Chapter 8

Hydrogen Production from Carbohydrates: A Mini-Review

Y.-H. Percival Zhang*[,1,2,3]

[1]Department of Biological Systems Engineering, Virginia Tech, 210-A Seitz Hall, Blacksburg, VA 24061, USA
[2]Institute for Critical Technology and Applied Science (ICTAS), Virginia Tech, Blacksburg, VA 24061, USA
[3]DOE BioEnergy Science Center (BESC), Oak Ridge, TN 37831, USA
***Tel: 540-231-7414. Fax: 540-231-7414.**
Email: ypzhang@vt.edu.

The hydrogen economy promises a clean energy future featuring higher energy utilization efficiency and fewer pollutants compared to liquid fuel/internal combustion engines. Hydrogen production from the enriched low-cost biomass carbohydrates would achieve nearly zero carbon emissions in a whole life cycle. In this book chapter, we present latest advances of hydrogen generation from biomass carbohydrates by chemical catalysis (e.g., gasification, pyrolysis, gasification in supercritical water, and aqueous phase reforming), biocatalysis (e.g., anaerobic fermentation, electrohydrogenesis, photo-fermentation, and cell-free synthetic pathway biotransformation – SyPaB), and their combinations. Since hydrogen yield or energy efficiency is the most critical economic factor for hydrogen generation, SyPaB that can produce 12 H_2 per glucose equivalent seems to be an ultimate winner. When more stable enzyme building blocks with total turn-over number (TTN) values of more than 3×10^7 mol of product per mol of enzyme and engineered redox enzymes that can use low-cost stable biomimetic cofactor are available, cell-free SyPaB would produce hydrogen at the overall costs of less than $2 kg of hydrogen.

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Introduction

"What will replace cheap oil -- and when?" was listed as one of top 25 questions by Science Magazine in the year 2005 (*1*). Crude oil is a modern industrial blood, which is converted to affordable liquid fuels (e.g., gasoline, diesel, jet fuel) for the transportation sector, and other derivatives (e.g., plastics, heat oil, and lubricants). Since more than 70% of crude oil is consumed in the transportation sectors, it is vital to find out sustainable transportation fuel alternatives to replace liquid fuels that are usually used in internal combustion engines (ICE).

Combustion of fossil fuels results in net emissions of greenhouse gases, air pollution, as well as concerns of energy security, wealth transfer, trade deficits, and health problems (*2*, *3*). Therefore, reconstruction of a sustainable energy system to remedy the depletion of oil and its negative environmental impacts have become two of the most critical issues to current scientists and engineers (*4*, *5*).

Hydrogen is the most common element in the universe, but there is nearly no dihydrogen source on the Earth. Hydrogen atoms are present in water (most) and other H-containing compounds (e.g., carbohydrate and hydrocarbons). Dihydrogen gas is considered as a promising energy carrier to replace fossil fuel-based liquid fuels, offering advantages through hydrogen fuel cell systems, such as nearly no air pollution, high energy conversion efficiency, diverse primary energy sources, plus recyclable water as a hydrogen source (*6*, *7*).

Low-cost renewable carbohydrate from biomass is the most abundant sustainable bioresource, where terrestrial plants fix CO_2 by using intermittent low energy density solar energy (~170 W/m^2) through photosynthesis. Although plants have low photosynthesis efficiencies of ~0.3% (*2*), the yearly chemical energy (phytobiomass) produced by photosynthesis is nearly six-fold of the total world's energy consumption (*3*). Utilization of a small fraction of renewable biomass carbohydrate would be sufficient to replace crude oil, especially in the transport sector.

Although prices of different energy sources or carriers range greatly, they in an increasing order are carbohydrates ($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), and biodiesel ($27.4 per GJ, $3.5 per gallon). Therefore, it is economically appealing to generate relatively high value hydrogen from low-cost biomass carbohydrates.

Hydrogen Production

Hydrogen can be produced from biomass carbohydrate by chemical catalysis featuring harsh reaction conditions, biocatalysis featuring modest reaction conditions, and their combinations (Fig. 1).

