recycled from PEM fuel cells or 8.33% H_2 mass based on a carbohydrate/water slurry; volumetric density of carbohydrate is >100 kg of H_2/m^3 [8].

The possibility of carbohydrate as a hydrogen carrier is due to a new technology—cell-free synthetic enzymatic pathway biotransformation (SyPaB). SyPaB is the implementation of complicated biological reactions through *in vitro* assembly of a number of enzymes and coenzymes [2,25–27]. The basic idea of SyPaB is to surpass microorganisms' metabolisms and does not require other microbial functions such as self-duplication, maintenance, and so on. As compared with microbe-mediated fermentations, SyPaB has obvious advantages, such as greater engineering flexibility (i.e., neither cellular membrane nor gene regulation), higher product yield, faster reaction rate (i.e., faster mass transfer and higher biocatalyst concentration), broader reaction conditions, easier process control, and so on [2,25–27]. Because of this new technology, breaking the Thauer limit for microbial biohydrogen fermentation (i.e., 4 H_2/glucose) comes true. Also, to our limited knowledge, hydrogen is generated by using room-temperature waste heat without a temperature gradient (i.e., a cool sink) for the first time [23,24].

In this perspective article, we argued the possibility of carbohydrate as a high-density hydrogen carrier, compared hydrogen production on the basis of aqueous phase reforming (APR) and SyPaB, analyzed the carbohydrate economy and the methanol economy, and presented the SyPaB roadmap toward the hydrogen economy based on renewable carbohydrate. Here we did not want to compare carbohydrate with other potential hydrogen storage compounds.

2. HYDROGEN PRODUCTION FROM CARBOHYDRATE

Hydrogen can be produced from biomass-derived carbohydrate by chemical catalysis featuring severe reaction conditions and fast reaction rates (e.g., gasification, pyrolysis, APR), biocatalysis featuring modest reaction conditions and low energy input (e.g., dark fermentation), and their combinations (e.g., ethanol fermentation followed by partial oxygenation). Hydrogen production through chemical and biological catalysis from carbohydrate has been reviewed elsewhere [6,28–31]. All of previous chemical and biological hydrogen-producing means based on carbohydrate except SyPaB suffer from low hydrogen yields, far from its theoretical yield (i.e., 12 H_2/glucose). Here we focused on the comparison of two newly developed technologies—APR and SyPaB (Table I).

APR is chemical reforming conducted in the liquid phase (e.g., ~200°C–300°C and ~50–70 atm) mediated by the catalysts below the critical point of water (647K and 218 atm). This process generates hydrogen without volatilizing water, which means major energy savings. Furthermore, it occurs at temperatures and pressures where the water–gas shift reaction is favorable so that it can generate hydrogen with low amounts of CO_2 in a single chemical reactor. At low temperature, this process also minimizes undesirable decomposition reactions [32]. In 2002, Dumesic and his coworkers demonstrated that hydrogen can be produced from biomass-derived carbohydrates at temperatures near 500K in a single reactor by using a platinum-based catalyst [33]. One of the disadvantages is leaching and instability of catalyst components into the aqueous phase. Although APR process may be attractive to produce H_2 from carbohydrate, hydrogen yields were low to date, owing to low selectivity and formation of coke and by-products [34].

Biocatalysis mediated by microorganisms or isolated enzymes has several advantages over chemical catalysis, such as higher selectivity, lower energy input (e.g., higher energy efficiency), and less costly bioreactor [35]. In nature, anaerobic microorganisms can produce 4 mol of hydrogen and 2 mol of acetate or 2 mol of hydrogen and 1 mol of butyrate per mol of glucose through the mixed acid pathway, called the Thauer limit [36]. In practice, natural or genetically modified microorganisms produce hydrogen yields lower than or close to this theoretical yield (i.e., 4 H_2/glucose) [28,36–39].

Table I. Comparison of carbohydrate-to-hydrogen technologies: aqueous phase reforming and synthetic enzymatic pathway biotransformation.

