NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Biomass to Hydrogen (B2H2)

Pin-Ching Maness (P.I.)
National Renewable Energy Laboratory
June 14, 2018

DOE Hydrogen and Fuel Cells Program
2018 Annual Merit Review and Peer Evaluation Meeting

PD038

Overview

Timeline and Budget

- Project start date: 10/1/2015
- FY16 DOE Funding: $1M
- FY17 DOE funding: $900K
- FY18 planned DOE funding: $800K
- Total DOE funds received to date: $2M*

* As of 3/31/18

Barriers

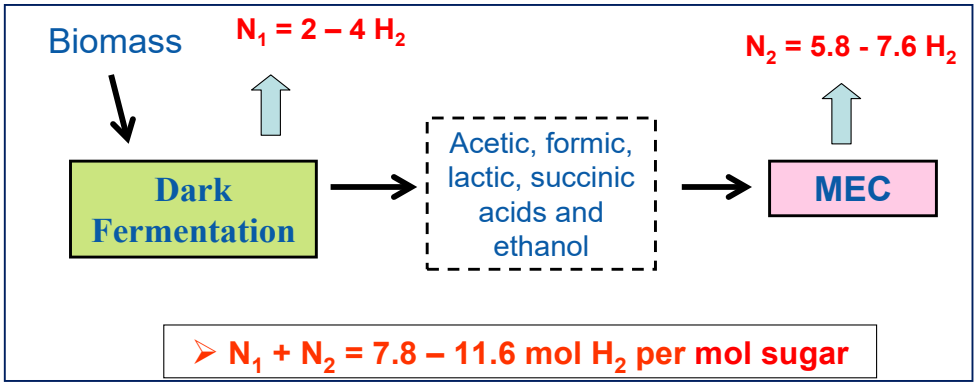
- H_2 molar yield (AX)
- Feedstock cost (AY)
- System engineering (AZ)

Partners

- Dr. Bruce Logan
  Pennsylvania State University
- Drs. Steven Singer, Lawrence Berkeley National Lab (LBNL) and Ken Sale, Sandia National Lab (SNL)
- Dr. James Liao at UCLA (no cost)

Relevance

Overall Objective: Develop ***direct*** fermentation technologies to convert renewable lignocellulosic biomass resources to H_2.



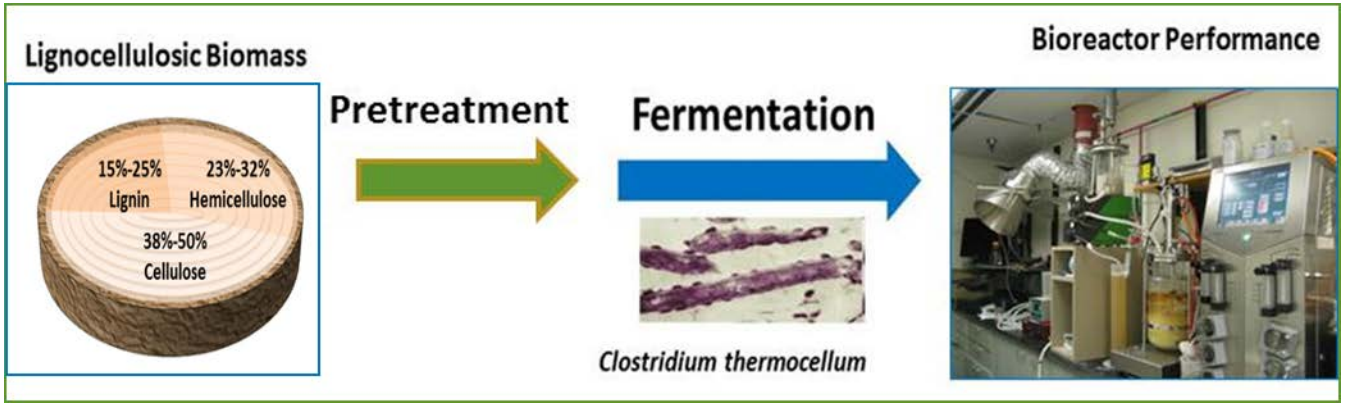
Current Project Year Objectives (April 2017 – April 2018)

- **Addressing feedstock cost barrier**
  - Increase solid substrate loading to obtain high rate of H_2 production leading to a smaller bioreactor footprint to lower the cost of H_2.
  - Improve biomass utilization by converting most of the sugars (5-carbon and 6-carbon sugars) to H_2 production either via co-culture systems or genetic engineering of *Clostridium thermocellum*.
- **Addressing H_2 molar yield barrier**
  - Identify electron carriers to boost H_2 production.
  - Determine the most important cellulose-degradation pathways to yield H_2.
  - Microbial Electrolysis Cells (MEC): Replace the costly platinum cathode with inexpensive materials while still obtaining high rate of H_2 production, ultimately using fermentation waste – also addressing waste removal.

This project addresses key DOE Technical Targets and leverages DOE Bioenergy Technologies Office (BETO) investment in biomass pretreatment.

Approach
Task 1: Bioreactor Performance

- **Approach:** Optimize bioreactor in batch and fed-batch modes by testing parameters such as corn stover lignocellulose loadings (DMR pretreatment), and hydraulic retention time (HRT), using the cellulose-degrading bacterium *Clostridium thermocellum* engineered to co-utilize both cellulose and hemicellulose.



Pretreatment – De-acetylated and Mechanically Refined (DMR)

0.4% NaOH

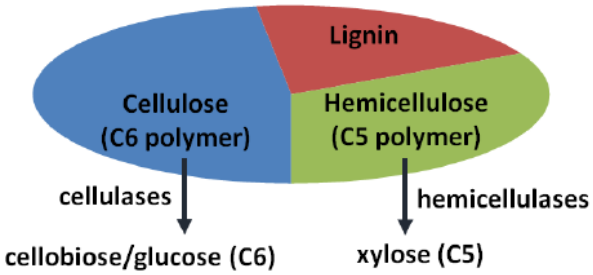
70°C

Solid: 44% cellulose
23% xylan
17% lignin
Ash, etc.

