Waste to Biohydrogen via Fermentation

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Waste to BioHydrogen

Feedstock

Agricultural Waste

Forest Residue

Aqueous Waste

Fermentation

Microbial Catalysts for H₂ Production
Why Biohydrogen

- **Renewable** – convert waste to renewable H₂: monetize waste and its removal
- **Scalable**
  - DOE-USDA **Billion-Ton Report** estimated one billion tons of waste biomass is available for fuels and chemicals, i.e., H₂
  - Bioreactors is a mature technology
- **Continuous Productivity** in the dark
- **Microbial Catalysis** – many microbes naturally can produce H₂ without using the expensive precious metals.
Relevance to US DOE HFTO and Hydrogen Shot

Portfolio Includes Hydrogen Production from Diverse Sources and Pathways

EERE HFTO areas of focus

FOSSIL RESOURCES
- Low-cost, large-scale hydrogen production with CCUS
- New options include byproduct production, such as solid carbon

BIOMASS/WASTE
- Options include biogas reforming and fermentation of waste streams
- Byproduct benefits include clean water, electricity, and chemicals

H₂O SPLITTING
- Electrolyzers can be grid-tied, or directly coupled with renewables
- New direct water-splitting technologies offer longer-term options

*SMR: Steam Methane Reforming
*ADR: Anaerobic Digester Gas

*Sourced from March 11, 2021, Sustainable Energy Council (SEC) World Hydrogen Summit by Dr. Sunita Satyapal
**Technical Challenges and Approaches**

- Lignocellulosic biomass has three polymers: cellulose (six-carbon glucose), hemicellulose (five-carbon xylose), and lignin.
- H\(_2\) yield via fermentation is low: 4 mol H\(_2\)/mol sugar if only acetate produced.
- In practice, fermentation effluent contains other compounds (alcohols and organic acids).

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<th>Challenges</th>
<th>Approaches</th>
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| Feedstock Cost | • Use microbe that can **directly** convert cellulose to H\(_2\)  
• Engineer cellulosic microbe to co-utilize hemicellulose |
| H\(_2\) Molar Yield (mol H\(_2\)/mol sugar) | • Metabolic engineering to redirect pathways toward more H\(_2\)  
• Integrate fermentation with MEC* to increase H\(_2\) yield and remove waste. |

*MEC: Microbial electrolysis cell*
Integrating Fermentation with Microbial Electrolysis Cell

A NREL-Penn State integrated system has reported a combined H₂ molar yield >10.

Bruce Logan of Penn State Univ. will elaborate MEC.

**Clostridium thermocellum** – the Microbe of Choice

- A fast cellulose-degrader, at 55-60 °C
- A good H₂ producer
- *C. thermocellum* can
  - generate its own enzyme cocktails
  - hydrolyze cellulose
  - ferment
- Consolidated BioProcessing (CBP)

CBP lowers feedstock and bioreactor costs
Breakthrough Achievements to Utilize Hemicellulose

- Yet *C. thermocellum* cannot utilize xylose nor hemicellulose.
- The microbe cannot be engineered genetically.

**A Game Changer**

- **1926 – 2016**: C. *thermocellum* utilizes cellulose (C6), but not hemicellulose (C5 sugars).
- **2017 – 2018**: NREL genetically modified strain *(xylAB)* to enable C5 sugar (xylose) co-utilization.
- **2018 - 2020**: NREL evolved strains (created strain 19-9) for improved growth on monomeric xylose and H₂ production rate on hemicellulose (HC) sugars.
- **2020 – 2021**: Currently working to file a provisional patent for Co-utilizing hemi-/cellulose for H₂ production.
- **Enabled the co-utilization of hemi-/cellulose (BX)**

Cellulose/hemicellulose co-utilization will lower feedstock cost.
Convert Xylose to H₂: a Ground-breaking Achievement!!

A Really Big Deal!!

- Enable xylose utilization by adding two foreign genes.
- Double H₂ production upon adding equal amounts of xylose and cellulose, vs. cellulose alone.

An achievement 92 years after the first discovery of this microbe, a critical first step toward lowering feedstock cost!