Feature	Aqueous phase reforming	Synthetic pathway biotransformation
H_2 yield (mol/mol)	~2–8	12
Energy efficiency	30–50%	122%
Product purity	Low, purification needed	High, no purification
Reaction condition	~200°C–300°C, ~50–70 atm	~20°C-80°C, ~1 atm
Reactor cost	High pressure	Low pressure
Catalyst cost	Modest	Very high → very low
Catalyst stability	Modest → high	Low → high
Reaction rate	Very high	Low → high

In order to break the hydrogen production limit for microorganisms, nearly 12 mol of hydrogen has been produced per glucose unit of starch or cellulosic materials and water by SyPaB [23,24]. In these cell-free synthetic pathways, a number of enzymes and coenzymes purified from different sources (e.g., bacteria, yeasts, archaea, animals, and plants) are assembled *in vitro* for the implementation of complicated biological reactions. The pathways contain five sub-modules: (i) polysaccharide or oligosaccharide conversion to glucose-1-phosphate (g1p) catalyzed by glucan phosphorylase, (ii) glucose-6-phosphate (g6p) generation from g1p catalyzed by phosphoglucomutase, (iii) NADPH production catalyzed by two dehydrogenases of the oxidative phase of the pentose phosphate pathway (PPP), (iv) g6p regeneration from ribulose-5-phosphate catalyzed by the four enzymes of the non-oxidative phase of PPP and four enzyme of the glycolysis and gluconeogenesis pathways, and (v) hydrogen generation from NADPH catalyzed by hydrogenase (Figure 2a). The overall carbohydrate (starch or cellulosic materials)-to-hydrogen reaction is shown as

$$C_6H_{10}O_5(aq) + 7\ H_2O\ (l) \rightarrow 12\ H_2(g) + 6\ CO_2(g)$$

These reconstituted non-natural pathways split water by using the energy in carbohydrate and (waste) heat from the environment [23,24]. These processes are similar to catabolism, where water rather than oxygen works as an oxidant receiving electrons and generates hydrogen and carbon dioxide [2], but have much higher energy conversion efficiencies than any natural catabolism. These enzymatic reactions are among rare entropy-driven chemical reactions because two final products are gaseous under the experimental conditions (~1 atm and <100°C) [23,24]. Great increases in the entropy from aqueous to gas phases enable these positive-enthalpy reactions to occur.

We have demonstrated the feasibility of high-yield spontaneous generation of hydrogen from starch or cellulosic materials and water in batch reactions (Fig. 2 b&c) [23,24]. Although the practical hydrogen yield is slightly lower than the theoretic yield (i.e., 12 H_2/glucose equivalent) in batch reactions, it is expected that 100% product yield is achievable in a continuous reactor.

Although the current production cost of enzymatic hydrogen by SyPaB is very high, it is expected to be decreased as low as $1.50/kg of hydrogen or lower [25–27]. This cost estimate is mainly based on three major cost components—carbohydrate, enzymes, and coenzyme ($NADP^+$). Economic analysis suggests that the hydrogen cost decreases rapidly with increasing total turn-over number (TTN; mol product per mol enzyme) of the enzymes in