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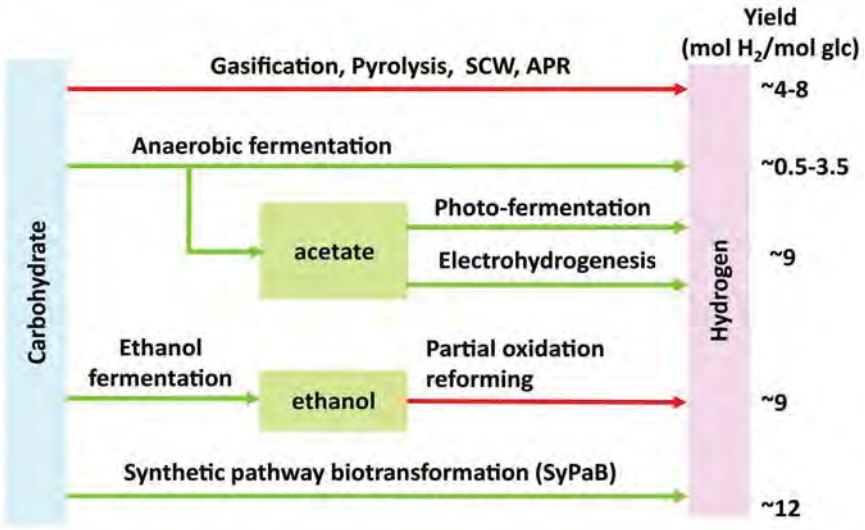

Figure 1. Comparison of different approaches to generating hydrogen from biomass carbohydrates.

Chemical Catalysis

Chemical catalysis for hydrogen generation can be classified based on a decreasing temperature order – gasification, pyrolysis, gasification in critical water, and aqueous phase reforming (APR). All of them suffer from relatively low yields of hydrogen (e.g., ~ 6-8 hydrogen per glucose).

Gasification is usually operated at the temperature above 1000 K and in the presence of oxygen and/or water. Coupled by water-gas shift reaction, gasification is more favorable for the production of hydrogen than pyrolysis (*8*). The gas generated from biomass gasification generally contain contaminants, such as particulates, ashes, alkali compounds, nitrogen-containing components, sulfur, and low-molecular-weight hydrocarbons (e.g. methane and ethane) (*9*). The need to remove the contaminants from the gas steam depends on the end use of the gas. Several reports and reviews are available (*9–12*). The integrated process containing an air-blown bubbling fluidized bed gasifier, a steam reformer, and a water-gas-shift membrane reactor has been reported to completely decompose tar and produce ultrapure hydrogen through biomass gasification (*13*, *14*).

Pyrolysis is chemical decomposition of a condensed substance induced by heat without oxygen (*9*). Pyrolysis of coal and biomass (primarily wood) was popular for producing fuel-related gas and smokeless solid fuel, e.g. charcoal, from 1700s to early 1900s (*15*). Now it is applied to convert biomass into syngas, to produce coke from coal, and to treat hazardous wastes. The product yield and composition through biomass pyrolysis depend on reaction temperature, heating rate, and particle size of biomass (*16*, *17*). High temperatures promote gas production, while lower temperatures favor the formation of char and tar (or heavy oil) (*18*). In the rapid heating experiments, the rapid removal of volatiles

from the reactor prevents the formation of secondary char and attributes to the higher volatile yield and lower char yield.

Gasification in supercritical water (SCW) is conducted at pressures and temperature above the critical point (221 Bar and 647 K) of water to supercritical conditions. Different from regular gasification that works on biomass with moisture contents of ~10-20% (*19*), SCW can gasify wet biomass with a moisture content of more than 35%. It is promising to gasify wet biomass because of the high gasification (100% conversion) and hydrogen ratios (50 vol %) (*20*, *21*).

