Biomass to Hydrogen (B2H2)

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Katherine Chou (Presenter)
National Renewable Energy Laboratory
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DOE Hydrogen and Fuel Cells Program
2019 Annual Merit Review and Peer Evaluation Meeting

P038

Overview

Timeline and Budget

- Project start date: 10/1/2015
- FY16 DOE Funding: $1M
- FY17 DOE funding: $900K
- FY18 DOE funding: $800K
- Total DOE funds received to date: $2.7M
- The project will be closed out in FY19.

Barriers

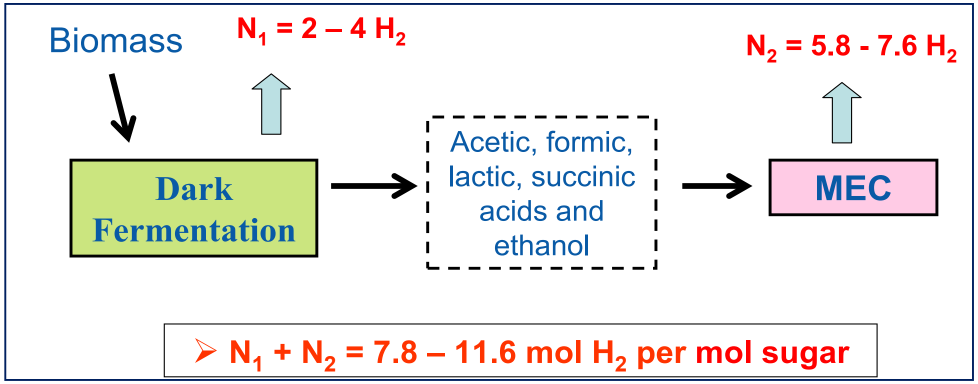
- H_2 molar yield (AX)
- Feedstock cost (AY)
- System engineering (AZ)

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.



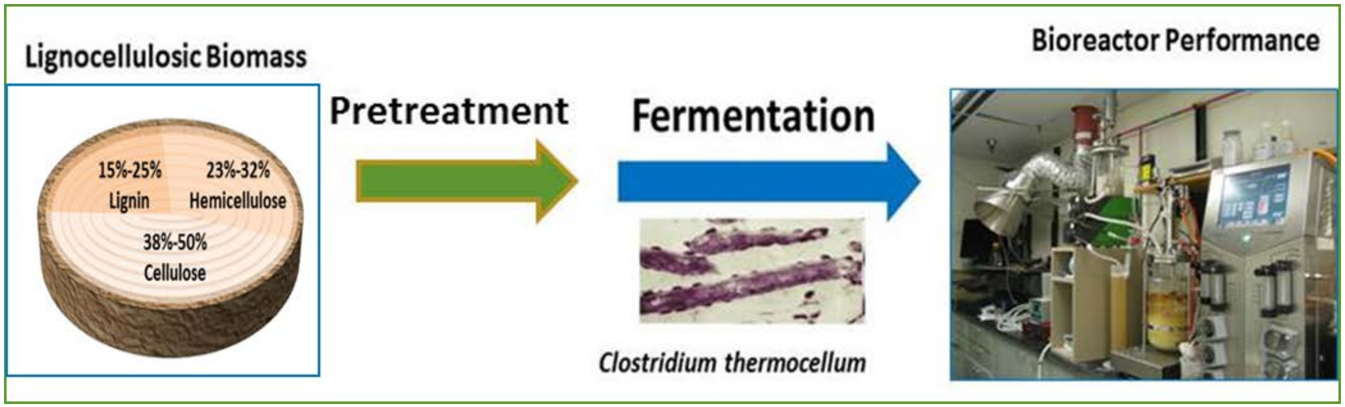
Current Project Year Objectives (May 2018 – April 2019)

- **Addressing feedstock cost barrier**
  - Improve biomass utilization by converting cellulose (6-carbon sugar) and hemicellulose (5-carbon sugar) to produce H_2 either via co-culture systems or genetic engineering of *Clostridium thermocellum*.
- **Addressing H_2 molar yield barrier**
  - Had generated a series of competing-pathway mutants with either increased rate or yield of H_2. Using ^{13}C-metabolic flux analysis, we aim to understand how these metabolic changes influence H_2 production to guide future genetic engineering strategies.
  - 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.