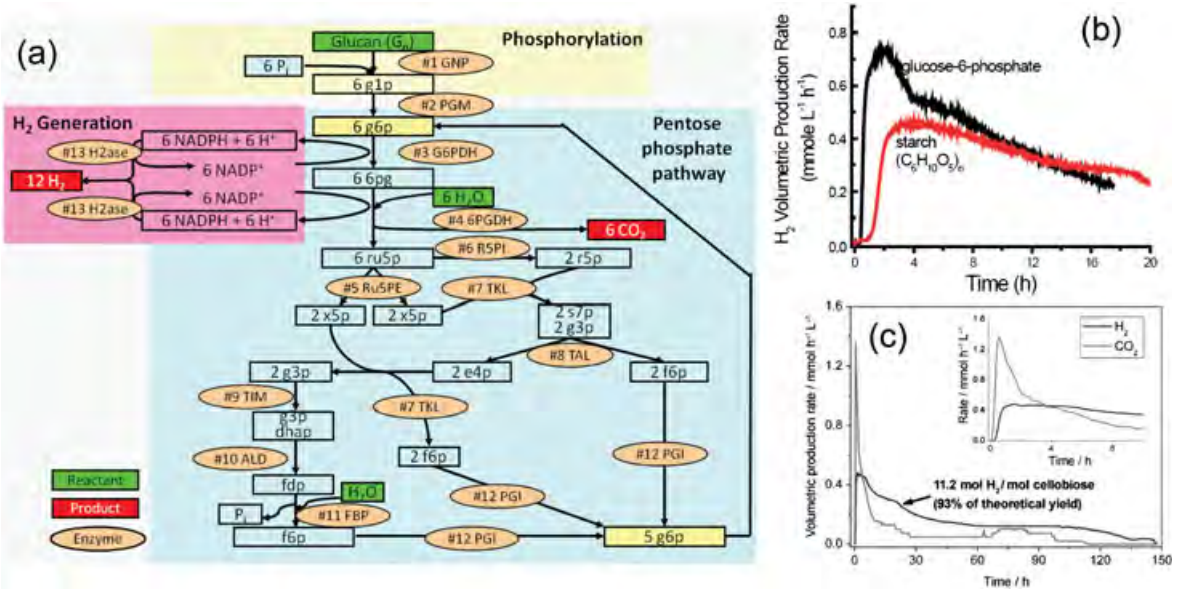

Figure 2. The cell-free synthetic enzymatic pathway (SyPa) (a), high-yield generation of hydrogen from starch (b) [23] or soluble cellodextrin (c) [24]. The enzymes are GNP, glucan phosphorylase; PGM, phosphoglucomutase; G6PDH, G-6-P dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; R5PI, phosphoribose isomerase; Ru5PE, ribulose 5-phosphate epimerase; TKL, transketolase; TAL, transaldolase; TIM, triose phosphate isomerase; ALD, aldolase; FBP, fructose-1, 6-bisphosphatase; PGI, phosphoglucose isomerase; and H2ase, hydrogenase. The metabolites and chemicals are g1p, glucose-1-phosphate; g6p, glucose-6-phosphate; 6pg, 6-phosphogluconate; ru5p, ribulose-5-phosphate; x5p, xylulose-5-phosphate; r5p, ribose-5-phosphate; s7p, sedoheptulose-7-phosphate; g3p, glyceraldehyde-3-phosphate; e4p, erythrose-4-phosphate; dhap, dihydroxacetone phosphate; fdp, fructose-1,6-bisphosphate; f6p, fructose-6-phosphate; and P_i, inorganic phosphate.

SyPaB and then levels off when all enzymes, regardless of their production costs, have total TTN values of more than 10^{7-8} mol product/mol enzyme [26]. When all enzymes have TTN values of 10^7 and each one has production costs of ~$40/kg, hydrogen production cost is anticipated to be $2.00/kg H_2. When TTN values of the enzymes are further enhanced to 10^8 or 10^9, the ultimate cost of hydrogen would be as low as $1.30/kg H_2 [27]. Such hydrogen production cost by SyPaB would be lower than those from natural gas (e.g., $2.00–$2.70/kg of hydrogen) [23].

In general, it is very feasible to have stable bulk enzymes with TTN values of more than 10^7 and the production costs of ~$40/kg of enzyme (neither membrane nor very complicated cellular enzymes). In our laboratory, we have obtained several thermostable enzymes with TTN values of more than 10^7, for example, *Clostridium thermocellum* phosphoglucomutase [40], *Thermotoga maritima* fructose-1,6-bisphosphatase [41], and *T. maritima* 6-phosphogluconate dehydrogenase [35]. In the literature, a large number of industrial and laboratory enzymes have TTN values of more than 10^7, including glucose isomerase. We have found that free *C. thermocellum* phosphoglucose isomerase (PGI) has relatively low TTN values but immobilized PGI becomes ultra-stable (TTN $> 1 \times 10^9$) [42]. Recently, Zhong and his co-workers obtained another recombinant thermoenzyme C–C bond forming transaldolase from *T. maritima* with TTN values of more than 3×10^7 [43].