Liquid: 79% acetate
2.9% xylan

→ H_2

Engineer Cellulose Microbe to Co-metabolize C5 Sugars



C. thermocellum → H_2

Ferment all the sugars to H_2 in one bioreactor

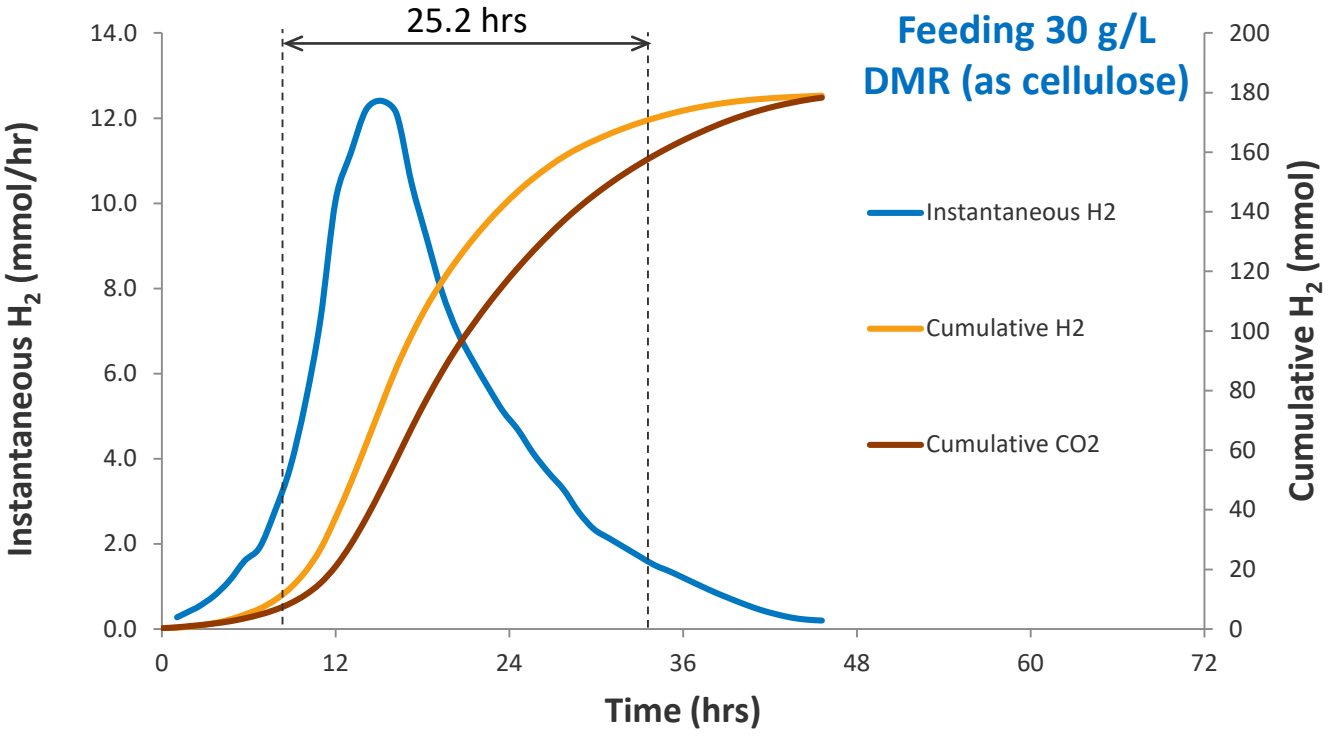
Task 1. Accomplishments and Progress: Achieved a H_2 Production Rate of 2.6 $L/L_{reactor}/d$

Lauren Magnusson

FY17 Q4 Milestone	Optimize and increase biomass substrate loading either in a sequencing fed-batch or batch mode operation, and obtain an average H_2 production rate of at least 2.5 $L/L_{reactor}$/day for a duration of minimally 24 h using DMR biomass as the substrate (NREL).	9/2017	**100% complete**

Strategies to increase rate of H_2 production:

- Tested 5-30 g/L of avicel (pure cellulose) loading with 30 g/L yielded the fastest H_2 production rate.
- Tested 5-40 g/L loadings of DMR (as cellulose), with 30 g/L yielded the highest rate (2.6L H_2 /L/d).
- Lower rate from higher DMR loading could be due to insufficient mixing, which warrants high-solid reactor development.

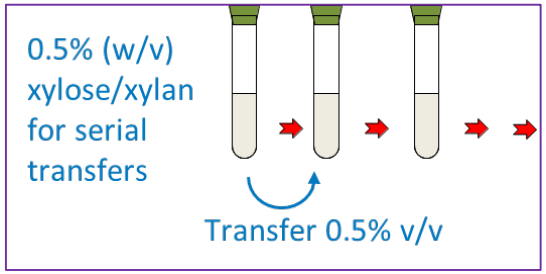


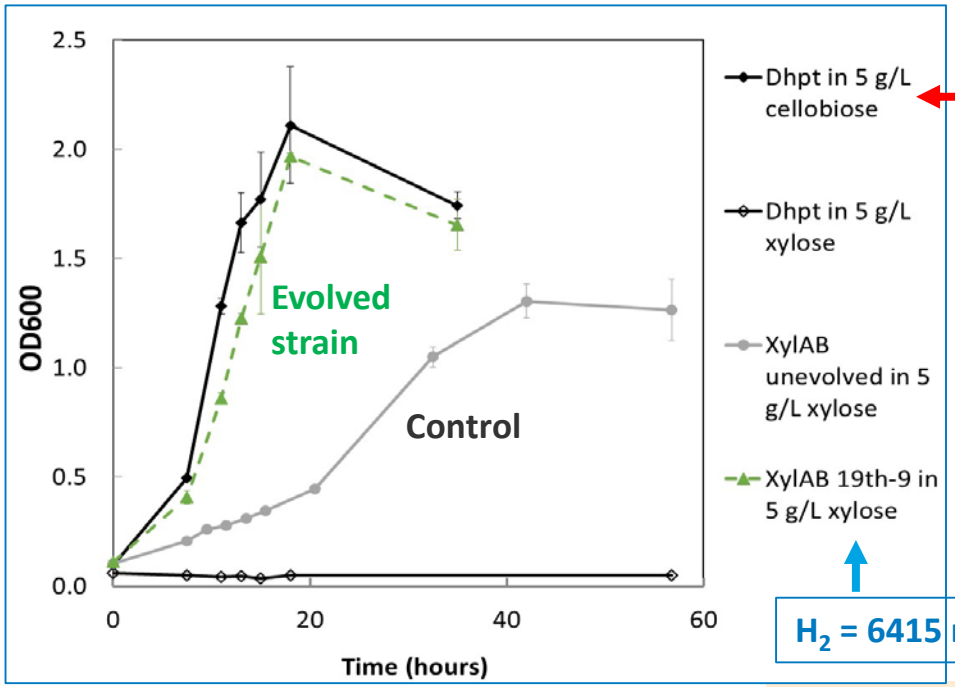
The most productive phase (25 h) yields a H_2 production rate of **2.6 $L/L_{reactor}/d$,** which is **2.4-fold** higher than that obtained in the last DOE review, benchmarking the progress.