<u>Pretreatment – De-acetylated and Mechanically Refined (DMR)</u>

0.4% NaOH → 70°C

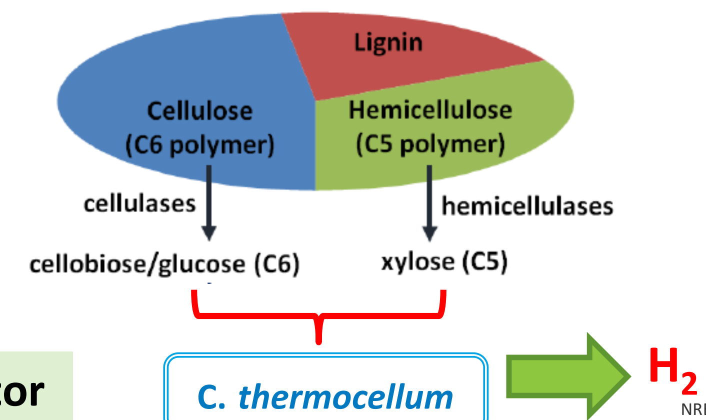
<u>Solid:</u> 44% cellulose
23% xylan
17% lignin
Ash, etc.

→ H_2

<u>Liquid:</u> 79% acetate
2.9% xylan

Ferment all the sugars to H_2 in <u>one</u> bioreactor

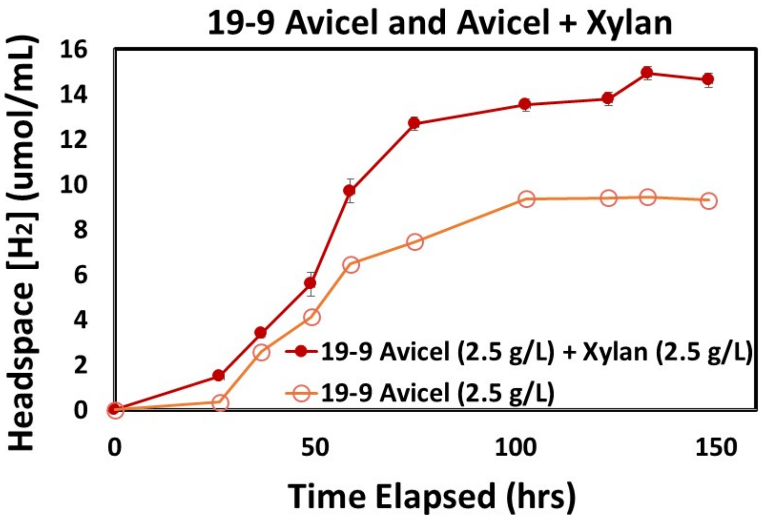
<u>Engineer a Cellulose-Degrading Microbe to Co-Metabolize C5 Sugars</u>

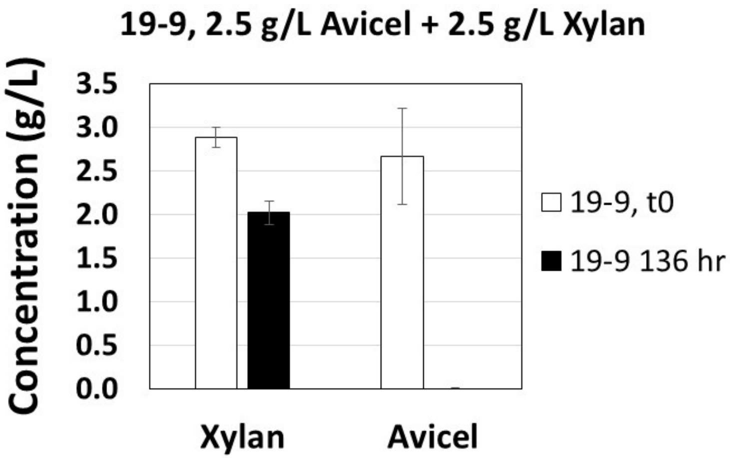


Task 1. Accomplishments and Progress: Increased Xylan Biomass Utilization by 29% and H_2 Production by 45%.