Numerous successful examples are available pertaining to modification of the coenzyme preferences of redox enzymes by rational design and directed evolution [44–46]. What is more important is that labile natural coenzymes can be replaced with low-cost and stable biomimetic coenzymes [47–49]. Ryan *et al.* have successfully engineered cytochrome P450 that can work on biomimetic cofactors better than natural cofactors [48]. Recently, an ultra-thermostable alcohol dehydrogenase has been modified to utilize small-size biomimetic coenzyme by rational design [50]. Furthermore, it is found out that the small-size coenzyme enables to improve electron transfer between the coenzyme and redox enzyme (Scott Banta, personal communication).

The SyPaB is a typical integrative technology platform based on the achievements of modern biotechnology during the past five decades [25]. SyPaB, which originated from cell-free ethanol (Nobel Prize Chemistry 1907), is evolving to a low-cost and high-yield biomanufacturing technology [51]. As partly discussed before and elsewhere [25,27], numerous mature biotechnologies, such as bulk enzyme production and purification, high-cell density fermentation, enzyme immobilization, thermoenzyme discovery and utilization, protein engineering through directed evolution, rational design and their combination, and so on, make the implementation of complicated reactors through synthetic cascade pathways feasible [25]. The SyPaB technology has successfully achieved some breakthroughs that neither microbes nor chemical catalysis can implement before, such

as production of nearly 12 mol of hydrogen from carbohydrate and water [23,24], regeneration of ultra-high-yield NAD(P)H based on carbohydrate and water [51], in-depth oxidation of organic compounds to electrons in enzymatic fuel cells [52,53], enzymatic conversion of ethanol and CO_2 to lactate [54], and so on. Although SyPaB is on its early stage, its unique features, such as high product yield, fast reaction rate, and easy process control, would allow it to play more important roles in several important fields, such as biofuel production and CO_2 fixation.

3. THE CARBOHYDRATE ECONOMY VERSUS THE METHANOL ECONOMY

Here we propose the carbohydrate as a hydrogen carrier rather than methanol, which is proposed by George Olah [22], as shown in Figure 3. Carbohydrate is the most abundant renewable bioresource. Each year approximately 100 billion tons of carbohydrate per year is synthesized by plants [7,27]. Renewable carbohydrate is used as sources for food (starch), feed (starch and cellulose), renewable materials (e.g., paper, lumber, poly lactic acid), and liquid biofuels (e.g., ethanol, butanol, and other advanced liquid biofuels) [7,27]. It is expected that carbohydrate would be a hydrogen carrier in the transport sector and utilization of a small fraction of renewable annual carbohydrate production (e.g., 5–10%) would be sufficient to nearly replace transportation fuels made from crude oil [6,8,55].

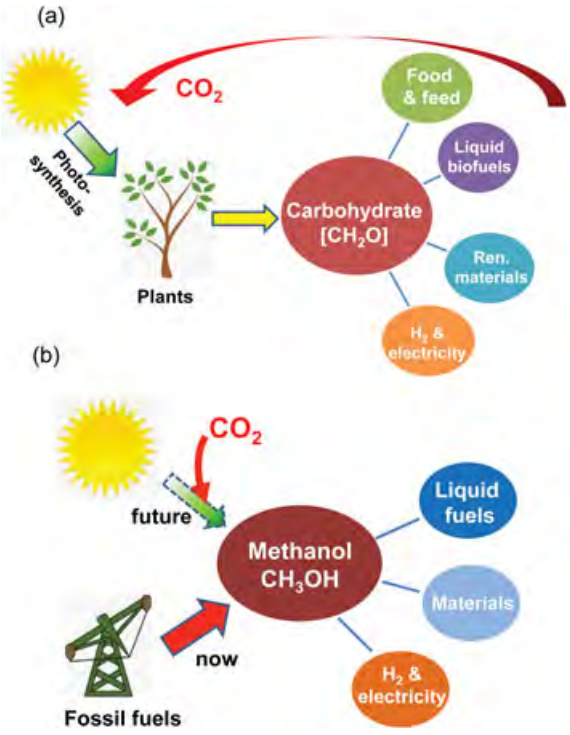

Figure 3. Comparison of the carbohydrate economy (a) and the methanol economy (b).