Accomplishment: 3.5-fold Increase in Rate of Xylose Utilization

FY18 Q1 Milestone	Using laboratory adaptive evolution, we will improve the rate of xylose utilization and identify one gene putatively responsible for faster xylose consumption. We will profile H_2 production, substrate consumption, and metabolite profiles in the adapted and evolved *C. thermocellum* strain (NREL).	12/31/ 2017	**100% complete**

- We engineered xylose utilization by inserting *xylAB* genes into the *C. thermocellum* **genome** for higher stability.
- Xylose is a hemicellulose-derived sugar. We increased rate of xylose consumption by adaptive evolution in xylose +/- xylan.





H_2 = 6470 nm/ml

H_2 = 6415 nm/ml

- **Increased xylose utilization by 3.5-fold** in the evolved strain compared to control strain (Dhpt) in xylose.
- Identified **6** gene mutations in the evolved strain with validation of a key mutation ongoing.

XylA: xylose isomerase; XylB: xylulokinase

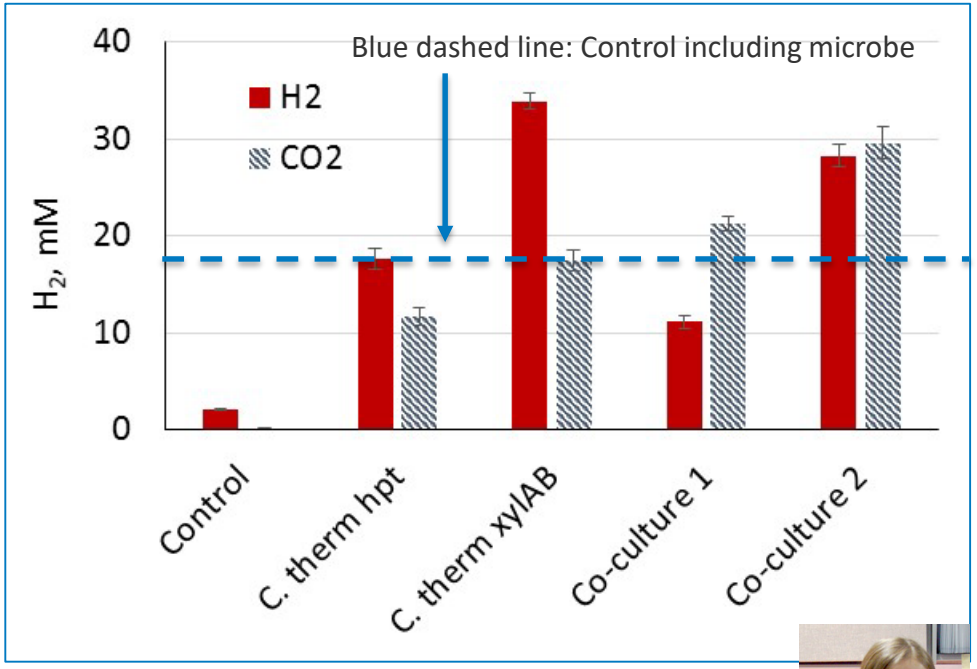
Xylose engineering published in Biotechnology Bioengineering, 2018.

Katherine Chou

Accomplishment: 1.9-fold increase in Total H_2 Production by Improving Biomass Utilization

FY18 Q4 Milestone	Optimize and improve biomass utilization by 20% by developing methods to convert both C6 and C5 sugars to H_2. We will achieve this by ether using *C. thermocellum* already engineered to co-utilize both sugars, or in a binary culture including another microbe to metabolize the C5 sugar of the biomass feedstock	9/2018	**50% complete**

- Use two strategies to improve biomass utilization:
  - One approach is to express foreign xylose-pathways genes (*xylAB*) to metabolize xylose.
  - Second approach is to co-culture *C. thermocellum* with two hemicellulose-degrading microbes: *Thermoanaerobacter ethanolicus* (co-culture 1) and *Thermoanaerobacterium saccharolyticum* (co-culture 2), at 55 °C.
- Reviewers suggested last year to test co-culture systems.



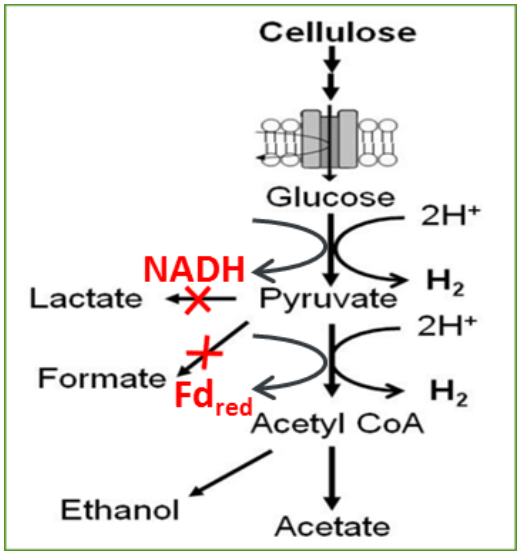
Lauren Magnusson

Increase total H_2 production by 1.9 and 1.6 fold in engineered strains and in a co-culture, respectively, reflecting dramatic improvements in biomass utilization.

