PD127 – Sweet Hydrogen: High-Yield Production of Hydrogen from Biomass Sugars Catalyzed by in vitro Synthetic Biosystems

Biological Systems Engineering

Virginia Tech — Invent the Future

Prof. Dr. Y-H Percival Zhang [1] **Prof. Dr. Mike WW Adams** [2]

[1] Biological Systems Engineering Dept, Virginia Tech, Blacksburg, VA 24061

[2] Biochemistry and Molecular Biology Dept., University of Georgia, Athens, GA 30602

E-mail: ypzhang@vt.edu, Phone: 540-231-7414

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Abstract

In vitro synthetic biosystems (IVSB) emerges as a new biomanufacturing platform by the in vitro assembly of numerous (stable) enzymes and/or (biomimetic) cofactors for the implementation of complicated reactions. We demonstrated the high-yield and high-speed hydrogen production from biomass sugars (glucose and xylose) and water by using IVSB [Ref. 1]. The stoichiometric reaction is CH_2O (sugar, L) + H_2O (L) → 2 H_2 (g) + CO_2 (g).

In the next two-year project supported by EERE, we will demonstrate the enzymatic hydrogen production on a liter scale and presents solid data to achieve the Fuel Cell Technologies Office's hydrogen production goal -- (distributed) hydrogen production at a cost of <$2/kg of hydrogen. We have three objectives in this project: (1) decreasing the production costs 1000-fold from $~10,000/kg (current estimated level) to $~10/kg of hydrogen estimated by using the H2A model by the end of the project; (2) increasing the volumetric productivity five-fold from current levels of ~150 mmol H_2/L/h to 750 mmol H_2/L/h, and (3) scaling up in vitro enzymatic hydrogen production 1000-fold from 1-mL to 1-L bioreactor.

Low-cost distributed green hydrogen could be produced from evenly-distributed renewable carbohydrate sources and carbohydrate would become a novel off-board or even on-board hydrogen storage compound (> 8% wt/wt H_2).

Novel Biomanufacturing Platform: IVSB

IVSB, without the constraints of living microorganisms (e.g., energetics, membrane, self-duplication), can implement complicated reactions that microbes and chemical catalysts cannot do. This platform features numerous advantages:

- high product yield without side pathways and cell mass synthesis,
- fast volumetric productivity without cell membrane,
- simple product separation from aqueous solution,
- easy access and control for an open system by optimizing enzyme ratios,
- tolerance of toxic compounds without labile cell membrane & a few sensitive enzymes,
- broad reaction conditions, and so on [**Ref. 2**].

Via it, we can implement a few reactions that microorganisms and chemical catalysts cannot do before, for example, the highest yield hydrogen from sugars (here), biotransformation of nonfood cellulose to starch and ethanol without sugar losses (PNAS 2013, 110: 7182), and the highest energy density biobattery (Nature Communications 2014:5:3026).

When enzyme building blocks are stable and biomimetic cofactor replaces natural NAD(P), the biosystems could produce a number of products at very low biomanufacturing costs. Sweet hydrogen production cost is estimated to be as low as $2.00/kg H_2 (see **Fig. 2, Ref 2**). Furthermore, sugar could be a new hydrogen carrier, better than methanol [**Ref. 3**].

Previous Work: High-Yield and High-Speed H_2 Production

Glucose and xylose are two most abundant sugar components of biomass. We designed the synthetic enzymatic pathway for co-utilization of biomass sugars (**Fig. 1**). The experimental data clearly suggested that both glucose and xylose from biomass were simultaneously converted to H_2 with a yield of two H_2 per carbon, the maximum possible yield [**Ref. 1**].

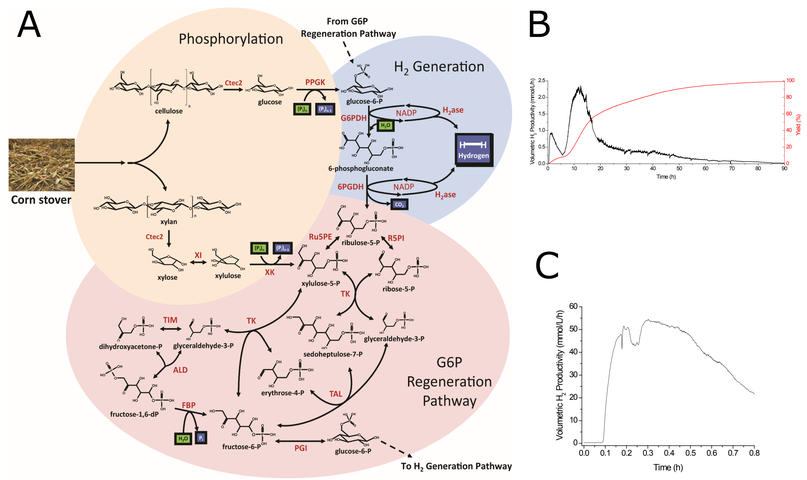


Fig. 1. In vitro synthetic pathway for co-utilization of biomass sugars (A) and nearly theoretical yield hydrogen production from pretreated biomass sample (B) and enhanced volumetric productivity (C) [1].