Aqueous-phase reforming (APR) is reforming under relatively high temperature (400-550 K) and high pressure (50-70 bar), where the reactions occurs in an aqueous phase (*22*). In 2002, Dumesic *et al.* firstly demonstrated that hydrogen can be produced from biomass-derived carbohydrates at temperatures near 500 K in a single-reactor by using a platinum-based catalyst (*23*). The advantages of APR over vapor-phase reforming of oxygenated carbohydrates are (i) favorable water-gas shift reaction under the reaction conditions, generating hydrogen with low levels of CO (around 100 ppm); (ii) major energy savings from separation of gas hydrogen and aqueous water; (iii) reduction in undesirable decomposition reactions when carbohydrates are heated under modest conditions; and (iv) a better control of the performance of the catalytic process. The disadvantage is leaching and destability of catalyst components into the aqueous phase. Although APR process may be attractive to produce H_2 from carbohydrates, hydrogen formed had very low yields (1.05-1.41 mmol per gram of carbohydrates) to date owing to formation of coke and by-products (*24*).

Biological Catalysis

Biological catalysis is mediated by microorganisms or enzymes at ambient temperature and around atmospheric pressure. Biocatalysis has advantage over catalysis, such as higher selectivity, lower energy input, and less costly bioreactor (*25*, *26*). But it suffers from lower reaction rates. For high-water content organic resources, such as wastewater, sewage sludge, etc., biological approaches mediated by microorganisms is the only cost-efficient way to produce hydrogen (*27*). Hydrogen can be produced by dark (anaerobic fermentation), light fermentation, their combination, microbial electrohydrogenesis, and cell-free synthetic enzymatic pathway biotransformation (SyPaB).

Most of biohydrogen or methane in nature is produced from carbohydrates or their metabolic products by anaerobic microorganisms without light -- dark fermentation. In principle, one mole of glucose can produce four moles of hydrogen and two mole of acetate or two hydrogen and one butyrate through the mixed acid pathway, called the Thauer limit (*28*). In practice, hydrogen yields are much lower than this theoretical yield (4 H_2/glucose). High H_2 yields are usually associated with acetate production, and low yields are related with the production of propionate or other reduced end products, like alcohols and lactic acid (*29*). *Enterobacter*, *Bacillus*, and *Clostridium spp. (e.g. Clostridium pasteaurianum*, *C. butyricum*, and *C. beijerinki)* are well-known species to produce biohydrogen (*30*).

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Due to poor hydrogen yields, dark-fermentation is far from practical solutions to hydrogen production based on relatively pure carbohydrate but is operative to produce hydrogen from waste water (*31*). In order to achieve the Thauer limit (4 mol H_2 per mol glucose), hydrogen production should be conducted under very low partial pressures of H_2 with very slow rates (*25*, *32*). In order to get close to the theoretical yield, intensive efforts have been made through process optimization, reactor design, and metabolic engineering (*25*, *33*, *34*).

To increase overall hydrogen yields, acetate can be converted to hydrogen by electrohydrogenesis or photo-fermentation (Fig. 1). Since hydrogen generation from acetate is thermodynamically unfavorable, extra energy needs input. Electrohydrogenesis is a process in which exoelectrogenic bacteria generate protons and electrons in modified microbial fuel cells by using chemical energy in acetate plus a small amount of electric energy (*35*). Experimental results show that hydrogen production is dependent on the voltage supplied. The minimum voltage for hydrogenesis is 0.11 V at the cathode based on the thermodynamic analysis. The yields are 2.01-3.95 mol H_2 per mol acetic acid by applying the voltages of 0.2 to 0.8 V. The overall yields are 8.55 mol H_2 per mol glucose on glucose and 8.20 mol H_2 per mol hexose equivalent of cellulose. The corresponding hydrogen production rates were 2.29 mmol/L/h and 0.20 mmol/L/h for glucose and cellulose, respectively (*35*). It is worth noting that capital investment for microbial fuel cells may be too high to prevent its scale-up as compared to mature anaerobic digestion (*36*).

Alternative, photosynthetic bacteria can utilize organic acids plus solar energy for H_2 production (*37*, *38*), but very slow hydrogen generation rates (e.g., one order of magnitude lower than those of dark fermentation) and high hidden costs in hydrogen collection from a non-point (large area) source may prevent it from potential applications. Integration of multiple processes lead to more challenges in reactor engineering, system design, process control, and operation and maintenance (*39*). For example, in order to obtain a maximum utilization of the substrate, the system should be well-controlled to provide optimum media composition and environmental conditions for the two microbial components of the process (*40–42*) . For example, ammonia concentration and C/N ratio in the effluent from the first stage should not inhibit the hydrogen production in the second stage (*41*, *43*). Dilution and neutralization is thus required before photo fermentation to adjust the organic acid concentration and the pH level (*42*). Other challenges include i) adjusting photosynthetic and respiration capacity ratio, ii) co-culture balance, and iii) pretreatment of cell biomass from dark fermentation for photo fermentation (*39*).