Now methanol is mainly synthesized from methane or coal, especially in China [56]. Also, methanol is expected to be produced from methane mediated by the enzymes (e.g., monooxygenase) under ambient conditions in the future [57–59]. In the long-term future, artificial photosynthesis would utilize solar energy to fix CO_2 to produce methanol [60–62]. Now methanol is mainly converted to formaldehyde, which can be used in making plastics, plywood, explosive, paints, and other chemical derivatives, such as dimethyl ether (DME) [22]. A large fraction of methanol was used to produce methyl *tert*-butyl ether (MTBE) as gasoline additive, but now MTBE has been replaced by ethanol. Methanol may be used as liquid fuel directly but it is corrosive, toxic, and has much lower energy density as compared with ethanol and gasoline [2]. In China, DME made from methanol is being used as home heating and cooking. It also can be used as a diesel replacement fuel. The energy content of methanol ($1.00/Gal of methanol) is approximately $16.8/GJ, higher than that of carbohydrate ($12.9/GJ, $0.22/kg of carbohydrate) [2,8].

Electricity can be produced from methanol or carbohydrate through different fuel cell systems (Figure 4). The scheme of direct methanol fuel cell (DMFC) is presented in Figure 4a. Because DMFC has much lower power density and requires higher loading of costly platinum as compared with PEM fuel cell (PEMFC), it is well suited for portable electronics, such as cellular phone and laptop, whose power requirements are low. Meanwhile, costumers are willing to pay high costs for better performance [63,64] because DMFC has much higher energy density (megajoule electricity per kilogram) and fuel refilling is faster, compared with rechargeable lithium batteries.

Carbohydrate can be converted to electricity through two pathways: enzymatic fuel cell (Figure 4b) and a hybrid of sugar-to-hydrogen and PEMFCs (Figure 4c). The former would have low power densities (e.g., 1–10 mW/cm^2 anode), so that it would be good for portable electronics, competing with DMFC or rechargeable batteries in the future. In contrast, integrated SyPaB biotransformer and PEMFCs featuring 500–1000 mW/cm^2 would be good for distributed electricity generators and light-duty vehicles [8].

Enzymatic fuel cells (EFC) are biologically inspired fuel cells where enzymes rather than chemical catalysts oxidize chemical compounds to electrical energy by mimicking biological metabolisms [65,66]. Enzymes are used to catalytically oxidize the fuels (e.g., sugars, alcohols, or organic acids) at the anode and reduce oxygen at the cathode (Figure 4b). Several companies (e.g., Sony and Nokia) are developing EFCs [67]. Sony Co. has increased the power density of enzymatic fuel cell to 5–10 mW/cm^2 anode surface and prolonged the lifetime of enzymes to more than 3 months, but such designs only extract two electrons in glucose (i.e., 1/12 of the theoretical maximum yield) [67,68]. Complete conversion of the chemicals to electricity would have fourfold benefits: high energy utilization efficiency, high energy storage density, low product inhibition, and high power density [25,66,69]. For fuel utilization

