# Replacing Crude Oil by Renewable Sugar through a Cell-Free Synthetic Biology Technology

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## ABSTRACT

Hydrogen holds great promise as a future transportation fuel, but problems with the storage and distribution of hydrogen gas make current schemes economically infeasible. Here we discuss the use of carbohydrates as a hydrogen carrier. This is possible through the use of an enzymatic synthetic pathway biotransformation (SyPaB), which produces hydrogen gas from a variety of renewable sugars. Other advantages of this conversion method include a compact size, moderate operating conditions, and greatly increased safety. Once enhancements such as improved enzyme stability and biomimetic cofactor use have been accomplished, SyPaB hydrogen has tremendous potential for use in the transportation sector, due to the unmatched yeild and enthalpic conversion efficiency of this process.

***Keywords***: biomass, synthetic biology, hydrogen storage, hydrogen carrier, synthetic pathway biotransformation

## 1 INTRODUCTION

A central question of the coming sustainability revolution is, "What will replace oil and when?" Since approximately 70% of crude oil is used for the transportation sector in the US, this question could be interpreted as, "what renewable energy source will be sufficient to drive tomorrow's vehicles?" Several special requirements for the transportation sector include: high energy storage capacity in a small container (e.g., ~50 liters), high power output (e.g., ~20-100 kW per vehicle), affordable fuel costs (e.g., $~20/GJ), affordable vehicles, low costs for rebuilding the relevant infrastructure, fast charging or refilling of the fuel, and safety. In this presentation, we will discuss a novel means of meeting these criteria – the sugar hydrogen fuel cell vehicle (sugar car).

The enabling technology for a hydrogen fuel cell vehicle that uses sugar as an energy storage medium is the cell-free synthetic enzymatic pathway biotransformation (SyPaB). SyPaB is the implementation of complicated biochemical reactions by in vitro assembly of numerous enzymes, called an enzyme cocktail. SyPaB has clear advantages over microbial fermentation, such as a high product yield, faster reaction rate, easy control, and so on.

The sugar car concept solves several of the major challenges of hydrogen fuel cell vehicles. In the place of current cumbersome, hazardous hydrogen containment systems, the SyPaB fuel cell vehicle requires only a small sugar container and an on-board bioreformer containing the enzyme cocktail. A biomass-to-wheel efficiency analysis we conducted suggested that the sugar car would have similar efficiency to battery electric vehicles and nearly four times the efficiency of cellulosic ethanol-internal combustion engines. A number of obstacles to commercialization of SyPaB and the sugar car are currently being addressed by international collaborators. If SyPaB were fully implemented, a feasible fraction of US biomass resources (approximately 5%) would be sufficient to replace crude oil in the light duty transportation sector.

## 2 HYDROGEN FROM BIOMASS

Carbon-neutral hydrogen gas is the best future energy carrier, especially for the transportation sector. This is due to the high efficiency of fuel cell vehicles and avoidance of non-point pollutats. Low-cost hydrogen can be produced

| Method | Theoretical Yeild | Practical Yeild | Chemical Energy of $H_2$ Out / Chemical Energy of Sugar In |
|---|---|---|---|
| Dark Fermentation (DF) | 4 | 1-3.2 | 10-30% |
| DF + Electricity-assisted microbial fuel cell | 12 | 9 | ~75% |
| Ethanol fermentation / partial oxidation reforming | 10 | 9 | ~60% |
| Gasification | 12 | 2-8 | 35-50% |
| Pyrolysis | 12 | 2.5-8 | 30-50% |
| Hydrolysis + Aqueous-phase reforming | 12 | 6-8 | 30-50% |
| Synthetic pathway biotransformation | 12 | ~12 | ~122% |

Table 1: Comparison of various methods for the conversion of carbohydrates to hydrogen [8].

from abundant, renewable lignocellulosic biomass through numerous methods, employing chemical catalysis, biocatalysis or a combination of both, but these technologies suffer from low hydrogen yields (far below the theoretical yield of 12 $H_2$ per glucose) and undesired side-products and/or severe reaction conditions [1]. Biofuels (e.g., ethanol, hydrogen, butanol) are low-value products where the cost of carbohydrates often accounts for ~50-70% of the price. Therefore, the yield of any biofuel-producing process is the most important cost factor [2]. See Table 1 for a comparison of the yeilds and enthalpic efficiencies for a variety of hydrogen generation methods.

## 2.1 Synthetic Pathway Biotransformations (SyPaB)

Our method of converting sugars to hydrogen is unlike any other. Harnessing the advantages of cell-free enzymatic conversion, SyPaB allows both a high level of engineering control and the high selectivities and yeilds of biological systems.

We have demonstrated the most efficient way to produce hydrogen from polysaccharides and water: $C_6H_{10}O_5$ (aq) + 7 $H_2O$ (l) → 12 $H_2$ (g) + 6 $CO_2$ (g) [3, 4]. The pathway that catalyzes this conversion is included as Figure 1. This pathway uses 13 enzymes and the NADPH cofactor, harnessing the pentose phosphate pathway to fully oxidize glucose and produce the maximum number of hydrogen molecules per glucose unit.