Cell-free synthetic enzymatic pathway biotransformation (SyPaB), a new direction of synthetic biology or *in vitro* metabolic engineering, is implementation of complicated biological reaction network by *in vitro* assembling a number of enzymes and coenzymes (*6*, *7*, *44*). To break the Thauer limit for hydrogen-producing microorganisms, the synthetic enzymatic pathways have been designed to produce 12 moles of hydrogen per mole of glucose equivalent of glucan (starch or cellulose) and water (Fig. 2a) (*45–47*). The reconstituted non-natural catabolic pathways degrade polysaccharides initially to glucose 1-phosphate (g1p) and eventually to CO_2, split water and finally release the

chemical energy in the form of hydrogen gas. These processes are like catabolism where water rather than oxygen works as an oxidant receiving electrons and generates hydrogen and CO_2 (*46*).

The pathways contain five sub-modules: (i) polysaccharide or oligosaccharide conversion to glucose-1-phosphate (g1p) catalyzed by phosphorylases, (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 eight enzymes of the non-oxidative phase of PPP, glycolysis and gluconeogenesis pathways, and (v) hydrogen generation from NADPH catalyzed by hydrogenase. The overall carbohydrate-to-hydrogen reaction can be summarized as

$$C_6H_{10}O_5\ (aq) + 7\ H_2O\ (l) \rightarrow 12\ H_2\ (g) + 6\ CO_2\ (g) \qquad [1]$$

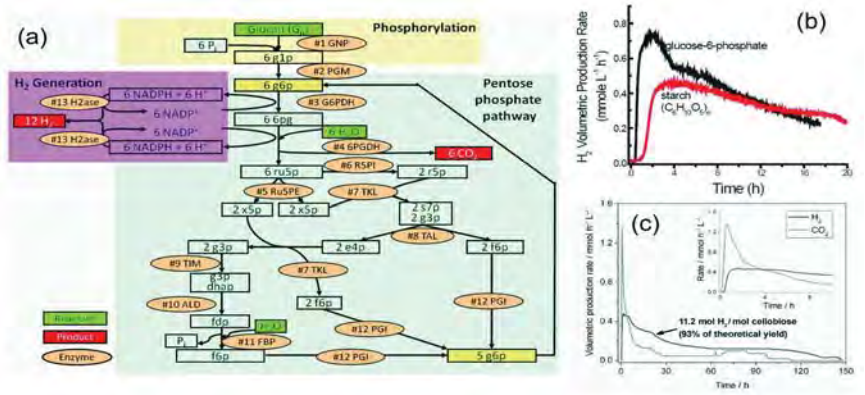

Figure 2. The cell-free synthetic pathway for high-yield hydrogen generation (a), high-yield generation of hydrogen from starch (b) (47) or soluble cellodextrin (c) (45). The enzymes are: GNP, glucan phosphorylase; PGM, phosphoglucomutase; G6PDH, G-6-P dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; 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-diphosphate; f6p, fructose-6-phosphate; and Pi, inorganic phosphate.

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Thermodynamic analysis suggests that the overall reaction (Equation 1) is spontaneous ($\Delta G°$ = -48.9 kJ/mol) and endothermic ($\Delta H°$ = +596 kJ/mol) (*45*, *47*). This enzymatic reaction is among rare entropy-driven chemical reactions because two final products are gaseous under experimental conditions (<100°C and ~1 atm) (*45*). Great increases in the entropy from aqueous to gas phases enable the negative-enthalpy reactions to occur. To our limited knowledge, the reactions (Equation 1) may be the first chemical reaction that can generate hydrogen energy by absorbing waste heat.