To increase reaction rates and prolong enzyme lifetimes, we obtained the first set of thermostable enzymes (**Table 1**). By elevating reaction temperature and optimizing enzyme ratios based on kinetic modeling, we increased hydrogen production to ~150 mmol H_2/L/h, among the highest biohydrogen production rates reported.

Table 1. Thermophilic enzymes used in sweet hydrogen production [Ref 1].

Enzyme	Abbreviation	EC no.	Source
Polyphosphate glucokinase	PPGK	2.7.1.63	*Thermobifida fusca*
Xylose isomerase	XI	5.3.1.5	*Streptomyces murinus*
Xylulokinase	XK	2.7.1.17	*Thermotoga maritima*
Glucose 6-phosphate dehydrogenase	G6PDH	1.1.1.49	*Geobacillus stearothermophilus*
6-phosphoglucanate dehydrogenase	6PGDH	1.1.1.44	*Moorella thermoacetica*
Ribose 5-phosphate isomerase	R5PI	5.3.1.6	*T. maritima*
Ribulose 5-phosphate epimerase	Ru5PE	5.1.3.1	*T. maritima*
Transketolase	TK	2.2.1.1	*Thermus thermophilus*
Transaldolase	TAL	2.2.1.2	*T. maritima*
Triose phosphate isomerase	TIM	5.3.1.1	*T. thermophilus*
Aldolase	ALD	4.1.2.13	*T. thermophilus*
Fructose-1,6-bisphosphatase	FBP	3.1.3.11	*T. maritima*
Phosphoglucose isomerase	PGI	5.3.1.9	*Clostridium thermocellum*
Hydrogenase	H2ase	1.12.99.6	*Pyrococcus furiosus*
Cellic Ctec2 cellulase	Ctec2	N/A	N/A

Research Plan and Objectives

Title: Sweet Hydrogen: High-yield Production of Hydrogen from Biomass Sugars Catalyzed by In Vitro Synthetic Biosystems

PI: Y-H Percival Zhang at Virginia Tech; Co-PI: Mike Adams at UGa

Award number: DE-EE0006968

DOE FOA: DE-FOA-0000966, Fuel Cell Technologies Office

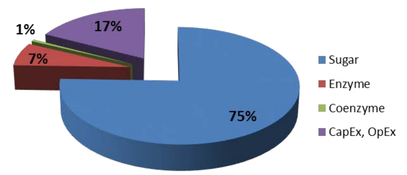
Project Officer: Katie Randolph, Ph.D.

Period: June 1, 2015 to May 31, 2017

Award amount: $750,000 (federal) and $187,602 (cost-sharing from VT and UGa)

Objectives	Summary of Tasks and Key Objectives
1 – Decrease enzymatic hydrogen production cost 1000-fold to $10/kg H_2 estimated by using H2A model	Task 1.1 Co-expression of multiple enzymes in one host (i.e., 10-fold reduction in enzyme costs) Task 1.2 Use of less-costly biomimics instead of NADP and coenzyme engineering Task 1.3 Discovery of high-stability and high-activity enzymes (i.e., to decrease enzyme use and prolong their life) Task 1.4. Detailed economic analysis of enzymatic H_2 production Key Objective: Production costs decreased by 1000-fold from $~10,000/kg of hydrogen
2 – Increase volumetric productivity five-fold to 750 mmol H_2/L/h	Task 2.1 Optimization of enzyme ratios by using modeling Task 2.2 Fitting of experimental data with model and validation of rate-limiting steps by experiments Task 2. Task 2.3 Use of synthetic metabolons Key Objective: Volumetric productivity increased to 750 mmol H_2/L/h
3 – Scale up 1000-fold to liter-level demonstration	Task 3.1 Mass enzyme production by high-density fermentation Task 3.2 Mass production of hydrogenase Task 3.3 Scale up for liter level demonstration Key Objectives: Liter-level demonstration

Ultimate Hydrogen Production Cost



Sugar	$	1.50
Enzyme	$	0.13
Coenzyme	$	0.01
CapEx, OpEx	$	0.35
Total	$	1.99

Fig. 2. Future hydrogen production cost from sugars [Ref 2].

Acknowledgment

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Key references

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