Convert *Hemicellulose* to H₂

### 1. Adaptive Laboratory Evolution

- 5 g/L xylose
- 10th transfer
- 19th transfer
- Transfer 0.5% v/v
- Grown on xylose

- Increase total H₂ by **67%** (to 3.5 LH₂/L)
- Increase rate of H₂ by **24%**

### 2. Gene X Hydrolyzing Hemicellulose to xylose

- Add Gene X**
- Enzyme X
- Monomeric Xylose

- Increase total H₂ by **95%** (to 4.1 LH₂/L)
- Increase rate of H₂ by **39%**

*from pretreated corn stover; **provisional patent underway*
Cutting-edge Research Drives New Frontiers of Science

Characterize gene regulatory network, which could be rewired to increase H₂ yield

Controller of genes expression
Genes regulated by the controller

Hebdon et al. (2021) *Frontiers in Microbiol.*

Probe how cells sense “food”, trigger gene expression, and convert more sugars to H₂

Sugar Sensing Systems

Gene Expression “On”

The knowledge is pivotal to increasing H₂ production and collaboration with Oak Ridge National Lab (left) and UCLA (right).
A Seminal Discovery: *C. thermocellum* Can Fix CO$_2$ While Converting Waste Biomass to H$_2$

- Tracking Carbons
- Machine Learning

13C-carbon tracer is a powerful tool to track the fate and flux of carbon inside the cells.

Flux map analysis revealed CO$_2$ fixation via a novel pathway, with ~15% increase in carbon efficiency.

This cross-cutting technology could reduce carbon emission.

Summary

• Engineer *C. thermocellum* to use xylose and hemicellulose, the outcomes increase rates and total amounts of H₂ and reduce feedstock cost.
• Probing gene regulatory network and sugar sensing will increase H₂ production
• Identify a novel CO₂-fixation pathway: build cross-cutting science toward carbon capture while producing H₂.

Acknowledgements

Collaboration

DOE HFTO and NREL LDRD for funding support
Biohydrogen Production using Microbial Electrolysis Cells

Bruce E. Logan, Penn State University
Cellulose to H$_2$: Getting past the fermentation barrier

In Theory: Cellulose $\rightarrow$ 12 H$_2$

1.34/2 billion ton/y of cellulose could produce $\sim 10^{11}$ kg/yr H$_2$

Need $10^{11}$ kg/yr H$_2$ for light duty vehicles
Cellulose to H₂: Getting past the fermentation barrier

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The cellulose/biomass “fermentation barrier”

- **Fermentation**
  - \( \text{C}_6\text{H}_{12}\text{O}_6 + 2 \text{H}_2\text{O} \rightarrow 4 \text{H}_2 + 2 \text{C}_2\text{H}_4\text{O}_2 + 2 \text{CO}_2 \)
  - Achieves $\leq 4 \text{H}_2$ (+ 2 Acetate)

- **Microbial Electrolysis Cells (MECs)**
  - \( 2 \text{C}_2\text{H}_4\text{O}_2 + 2 \text{H}_2\text{O} \rightarrow 8 \text{H}_2 + 4 \text{CO}_2 \)
  - Achieves $\leq 8 \text{H}_2 \rightarrow$ total possible = 12H₂

Need $10^{11}$ kg/yr H₂ for light duty vehicles

Cellulose; Wastewaters; Any biodegradable organic matter

Hydrogen Shot Summit

Hydrogen Consumption per year for US LDV Transportation (Metric tonnes/year)

10,000,000

2000 2020 2040 2060 2080 2100

[Graph showing hydrogen consumption over time]
Fuel cells versus (PEM) water electrolyzers

**Fuel Cell:**
Produces electricity using H₂ (+ O₂)

**Water Electrolyzer:**
Produces H₂ using electricity
Microbial Fuel Cells (MFCs) make electricity using microorganisms

**MFCs**

Bacteria that produce electricity

**Fuel = Acetate** (+organic wastes)

Anode (Bacteria on Carbon)  Cathode (Activated Carbon)

Membrane not used

Microbial Electrolysis Cells (MECs) produce $\text{H}_2$

- Fuel = Acetate (+organic wastes)
- Membrane optional
- No oxygen in cathode chamber

**Cathode**
- (Pt/Ni/SS catalysts)

**Bacteria** (bioanode)

**CO}_2$

**H}_2$

\textbf{Need >0.11 V}
(vs 1.2-1.8 V for water electrolysis)

What microorganisms produce current = exoelectrogenic?