We have demonstrated the feasibility of high-yield spontaneous generation of hydrogen from starch or cellulosic materials and water in batch reactions (Fig. b&c) (*45*, *47*). It is expected that 100% product yield (i.e., 12 H_2/glucose equivalent) is achieved in a continuous reactor. During the past three years, we have increased the reaction rates by nearly 20-fold through optimization of rate-limiting enzyme loadings, increasing substrate concentrations from 2 to 8 mM, and elevating reaction temperatures slightly from 30 to 32°C. The current production rate of H_2 is 3.92 mmol H_2/L/h (*45*), higher than those of photobiological systems and comparable to those reported in dark fermentations and electrohydrogensis (*4*, *35*).

A Combination of Catalysis and Biocatalysis

Another carbohydrate-to-hydrogen production process is a hybrid of biological and chemical catalysis, both of which have high selectivity under their conditions. First, polysaccharides are hydrolyzed to glucose by using hydrolases, such as amylases and cellulases (*48*, *49*). Then ethanol-producing yeasts or bacteria can convert glucose to ethanol with nearly theoretical yields (i.e., two ethanol per glucose) (*48–51*). Alternatively, consolidated bioprocessing (CBP) microorganisms that can produce cellulase, hydrolyze cellulose, and ferment ethanol can convert solid cellulose to ethanol in a single step (*50*, *52–55*). Second, ethanol after distillation can be converted to hydrogen by partial oxidation reforming (*56*, *57*). But the reformed product still contain a small amount of CO, which must be removed before entering proton exchange membrane fuel cells. The overall theoretical hydrogen yield of this hybrid is 10 H_2 per glucose. But considering energy conversion losses (e.g., carbohydrate use for cell mass synthesis, and partial oxidation reforming), the practical hydrogen yield through this hybrid is approximately nine hydrogen per glucose.

Opportunities and Obstacles of SyPaB

Among different carbohydrate-to-hydrogen technologies, SyPaB is the only way that can produce nearly theoretical hydrogen yield. SyPaB is a new direction of synthetic biology. Synthetic biology applies engineering principles (e.g., design, extraction, and standardization) and combines science (biology and chemistry) for designing and building novel biological functions and systems that function unnaturally or function much better than natural counterparts. Synthetic biology can also be interpreted as the engineering-driven building of increasingly

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complicated biological entities (parts, devices, and systems) from simple and basic building blocks. The design principles of cell-free synthetic biology are so clear that we are able to assemble a new system much more easily than to modify a living system without constraints from cellular viability, complexity, physiology, and the presence of membranes and /or walls (*44*).

Microbial fermentation is different from SyPaB in that microbes can duplicate themselves but enzymes cannot. Since microbes can self-duplicate and self-repair, the costs associated with microbe production are low and there is no cost for enzyme separation, stabilization, and co-factors. In contrast, SyPaB requires production, purification, and stabilization of enzymes based on microbial fermentation as well as the addition of costly co-enzymes.

Figure 3 shows comparison of microbial fermentations and SyPaB for biofuel production. A typical one-step microbial fermentation where the formation of product is associated with cell growth (Fig. 3a), for example, production of membrane lipids from carbohydrate (*58*). When the formation of desired product is not dissociated with cell growth, two-step microbial fermentation is usually conducted. For example, ethanol fermentation can be carried out in two steps: first, cell growth under aerobic conditions; second, ethanol production under anaerobic conditions. SyPaB may be regarded as atypical two-step fermentation. In the first step, several mesophilic microorganisms (e.g., *E. coli*) are cultivated separately for producing recombinant high-yield thermostable enzymes. After cell lysis, thermostable enzymes can be purified by low-cost approaches, such as, simple adsorption (*59*, *60*) or heat precipitation (*61*) because most of the *E. coli* cellular proteins, which are not stable at high temperature, can be precipitated by heat treatment (*44*). In the second step, numerous purified enzymes are reconstituted for high-speed biotransformation. If necessary, thermostable enzymes may be immobilized for higher turn-over number (TTN, mol product/mol enzyme) and/or better product/enzyme separation. Because the enzymes have several orders of magnitude total turn-over number higher than those of microbes (*6*, *7*), microbial fermentation-SyPaB would show economically advantageous over two-step microbial fermentation in long-term operation.