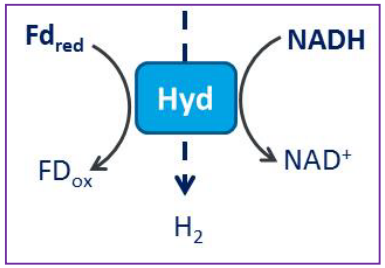
Approach
Task 3: Generate Metabolic Pathway Mutants

Approach: Redirect metabolic pathways to improve H_2 molar yield via developing genetic methods.

- Blocking carbon-competing pathways led to 86% increase in rate of H_2 production (2016 AMR accomplishment).
- Manipulating cofactor interconversion (NAD[P]H and reduced ferredoxin) led to 35% increase in total H_2 production (2017 AMR accomplishment).



Hydrogenase

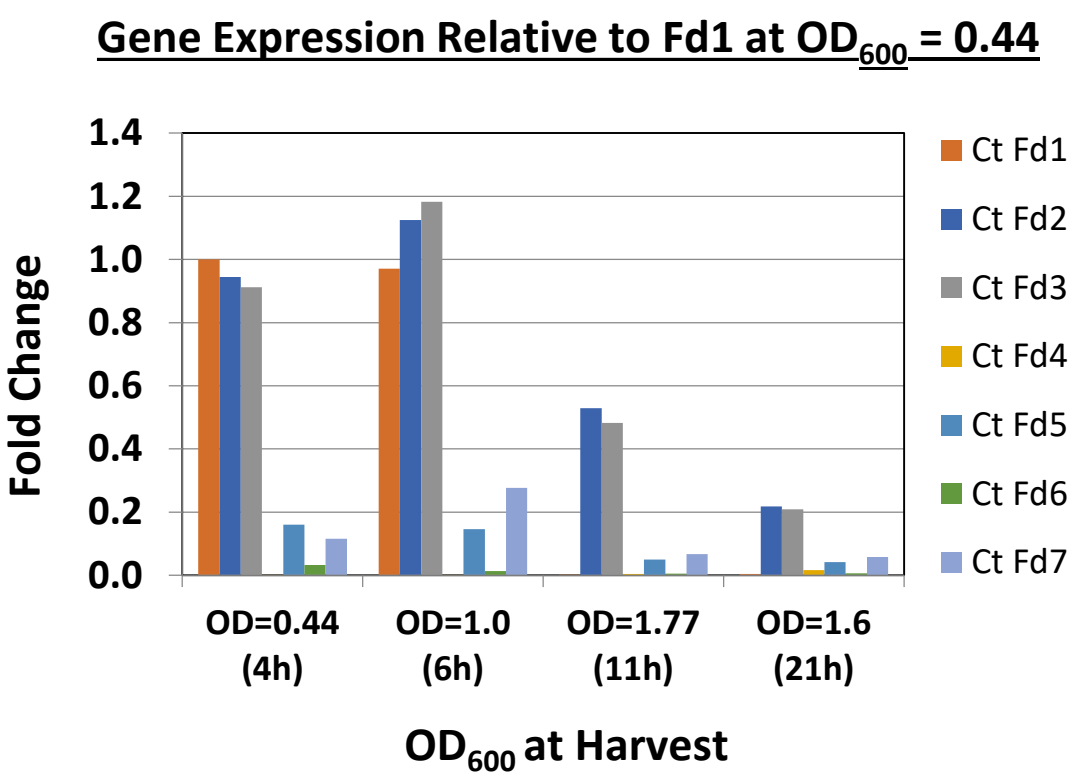


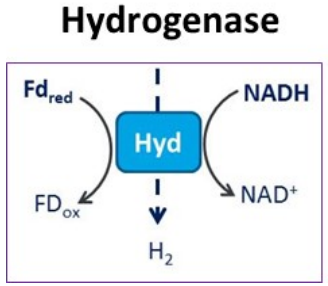
- Hydrogen production in *C. thermocellum* is likely mediated by both NADH and reduced ferredoxin.
- Identifying which ferredoxin(s) is important in H_2 production will guide strategy for improvement.

FY18 Q3 Milestone	Using qRT-PCR, we will profile expression of the various ferredoxins in *C. thermocellum* and their correlations with peak H_2 production in order to identify ferredoxin(s) important in H_2 production. The findings will guide the knockout of the ferredoxin to probe its functionality and its over-expression aimed to increase H_2 production (NREL).	6/30/ 2018	**65% complete**

Task 3 Accomplishments and Progress:
Identified 3 Ferredoxins Important in H_2 Production

- The goal is to identify the important ferredoxin(s) in H_2 production to guide genetic strategy for improvement.
- *C. thermocellum* contains up to 7 ferredoxin (Fd1-7) that potentially could donate electrons toward H_2 production.
- **H_2 production peaked between OD of 0.44 and 1.0 in *C. thermocellum,* correlated with higher expression of Fd1, Fd2, and Fd3 quantified via the qRT-PCR technique.**