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	**Complete**

- Use two strategies to improve biomass utilization:
  - The first approach is to use a binary co-culture system (reported in 2018 AMR).
  - A second approach is to express foreign xylose-pathways genes (*xylAB*) to metabolize xylose, followed by adaptive evolution to utilize xylan (complex xylose).
- *C. thermocellum* evolved *xylAB* strain (19-9) indeed utilized **29%** more xylan and produced 45% more H_2 by co-utilizing both cellulose and **xylan**.





Katherine Chou

Utilizing the hemicellulose portion of biomass will lower feedstock cost.

Accomplishments and Progress : Increased H_2 production Rate by 15% and total H_2 by 16% in pH-controlled Bioreactor

FY19 Q1 Milestone	Via laboratory evolution, we have evolved the xylose-engineered strain to also degrade the more complex xylan, yet its performance in H_2 production has not been demonstrated in bioreactors. We will quantify H_2 production in pH-controlled bioreactor and obtain up to 10% increase in H_2 production (over a baseline rate of 1 L/L/d at 5 g/L substrate loading) as an indication of xylan utilization. We will also determine xylan utilization and profile metabolites to guide additional engineering strategies to improve xylan utilization.	12/2018	Complete

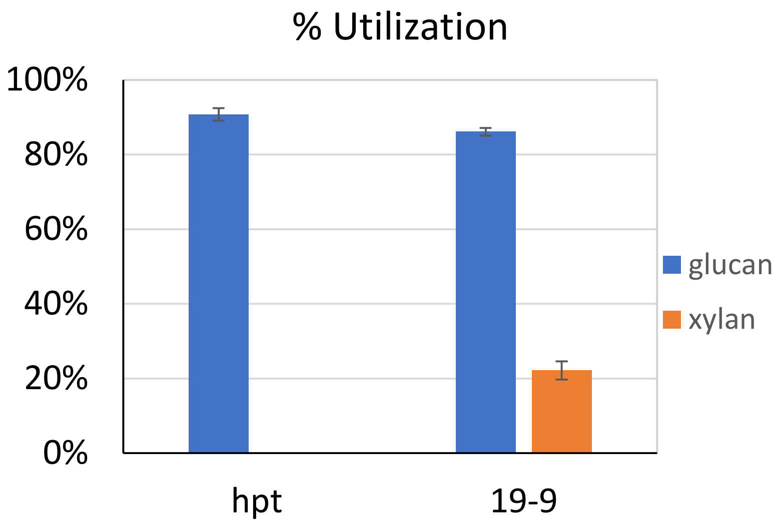
- The 19-9 evolved strain yielded an average H_2 production rate (over 43 h of fermentation) of 1024 mL/L-d using both cellulose and xylan, **a 15% increase** over the parental controlled strain (Δ*hpt*) using cellulose only, and with a rate of 871 mL/L-d.
- The 19-9 evolved strain also produced **16% more** total H_2.

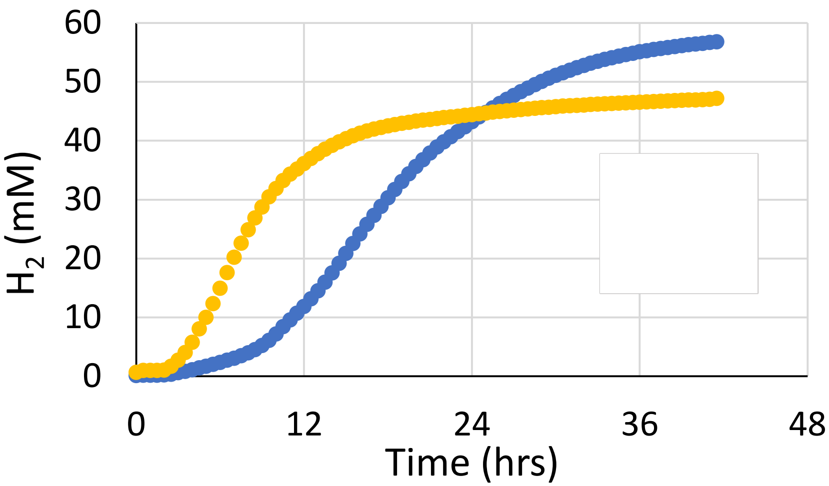
Strain	H_2 Production per Batch	Average H_2 Production Rate	Maximum 24 hr H_2 production rate
	$(mL\ L^{-1})$	$(mL\ L^{-1}\ d^{-1})$	
hpt	1506.04 +/- 32.08	870.94 +/- 18.54	1409.07 +/- 46.84
19-9	1792.61 +/- 20.41	1024.34 +/- 11.66	1504.35 +/- 46.42
% improvement	16%	15%	6%