Scaling up MFCs: from laboratory to pilot scale

MFCs

Gen 0: 0.025 L, 25 m²/m³

Gen 1: 0.13 L, 25 m²/m³

Gen 2: 2 L, 20 m²/m³

Gen 3: 6.1 L, 20 m²/m³

Pilot-Scale MFC:
850 L active volume, 25 m²/m³
Scaling up MECs: from laboratory to pilot scale: Part I

MECs

5 mL mini-MEC → 28 mL MEC → 2.5 L MEC

Single-Chamber MECs: \( \text{H}_2 \rightarrow \text{CH}_4 \)

Two-Chamber MECs: \( \text{H}_2 \) recovery

Cathode – Pt/C on SS Mesh

Cathode Chamber – modular

Catholyte effluent and \( \text{H}_2 \)

Anode Chamber – Graphite brush & attached microbial community

Anion exchange membrane

Power Supply

Catholyte influent

1000 L MEC (X)
Scaling up MECs: Part II, capturing H₂

- **Flow by brushes**
  - SS cathode
  - 60 A/m³
  - 1.3 L/L-d

- **Flow through brushes**
  - SS wool cathode
  - 200 – 400 A/m³
  - 2.6 – 5.2 A/m²
  - 3.8 L/L-d
Applying lessons learned from MFCs to MECs

- Improved MECs (in progress)
  - Avoided solution resistance by using a solid electrolyte anion exchange membrane (AEM) with gas phase electrolyte
  - Unique AEM design reduced anode and cathode resistances by balancing pH

- Preliminary MEC results: 17x increase in performance
  - 42 A/m²-d (versus 5 A/m²)
  - 63 L/L-d (versus ~3.8 L/L-d)
  - Highest H₂ production rate achieved under these solution conditions

Ultra-compact MFC design increased current densities from 8 to 50 A/m²
Avoiding the use of precious metals in MECs

Stainless Steel wool cathodes

Nickel particles, pNi on activated carbon

Nickel Phosphide Ni₂P

![Diagram of MEC setup and reaction process]

![Graphs showing hydrogen production rates and current densities]

Effluent  Flow out
Anode Chamber (with 8 brushes)
Cathode Flow Chamber

Influent  Flow in

H₂ production rate (L-H₂/L-d)

Average current density (A/m²) & Time (h)

Comparison of different catalysts:
- Ni₂P/C
- Ni/C
- Pt/C

Hydrogen Shot Summit
Why use biomass (electrolyzers) to achieve $1\text{H}_2/\text{kg}$?

**Water electrolyzers** require 2 steps
- Water purification (reverse osmosis + deionization)
- Electrolyzer operation using electrical power

**Electricity use is high**
- Minimum of electrical energy for water splitting is 33 kWh/kg H$_2$ (thermodynamics)

**$1$ kg H$_2$ requires for electricity:**
- $0.03$/kWh for electricity (thermodynamic limit)
- $0.02$/kWh considering current efficiencies (70%)

**Precious metals may be required.**
- PEM uses Ir, Pt; AEM does not (Ni-based)

**Small, compact reactors, high electricity demand**

**Biomass (with electrolyzers) requires 2 steps**
- Biomass fermentation
  - Fermentation is spontaneous, so no energy input needed during process (neglecting reactor stirring, pumps)
  - Produces 4 moles H$_2$ per cellulose (of maximum = 12)
- Microbial electrolysis Cells (MECs)
  - Minimum electrical energy is only 1/10th electrical energy compared to water electrolyzers

**$1$ kg H$_2$ requires for electricity**
- $0.30$/kWh for electricity (thermodynamic limit) for 8/12 moles of H$_2$
- $0.45$/kWh for 12/12 moles of H$_2$.

**Precious metals not required.**
**Large reactors used, need transport of biomass, low electricity demand**
CONCLUSIONS

• MECs use bacteria as the “catalyst” to produce an electrical current,
  – Fuel = waste organic matter
  – H₂ produced electrochemically (as in a water electrolyzer) using biomass electrons

• MEC designs have lagged those of MFCs... but innovations can improve both systems

• Recent MEC designs achieved 63 L/L-d, with 100 L/L-d on the horizon without using precious metals

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Two-chamber brush MECs and HER catalysts
QUESTIONS?