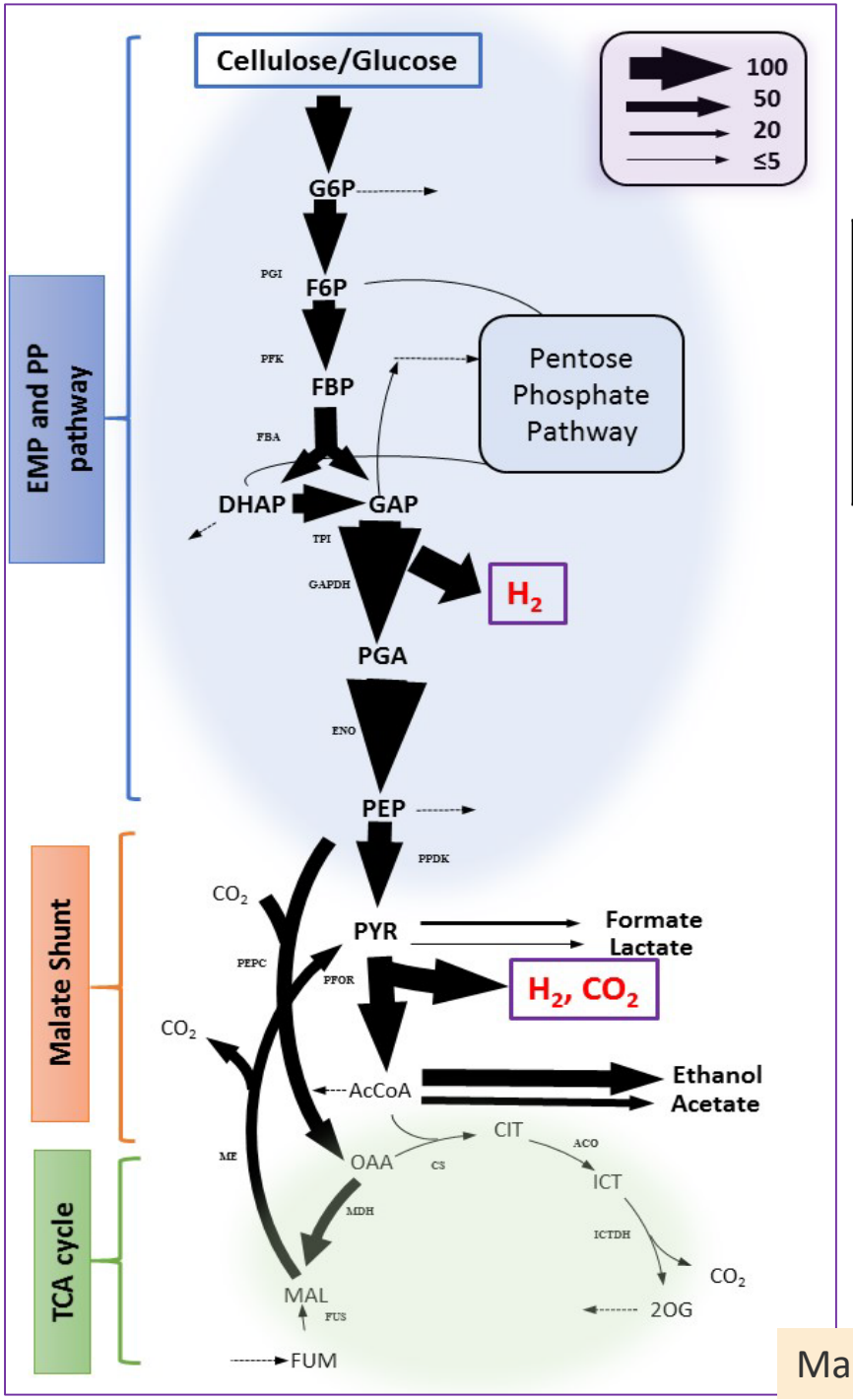


Over-expression of Ferredoxin 1, 2, 3 is a valid strategy leading to increased H_2 production.

Katherine Chou

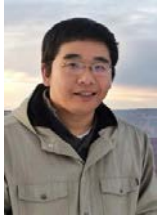
Accomplishment: Identified EMP Flux Contributes Most to H_2 Production

FY18 Q2 Miles-tone	Using isotope-tracer metabolic flux analysis, construct a high-resolution cellular carbon flux map and identify the most important glycolytic route(s) utilized by *C. thermocellum* to metabolize cellulosic sugars in support of cell growth and H_2 production (NREL).	3/31/2018	**100% Complete**



- The goal is to identify which cellulose-degrading pathway is important for H_2 production to guide improvement.
- **Use ^{13}C-labeled glucose, we constructed a high-resolution cellular carbon flux map to track electron/energy flow in *C. thermocellum*.**
- Identified that EMP (Embden-Meyerhof-Parnas) pathway has the highest flux toward H_2 production (**heavy arrow**).

The outcomes suggest EMP pathway is a target for manipulations to increase H_2 production.



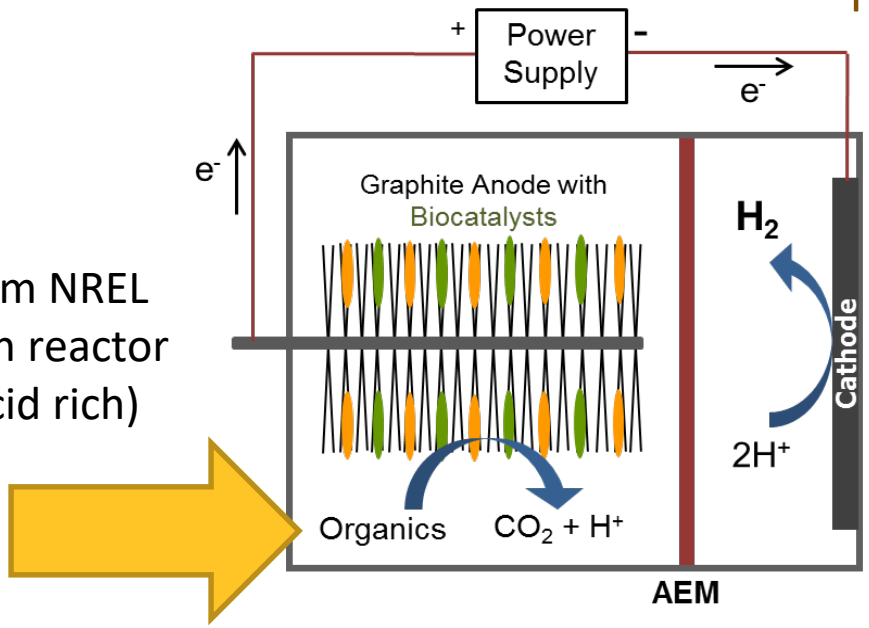
Manuscript submitted to J. Biological Chemistry

Wei Xiong

Approach
Task 4: Electrochemically Assisted Microbial Fermentation

Microbial Electrolysis Cell (MEC) — Conversion of Organic Waste to Hydrogen Gas

Effluent from NREL fermentation reactor (Organic acid rich)