Lauren Magnusson

Accomplishments and Progress : Increased Xylan Consumption by 22% in pH-controlled Bioreactor

- The 19-9 evolved strain utilized **22%** of the xylan, consistent with the increases in both rate (15%) and total (16%) H_2 production.
- Strain 19-9 displayed a lag in H_2 production which can be improved via further adaptation.
- Both the control (Δ*hpt*) and 19-9 mutants were derived from *C. thermocellum* DSM 1313 which can be manipulated genetically. The benchmark rate (1 L/L-d) was obtained from *C. thermocellum* ATCC 27405 which lacks a genetic system.



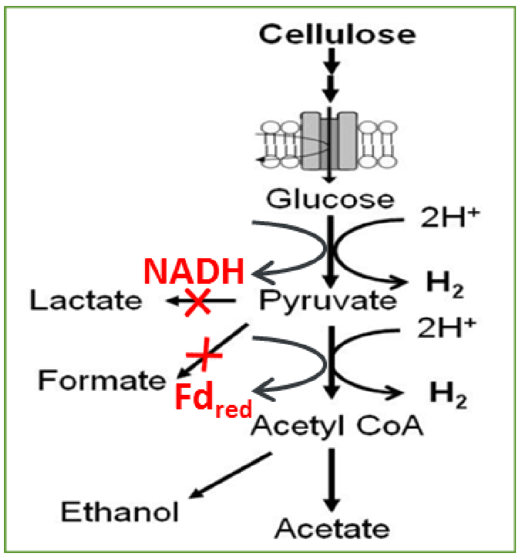


Results demonstrated bacteria’s capacity to utilize xylan, which makes further improvement promising.

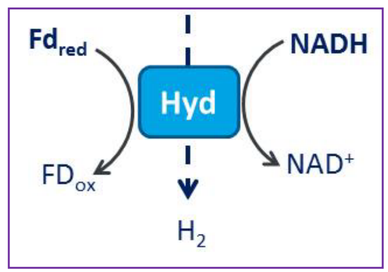
Approach
Task 3: Generate Metabolic Pathway Mutants

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

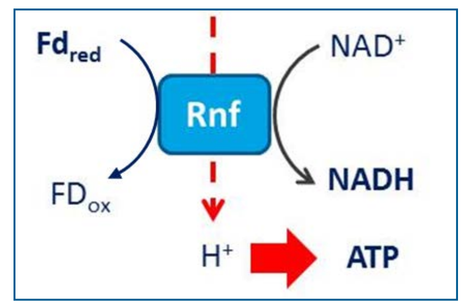
- Δ*pfl*: Blocking formate carbon-competing pathway led to **57% increase** in <u>rate</u> of H_2 production (2016 AMR accomplishment).
- Δ*rnf*: Manipulating electron-competing pathway led to **35% increase** in <u>total</u> H_2 production (2017 AMR accomplishment).