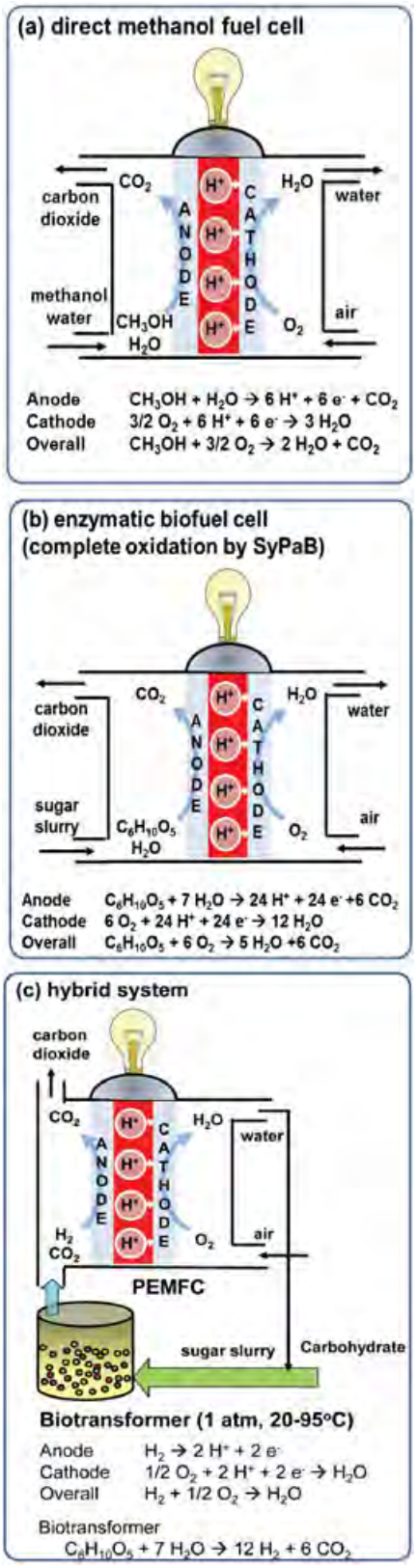

Figure 4. Comparison of direct methanol fuel cell (a), enzymatic biofuel cell (b), and a hybrid of biotransformer and PEM fuel cell (c).

efficiency to be increased, enzyme cascades are employed. Three cascade redox enzymes have been employed in an anode for the complete oxidization of one-carbon methanol to CO_2 [70]. Similarly, two-carbon ethanol has been deeply oxidized for generating more electrons by using an 11-enzyme pathway [53]. Three-carbon glycerol and pyruvate have been oxidized by using two cascade dehydrogenases [71] and the enzymes in the Kreb cycle [72,73], respectively. Figure 4b presents the scheme of EFCs that can completely oxidize carbohydrate by utilizing the cascade enzyme pathway through the modified NAD-preferred pentose phosphate pathway [51] or the citric acid cycle [69]. Although current EFCs have lower power densities than DMFCs, EFCs are on its early development stage. It is expected that further improvements of enzymatic fuel cells in prolonging enzyme stability and enhancing power density would enable EFCs to be competitive with DMFCs and rechargeable batteries [9,25].

For high-power stationary and transportation applications, fuel cell technologies face more stringent requirements in cost, power output, and durability. Figure 4c presents the scheme of an integrated biotransformer containing the enzyme cocktail responsible for generating high-purity hydrogen from the carbohydrate/water slurry and PEMFCs. A hybrid of the endothermic biotransformer and exothermic PEMFCs would increase the overall energy utilization efficiency and improve heat control of the PEMFC stack. For stationary power applications, there is no strict requirement in the size of biotransformer so that the reaction rates of biotransforming are not so important [8]. We envision that local biotransformers will utilize local low-cost biomass sugars, generate hydrogen for PEMFC stacks, and provide electricity and hot water to local users. For the light-duty passenger vehicles, it is vital to increase the hydrogen generation rates by three orders of magnitude due to a small room in vehicles [6,8]. The hydrogen-generating rates will be increased by using combined efforts in (i) increasing reaction temperature, (ii) increasing the rate-limiting step enzyme loading, (iii) increasing the substrate concentration, (iv) increasing the overall enzyme loading, (v) forming metabolite channeling among the cascade enzymes, and (vi) increasing the catalytic efficiency of enzymes [6,8]. Our previous analysis suggested the technological feasibility of three orders of magnitude enhancements in reaction rates [8]. In partial support to this prediction, power densities of microbial fuel cells have been increased by more than one million-folds through intensive research and development efforts during the past 15 years [74].