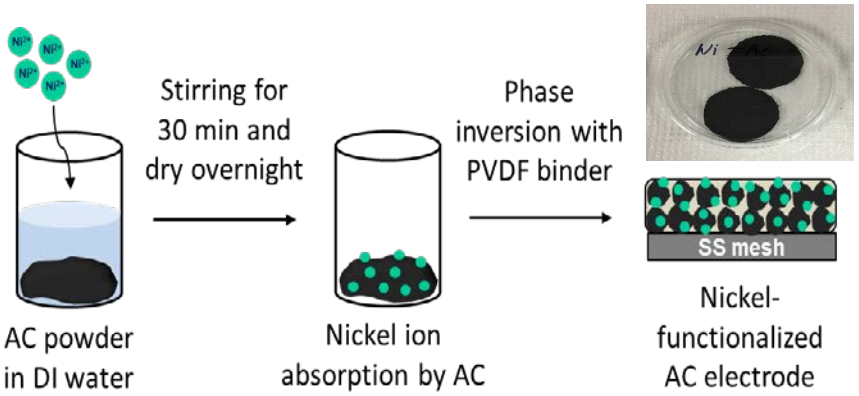
Goal:
Achieving high rate of H_2 production with non-Pt based cathode using NREL fermentation effluent.

Bruce Logan

	Milestones (PSU)	Completion Date	Status
FY17	Improve performance and increase electrode loading to double reactor performance to 2.4 L/reactor-d (PSU).	11/2017 – Q4 for Penn State	Complete
FY18	Evaluation of alkaline pH cathode catalysts and select new alkaline-optimized anion exchange membranes. Using a thinner cathode chamber and optimizing hydroxide ions crossover should improve overall performance by 30% (FY18 Q4; PSU*).	*1/2019 – Q4 of Penn State	On track

Task 4 Accomplishments and Progress:
Cathode Chamber Optimization: replaced Pt with alternative materials

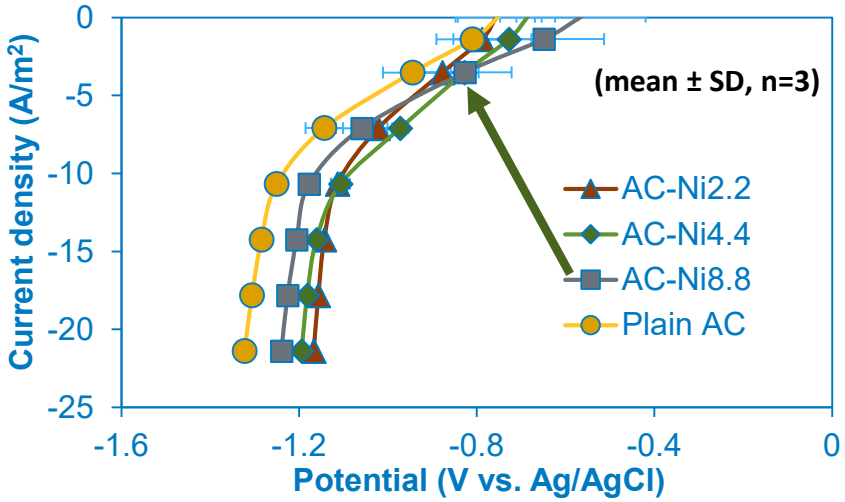
Ni-functionalized activated carbon (AC) cathode preparation-by simple adsorption



Abiotic electrochemical test (no catholyte flow)

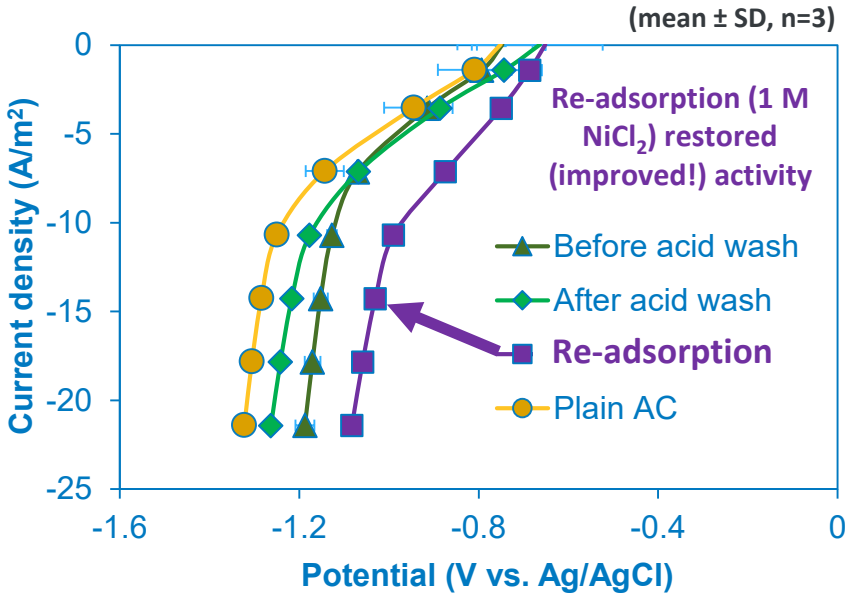
($NiCl_2 \cdot 6H_2O$ loadings: 2.2%, 4.4%, 8.8%)

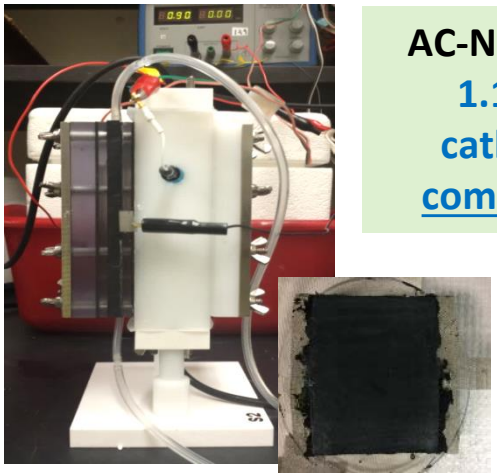
Ni adsorption was effective to lower potentials for HER.