Furthermore, because the overall biochemical reaction is spontaneous but endothermic (i.e., entropy-driven), ambient thermal energy is effectively converted to a useful form: chemical energy output (hydrogen) / input(carbohydrate) > 1. This is possible in part because gaseous products are evolved from solid and liquid reactants, causing a large increase in entropy.

## 2.2 Sugar Car

The most far-reaching application for SyPaB hydrogen production is on-board a fuel cell vehicle. Fuel cell vehicles have great advantages in efficiency and lack of tailpipe emissions, but major problems have prevented widespread use. A few of the challenges to be overcome include expensive and dangerous onboard hydrogen storage and a prohibitively expensive infrastructure cost. In

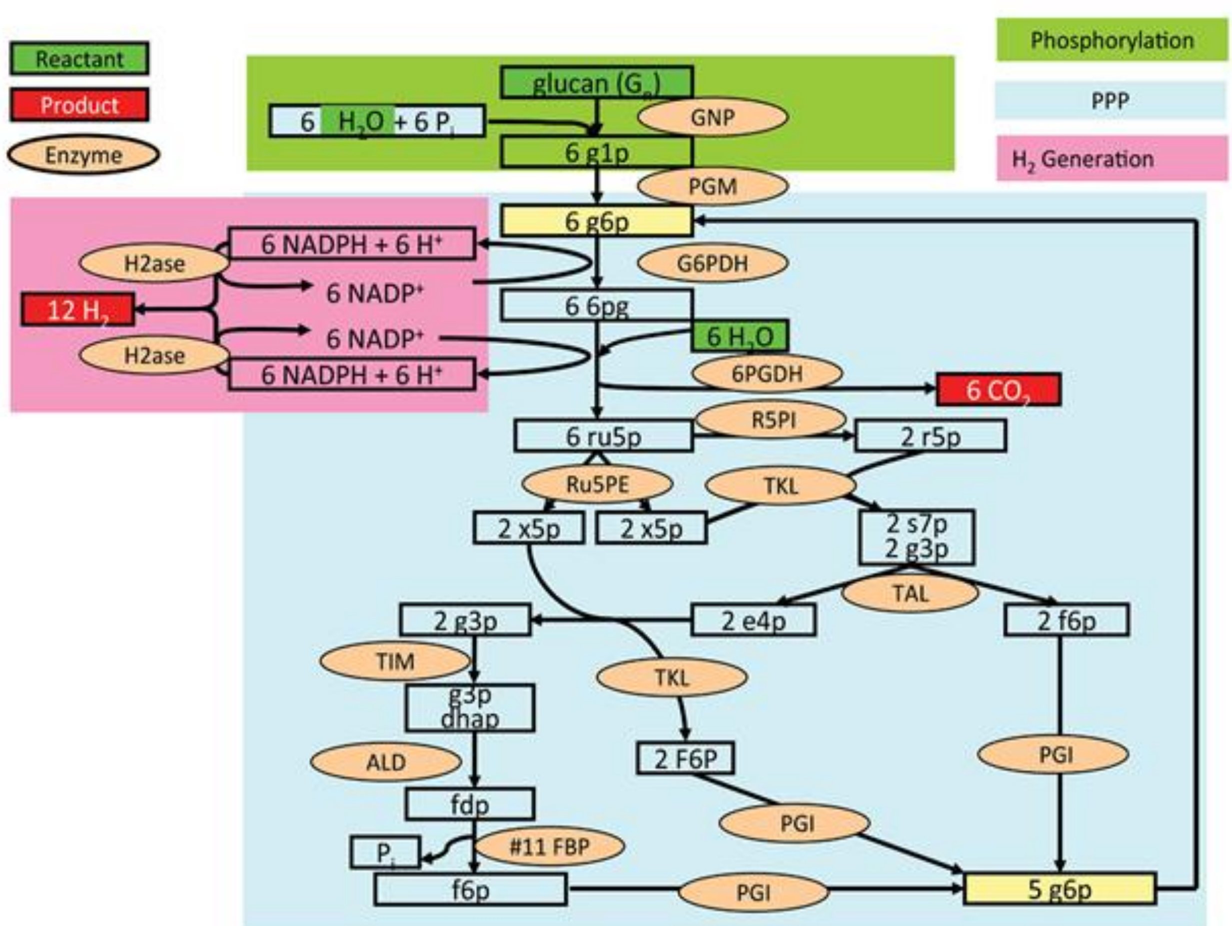


Figure 1: Enzymatic conversion of cabohydrates (glucan) to hydrogen gas, using a series of 13 enzymes. Detailed information is available in references [3] and [4]. PPP in the figure legend above stands for the Pentose Phosphate Pathway.