Production costs for hydrogen through SyPaB are mainly based on three major cost components – carbohydrate, enzymes, and coenzyme (NAD). Figure 4a shows the effects of the costs of enzyme ($40 or $4000/kg enzyme) on hydrogen production costs. The cost decreases rapidly with increasing total turn-over number (TTN, mol product per mol of enzyme) of the enzymes in SyPaB, and then levels off when all enzymes regardless of their production costs have TTN values of more than $10^{8\text{-}9}$. When all enzymes have TTN values of 3×10^7 and each one has production costs of $~40/kg, hydrogen production cost is anticipated to be $1.87 per kg H_2, where carbohydrate ($0.18/kg carbohydrate) accounts for approximately 66% of hydrogen production costs. 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 per kg H_2. The above hydrogen production by SyPaB would be lower than its generation from natural gas (e.g., $2.00-2.70 per kg hydrogen).

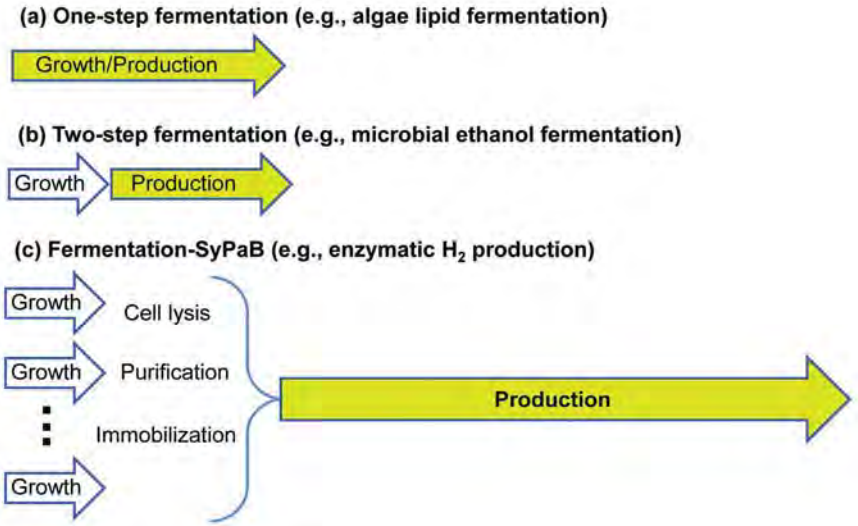

Figure 3. Comparison of microbial fermentation and cell-free synthetic pathway biotransformation.

The above economical analysis is based on two important assumptions: (i) enzyme production costs and (ii) enzyme TTN values. Typical industrial enzymes have production costs from $~5-40/kg dry protein weight, for example, protease, cellulase, amylase and so on. Fig. 4b shows typical TTN values of the enzymes in industrial applications and obtained in our laboratory. Very low TTN values for cellulase result in poor economical viability of biomass saccharification (*6*). Amylase has much higher TTN values than cellulase so that the enzymes costs are much more lower in starch ethanol biorefineries. For fructose production from glucose, ultra-stable immobilized themophilic glucose isomerase leads to enzyme costs to minimal levels. In our laboratory, we have obtained three thermostable enzymes with TTN values of more than 10^7, for example, *Clostridium thermocellum* phosphoglucomutase (*62*), *Thermotoga maritima* fructose-1,6-bisphosphatase (*63*), and *T. maritima* 6-phosphogluconate dehydrogenase (*61*). It is found that free *C. thermocellum* phosphoglucose isomerase has low TTN values but it becomes ultra-stable (more than 10^9) after simple immobilization through adsorption on cellulose surface by using cellulose-binding module (*64*). Clearly, it is highly operative to obtain numerous high-TTN non-membrane enzymes suitable for biocommodity production by using SyPaB.

With developments in (i) engineered oxidoreductases that can use biomimetic NAD factors (*65–67*) and (ii) stable enzymes as building blocks of SyPaB (*61–64*, *68*), we estimate that hydrogen production costs may decrease to ~$1.30 per kg of hydrogen (Fig. 4), where carbohydrate accounts for ~95% of its production costs.