Great advantage of AC-Ni:

Catalyst can be regenerated by adsorption





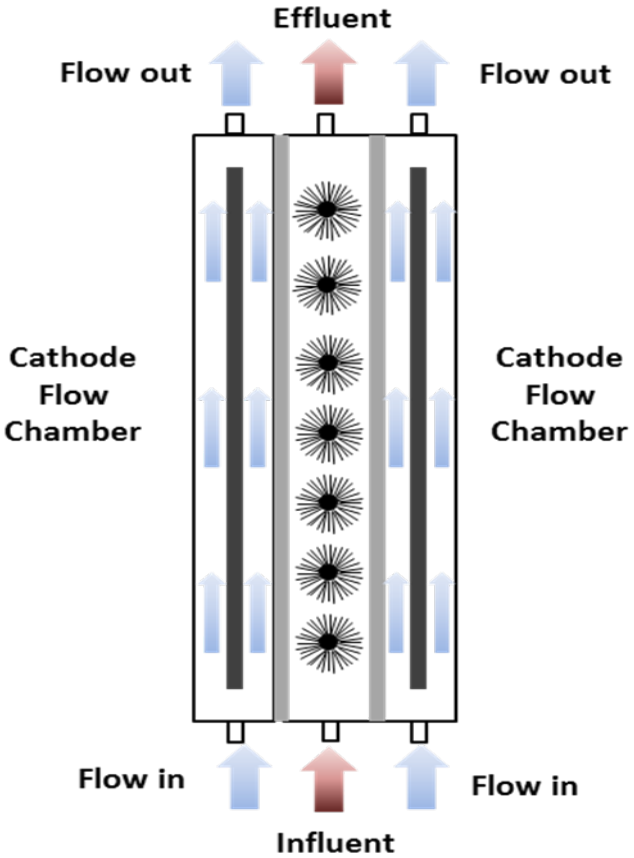
AC-Ni8.8 cathode produced 1.1±0.1 L-H_2/L-d (with catholyte flow) which is comparable to Pt cathode

Kyoung-Yeol Kim

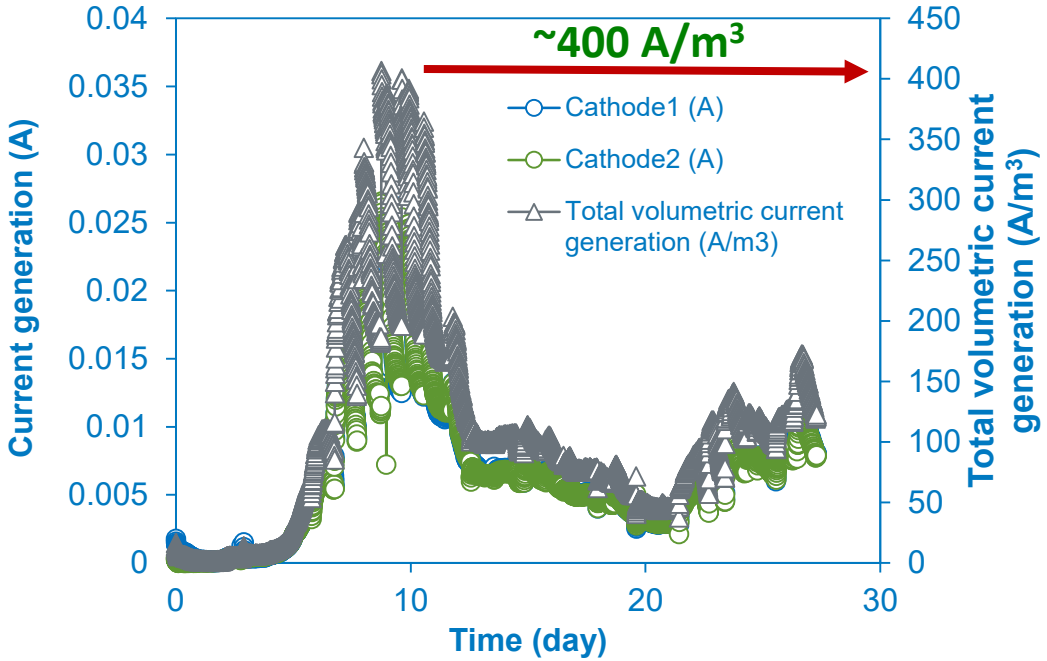
Accomplishment: Achieve 2.4 L H_2/L-d with Low Cost non-Pt Cathode

New MEC design: Reduce anode volume and add 1 more cathode chamber (total 130 mL)

Anode: 7 brush anodes
Cathode: 2 stainless steel (SS) wool cathodes (1 each side)
Both anolyte and catholytes were recirculated



Volumetric current densities and H_2 production rates



- Max. current density: ~400 A/m³ (~0.7 day cycle)
- Measured maximum H_2 production: 2.8±0.3 L-H_2/L/d (~0.7 day cycle); 4.7 L-H_2/L/d (~0.3 d cycle)
- Current drop occurred after day 10, but increasing again after re-inoculation.

H_2 production rate meets 2017 milestone (2.4 L/L-d) with low cost SS wool (a non-Pt based cathode) but stability needs to be further studied.

Accomplishments and Progress: Responses to Previous Year Reviewers' Comments

- Overall this is an exciting project, and future work is pleasantly anticipated.
  - *We thank the reviewers for the positive comments. We strive to address technical barriers in order to reach the FCTO H_2 cost goal.*
- The project was carried out outstandingly. However, …the use of pure culture of (engineered) bacteria may not be a low-cost process because of contamination issues…mixed microbial culture might be preferable.
  - *The optimal growth temperature of C. thermocellum is 60 °C at which contamination is minimized. Moreover, we have tested successfully a co-culture system including C. thermocellum with T. saccharolyticum, a thermophile. The co-culture exhibited a 60% increase in H_2 production. We routinely used unsterilized* corn stover *feedstock to lower cost with no contamination issue – another reviewers' concern.*
- The work proposed will continue to build on the progress of the different tasks…it is not clear when these improvements will be combined…i.e., xylose/glucose utilization, media cost reduction, and the enzyme mutations.
  - *This is a low TRL research, and we have made improvements in several areas followed by detailed characterizations. We do plan to eventually combine all the beneficial changes in one strain, feeding unsterilized pretreated biomass, in less costly growth medium in mutants with minimal carbon-competing pathways to determine H_2 production and meet FCTO cost goal.*

Collaboration and Coordination

- **Task 1 (Bioreactor)**

  Drs. Ali Mohagheghi and Melvin Tucker, National Bioenergy Center at NREL: provide DMR pretreated corn stover and their characterizations - leveraging DOE BETO funding.