4. ROADMAP AND CHALLENGES

Use of carbohydrate is a more appealing solution to the hydrogen economy than use of methanol according to numerous factors such as substrate cost based on energy content, energy conversion efficiency, catalyst cost and availability, sustainability, safety, toxicity, and applications (Table II). Hydrogen generation and storage based on

Table II. Comparison of two hydrogen carriers—methanol versus carbohydrate.

	Methanol	Carbohydrate
Phase	Liquid	Solid powder or slurry
Source	Depletable fossil fuels, made from natural gas or coal	Renewable, isolated from biomass
Cost ($/GJ)*	16.8 [1]	12.9 [2]
H_2 generation condition	Modest high	Modest
Application	Hydrogen carrier	Food and feed
	Liquid fuel	Hydrogen carrier
	Precursor of chemicals	Precursor of liquid biofuels
		Precursor of chemicals
		Precursor of materials
		Electricity storage compound
Toxicity	High	No
Electricity generation	Direct methanol fuel cell	SyPaB + PEM fuel cell
		Enzymatic fuel cell

*methanol, $1.00/Gal and delivered carbohydrate, $0.22/kg.

carbohydrate mediated by SyPaB is a disruptive innovation, which would improve a product or service in ways that the market does not expect, typically by lowering price or designing for a different set of consumers [75]. But there are three major obstacles ahead: a lack of low-cost stable enzymes as building blocks (such as parts of computers, CPU, monitor, keyboard, mouse), high cost and labile NAD(P) cofactors, and low reaction rates for sugar fuel cell vehicles [25,26]. But it is not necessary to solve the aforementioned three obstacles at the same time before the innovation of SyPaB.

Figure 5 presents the application roadmap of hydrogen/electricity generation mediated by SyPaB from high-end (high-value) applications at the beginning to low-end (low-value) applications in the future. Biohydrogenation catalyzed by enzymes and NAD(P)H is becoming more accepted, especially for the synthesis of chiral compounds in the pharmaceutical industry with a market size of billions of dollars per year [76,77]. Now we have developed a synthetic pathway responsible for the stoichiometric reaction of $(C_6H_{10}O_5)_n + 12\,NADP^+ + 7\,H_2O \rightarrow (C_6H_{10}O_5)_{n-1} + 12\,NADPH + 12\,H^+ + 6\,CO_2(g)$ [51]. The NADPH yield is as high as 11.4 mol NADPH per cellobiose (i.e., 95% of theoretical yield—12NADPH per glucose unit) in a batch reaction [51]. The complete oxidation of biomass sugars for biohydrogenation has the lowest substrate cost ($1.35/kg H_2 added) [74], much lower than the costs of hydrogen generated from natural gas (e.g., ~$2.7/kg H_2) [78,79], ethanol ($2.50/Gal), formic acid ($0.90/kg) [80], phosphite ($1.00/kg) [81,82], and one-NADH regeneration from glucose [35,83,84]. For this application, the SyPaB is very competitive based on substrate costs, whereas the stability of the enzymes and coenzymes are not problematic for the synthesis of high-value products. Furthermore, the use of renewable carbohydrate for NAD(P)H regeneration/biohydrogenation would achieve nearly zero greenhouse gas emissions based on the whole life cycle.

The second high-end application is electricity generation by EFCs (Figure 4b), where clients care more about the performance, such as high energy storage density, user safety, biodegradability, refilling or recharging rate, and so on. It is anticipated that sugar batteries with enhanced power density (e.g., ~5–10 mW/cm^2), prolonged enzyme lifetime (e.g., several months to years), and complete oxidation of sugars (i.e., energy density of ~500–1000 Wh/kg of 20% sugar/water slurry) would be demonstrated within several years. Now Sony and Nokia are developing sugar batteries for portable electronics and cellular phones, respectively. Now the market size of rechargeable batteries for mobile electronics is more than $10bn/year. Therefore, low-cost EFCs based on sugars are expected to be available in the market soon (Table III).