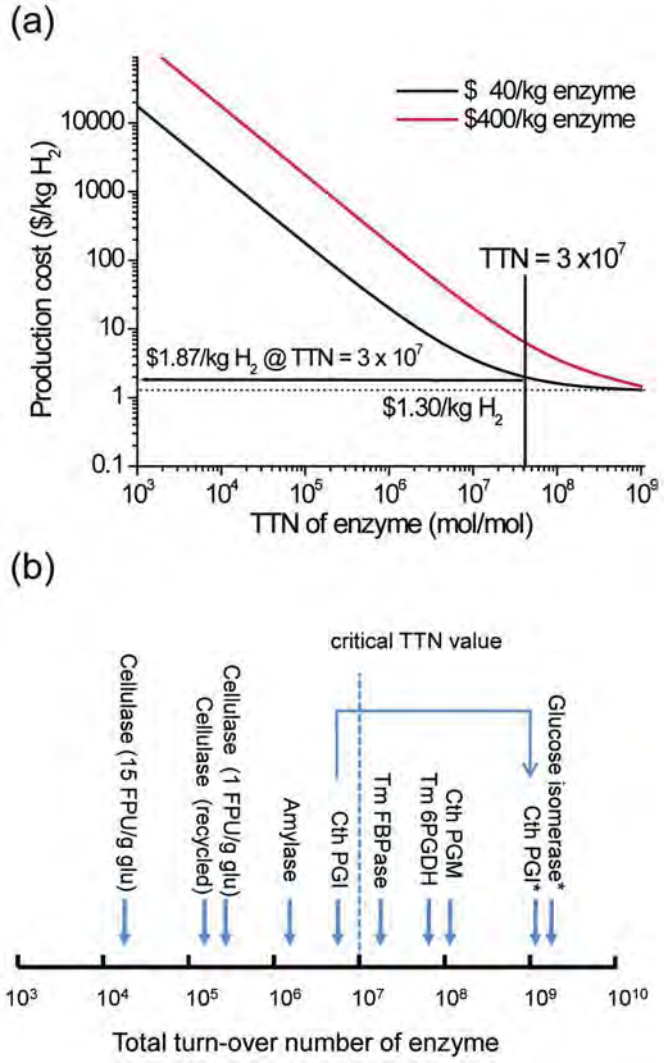

Figure 4. Hydrogen production cost analysis (a) and typical total turn-over number (TTN) values of enzymes.

Concluding Remarks

Hydrogen production costs are highly based on its yield on carbohydrate. The USA DOE report has set a bottom conversion goal at 50% efficiency (e.g., 6 H_2/glucose) for hydrogen production from biomass (*69*). This mini-review presents different yields for different carbohydrate-to-hydrogen technologies (Fig. 1). Several new technologies have been achieved for producing more than 8 mol H_2 per mol hexose equivalent. Among them, SyPaB is highly promising because of its highest yield (plus extra hydrogen generation by utilizing waste heat),

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modest reaction condition, acceptable reaction rates, and low-cost bioreformers or bioreactors because in long terms thermodynamics (efficiency) decides economics (i.e. cost) (*58*, *70*). The obstacles to commercial hydrogen production by SyPaB – (i) a lack of thermostable enzymes and (ii) modifying oxidoreductases that can work on low-cost and stable biomimetic cofactors – are being addressed through international collaboration. In the past, strong motivations have driven to discover and engineer thermostable enzymes with obvious applications, such as DNA polymerase, amylase, glucose isomerase, cellulase, and so on. When the concept of SyPaB is accepted and more stable enzyme building blocks and stable biomimetic cofactor analogues are available, cell-free SyPaB would compete with microbial fermentations for the production of low-value biocommodities (Fig. 3).

In addition to high-yield hydrogen generation, this carbohydrate-to-hydrogen technology by SyPaB would address more challenges associated with the hydrogen economy, such as, storage, safety, distribution, and infrastructure of the hydrogen economy (*3*, *44*, *47*). But in short terms, hydrogen production through catalysis based on lignocellulosic biomass or dark fermentation based on waste may be more practical.

Acknowledgments

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