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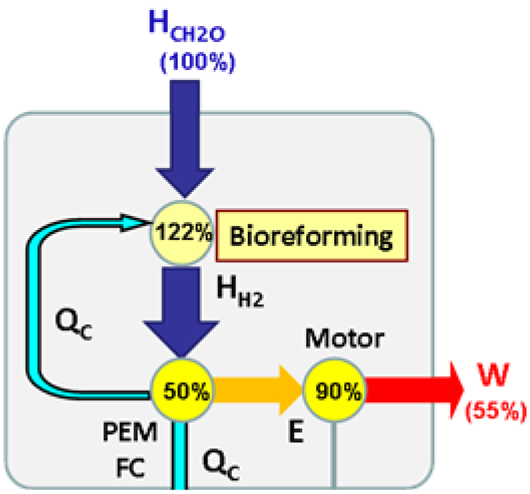


Figure 2: Diagram of the hydrogen fuel cell vehicle. Efficiencies labelled are enthalpic; bioreforming is >100% due to use of waste heat from the fuel cell stage [6].

addition, hydrogen is currently generated from fossil sources, meaning that these vehicles remain net carbon producers. An onboard SyPaB bioreformer would allow hydrogen to be generated when needed, in a safe compact manner. Fuel sugar could be distributed via networks analagous to current grain infrastructure; such sugars are also completely stable, non-toxic and without any specialized storage requirements. Finally, sugars produced from biomass as envisioned would mitigate any potential food versus fuel conflict.

When combined with current proton exchange membrane (PEM) fuel cells and an electric motor, the SyPaB bioreformer completes a highly efficient conversion system. A diagram of this is shown in Figure 2.

We have also conducted a comparison study to examine the conversion efficiency of biomass to kinetic energy, for several biomass utilization routes. The findings suggest that ethanol is a reasonable intermediate fuel, but that ultimately the unique thermodynamics of the hydrogen-evolving SyPaB reactions will enable efficiencies as high as battery electric vehicles (BEVs) [7].

The energy storage density of carbohydrates is more than an order of magnitude higher than the most futuristic battery. For this reason, long-distance travel with BEVs is not feasible. Based on the reaction outlined above, sugars used in the SyPaB process have an effective hydrogen storage density of 14.8 $H_2$ mass%, which is also superior to any other current hydrogen storage technology [8]. In addition, due to the use of PEM fuel cells, the power output of a SyPaB fuel cell vehicle has the potential to be in the 10 kW range or greater, more than enough to power a passenger vehicle moving at highway speeds.

## 2.3 Scalability

One of the major concerns regarding the scalability of cell-free SyPaB is the potential cost of using an in vitro enzymatic system to produce a biocommodity such as hydrogen. However, there are several current industrial examples of low-cost enzymatic conversion. Protease enzymes have been produced on a large scale for years, as a major component of laundry detergents, and the food processing industry currently uses engineered thermostable glucose isomerase enzymes to produce high-fructose corn syrup. The key to a similarly viable cost for SyPaB-produced goods would be the development of low-cost, stable building blocks (enzymes or their complexes) and biomimetic cofactor analogues [1]. Other process improvements, such as coenzyme recycling, immobilization, and engineered substrate channeling, would improve SyPaB economics further, resulting in low final production costs. The ultimate SyPaB hydrogen production cost is estimated to be ~1.5 dollars per kg of hydrogen, based on sugar prices of ~0.18 dollars per kg [6]. rices of ~0.18 dollars per kg [6].

# 3 ADDITIONAL SYPAB APPLICATIONS

The SyPaB platform has the potential to produce a variety of useful biocommodities with great efficiency. Figure 3 shows three of the possible applications. In addition to vehiclular use discussed thus far, stationary biohydrogen generation and stationary power generation are important uses of the SyPaB pathway presented here. In addition, slight modifications to the pathway enable adaptation of the system for the production of electricity directly (Figure 3), or for biohydrogenation, of potential use in the preparation of feedstocks for aqueous-phase reforming, or for biopharmaceutical production [2].

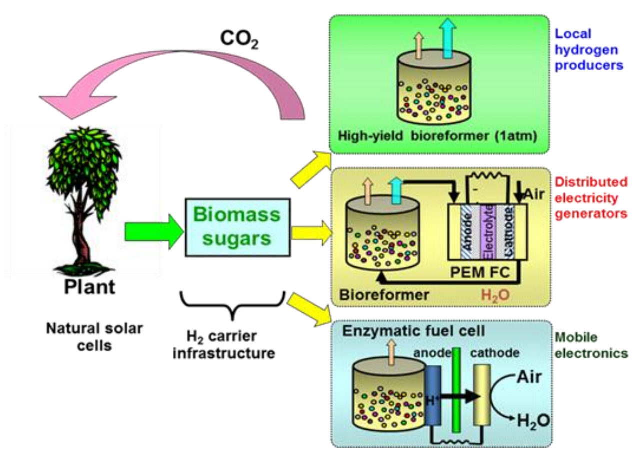


Figure 3: Various SyPaB conversion routes for producing hydrogen and electricity from biomass sugars [5].

## 4 CONCLUDING REMARKS

The production of commodities fuels and chemicals by engineered, synthetic enzymatic pathways is an emerging field with great promise. The SyPaB technology is a platform capable of a variety of industrially useful conversions; due to the unique thermodynamics of SyPaB hydrogen production, this application has particularly high potential. We are currently pursing both industrial partners and academic collaborators, to continue the development of this extremely promising technology.

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