- **Task 2 (Ionic Liquid) – discontinued in FY17**

  Drs. Steve Singer (LBNL) and Kent Sales (SNL): conducted biomass pretreatment using ionic liquid as a complementary pretreatment approach to lower feedstock cost.

- **Task 3 (Genetic Methods)**

  Dr. James Liao of UCLA in pathway engineering of *C. thermocellum* – leveraging DOE Office of Science funding.

- **Task 4 (MEC)**

  Dr. Bruce Logan at Penn State University: microbial electrolysis cells to improve H_2 molar yield.

Remaining Challenges and Barriers

Task 1. Bioreactor Performance

- High solid-substrate loading (175 g/L) is needed to lower H_2 selling price, which might present a challenge to ensure sufficient mixing.
  - Novel impeller design with high power/low torque may be able to address this challenge.

Task 2. Fermentation of Pretreated Biomass using Ionic Liquid (LBNL/SNL)

- This task was closed out in FY17/Q1.

Task 3. Generate Metabolic Pathway Mutant in *C. thermocellum*

- Improve the rate of xylan utilization in engineered strain to improve biomass utilization.
  - Continue with adaptive evolution strategy feeding xylose/xylan and select fast grower in xylan.
  - Targeted insertion of foreign genes to overcome the rate-limiting step(s) of xylan utilization.

Task 4. Electrochemically Assisted Microbial Fermentation of Acetate (PSU)

- Maximizing current generation with non-precious metal catalysts
  - Already demonstrated good performance with SS wool;
  - New data suggests Ni adsorption into activated carbon may be better.
- Further reducing reactor size, which will increase overall reactor production rates; examining new anode configurations which may help.

Proposed Future Work: project is scheduled to close out in FY19/Q1

Any proposed future work is subject to change based on funding levels

Task 1 (NREL)

- Optimize cellulose/hemicellulose co-fermentation to improve biomass utilization by at least 20%, in scale-up bioreactors for long-term durability, using either engineered *C. thermocellum* strain or in co-culture systems (FY18/Q4 milestone).
- Test 40-50 g/L DMR biomass substrate loadings (based on cellulose) in the engineered xylan strains (FY19/Q1).

Task 2 (LBNL/SNL): none.

Task 3 (NREL)

- Redesign primers to be more specific to Fd1, Fd2, and Fd3 via qRT-PCR to identify the most important ferredoxin(s) to improve H_2 production as *C. thermocellum* contains multiple ferredoxins with unknown functions (FY18/Q3 Milestone).
- Coordinate with Task 1 in bioreactor work testing engineered *C. thermocellum* expressing a foreign xylosidase enzyme to accelerate xylan conversion to H_2 (FY18/19 Q1).

Task 4 (Penn State):

- Further improve reactor performance using the MEC to produce more than 2.4 L-H_2/$L_{reactor}$/d (surpassing the FY17 goal) using synthetic effluent (FY19/Q1).
- Examine if performance can be improved using flat anodes (FY18)
- Improved Ni^{+}-AC cathode catalyst performance; examine alternative catalysts at high pH (FY18).

Complete a Close-out Report of the project (FY19/Q1)

Technology Transfer Activities

Technology-to-market or technology transfer plan or strategy

- Air Product and Chemicals, Inc.
  - Main interest in H_2 from biomass can be low carbon or even potentially carbon neutral; have funded the Logan lab in the past for work on MECs and RED for H_2 production from wastewaters
  - Large-scale process of greatest interest, but currently there are no larger reactors.
  - Cost needs to be near to, or lower than, making H_2 from alternative sources (natural gas).

Plans for future funding

- Pursue opportunities to collaborate with other national Labs and industries for potential future funding support.
- Network with biofuels industry to expand the use of H_2.
- Advocate the advantages of "green" H_2 rather than fossil-fuel derived H_2

Patents, licensing

- A Record of Invention (ROI-14-70) is filed for developing the proprietary genetic tools tailored for *C. thermocellum*.
- A second ROI-15-42 has been filed for generating xylose-metabolizing strain, leading to enhanced biomass utilization.

Summary

Task 1

- Achieved an average (25 h) H_2 production rate of **2.6L H_2/$L_{reactor}$/d** fermenting DMR-pretreated corn stover directly to H_2, which is **2.4-fold** higher than that obtained in the last DOE review, benchmarking the progress.
- Improved xylose utilization by **3.5-fold** via adaptive evolution in xylose and obtain fast grower.
- Determined **1.9-fold increase** in total H_2 production via improved biomass utilization (both cellulose and hemicellulose) either in engineered *C. thermocellum* or in co-cultures, which could lower H_2 selling price.

Task 2: Closed out in FY17/Q1, not meeting GNG.

Task 3

- Via qRT-PCR, we determined Fd1, Fd2, and Fd3 are most important for H_2 production, which guides genetic strategies for improvement.
- Identified that EMP is the dominant pathway in cellulose hydrolysis, which guides genetic engineering strategies for improvement.

Task 4

- New Ni-functionalized AC cathodes produced comparable H_2 production rate (1.1±0.1 L/L-d) to Pt or SS wool cathodes and catalytic activity of the cathodes was simply restored by re-adsorption of Ni salts.
- H_2 production rate meets 2017 milestone (**2.4 L/L-d**) with SS wool with a newly-designed MEC, and further study is ongoing to achieve long-term stability.

Thank You

www.nrel.gov

Publication Number

NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

NREL
NATIONAL RENEWABLE ENERGY LABORATORY