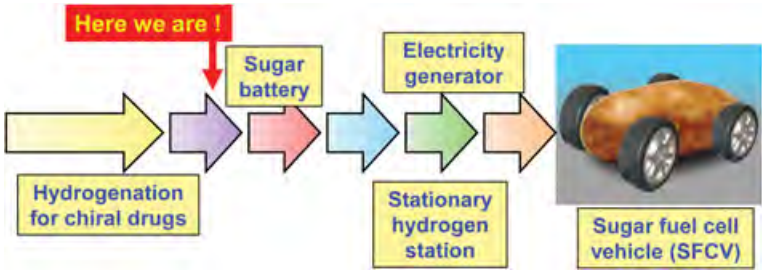

Figure 5. Application roadmap of the carbohydrate-to-hydrogen/electricity technologies.

Table III. Comparison of different electricity generation systems from methanol, carbohydrate, and hydrogen made from carbohydrate.

	DMFC	EFC	PEMFC
Fuel	Methanol	Carbohydrate	Sugary hydrogen
Catalyst	Platinum	Enzymes	Platinum
Catalyst cost	Very high	High → Low	High
Efficiency	Modest	Low modest	High
Power density (mW/cm^2 anode)	Modest (~10–100)	Low modest (~0.1–10)	High (~500–800)
Major application	Portable electronics	Portable electronics	Hydrogen fuel cell vehicles

The first low-end market is local satellite hydrogen generation stations and distributed electricity generator. The hydrogen market size is approximately $20bn in the USA alone [16]. For this application, we need to solve two obstacles—stability of enzymes and use of low-cost biomimetic coenzymes replacing costly and labile NAD (P) cofactors—but accelerating reaction rates is not so important because we can build very large-size bioreactors at low costs, such as waste water treatment facilities. The ultimate hydrogen production costs from sugars would be as low as $1.30/kg, where carbohydrate would account for 90–95% of production costs [26,27].

The most ambitious application is sugar fuel cell vehicles (SFCVs) [6,8]. Four obstacles related with (i) enzymes, (ii) coenzymes, (iii) reaction rates, and (iv) power train system configuration and control must be solved before its implementation. Although so many obstacles seem challenging, huge potential markets (e.g., trillions of dollars per year, including SFCV production as well as the production of sugars and enzymes) mean a strong motivation. It is estimated that 1 kg of sugar through the biotransformer and PEMFC can generate the same kinetic energy as 1.1 kg of gasoline through internal combustion engine [2,55]. But the operation costs for the SFCV [sugar, $0.22/kg sugar, plus enzymes as well as coenzymes (50%, equaling the costs of sugar)] would be less than a half of gasoline ($3.00/Gal). Along with other potential benefits, such as the nearly net-zero carbon emissions, national energy security, and local job creation, SFCV would be the holy grail of the future hydrogen economy. We envision that the SFCV would come true as in the movie 'Back to the Future Part II' eventually.

In a word, hydrogen/electricity generation from renewable carbohydrate mediated by SyPaB may be an out-of-the-box to the hydrogen economy [6]. The obstacles associated with stable enzymes, use of biomimetic coenzymes, and enhancement of reaction rates can be addressed according to the current knowledge and technologies. With regard to unstable enzymes, a number of recombinant (hyper)-thermostable enzymes can be produced by *E. coli*, including the *Clostridium themocellum* phosphoglucomutase [40], *Thermotoga martima* 6PGDH [35], and fructose bisphosphatase [41]. Currently the key recombinant hyperthermophilic NiFe SH1 hydrogenase has been produced in *E. coli* [85]. So all of the enzymes involved in the sugar-to-hydrogen generation can be heterologously produced by low-cost *E. coli* fermentation. In order to decrease enzyme purification costs, simple and scalable protein purification approaches by adsorption/desorption [35,86,87] or heat precipitation [27,40] have been developed. With regard to costly labile cofactors, biomimetic NAD analogs have been used by redox enzymes [47,48,50,88]. With regard to reaction rates, the hydrogen generation rates have been improved by 20-fold during the past 3 years [24] and the further enhancement is on the way.

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