Hydrogenase



Transhydrogenase

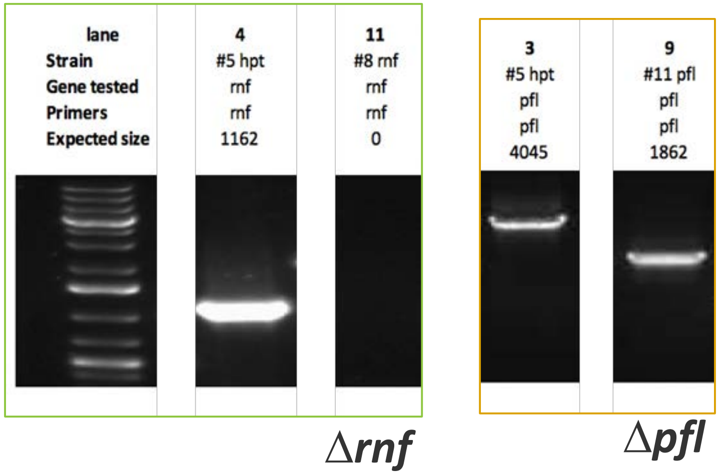


^{13}C-based metabolic flux analysis could probe metabolic changes responsible for increased H_2 production and guide genetic engineering.

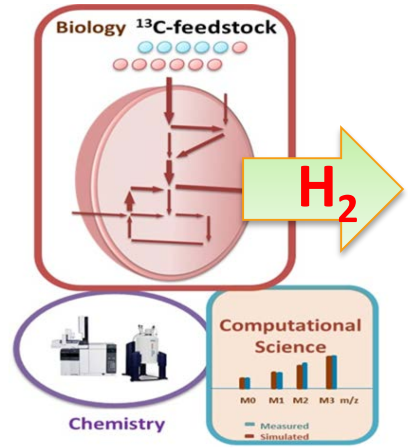
FY19 Q2 Milestone	Compare growth patterns of the wild-type and mutant strains lacking either the carbon (lactate, formate) competing pathway or deficient in electron inter-conversion (ferredoxin to NADH) by growing them in two different substrates, glucose and cellobiose of different energetics. The outcome will reveal how microbe manage carbon and electron flow toward increasing H_2 production. (NREL).	3/2019	Complete

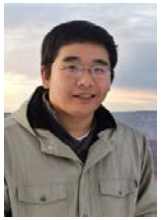
Task 3 Accomplishments and Progress:
High-throughput Phenotyping Analysis on H_2 Production Mutants

Genotype



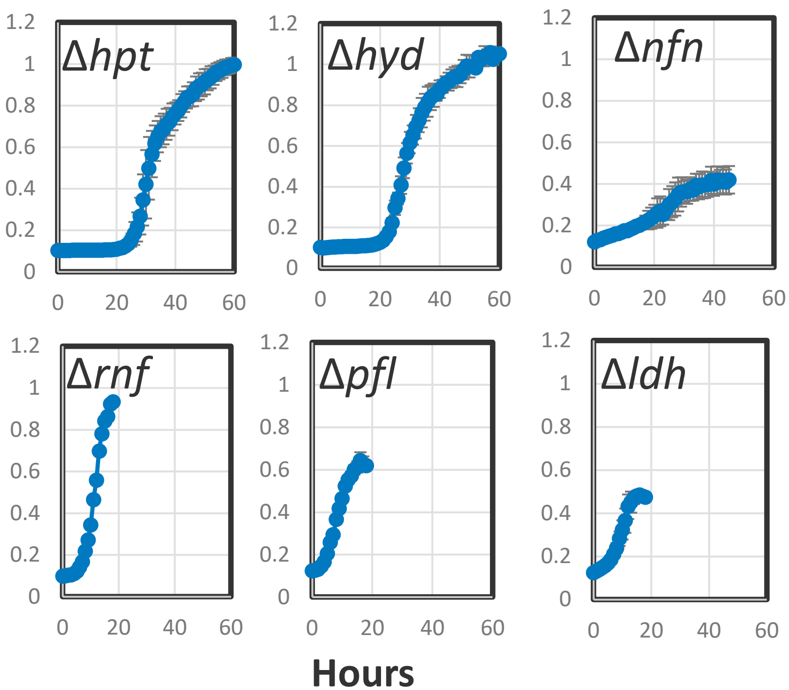
Fluxomic Phenotype





Wei Xiong

Growth Phenotype



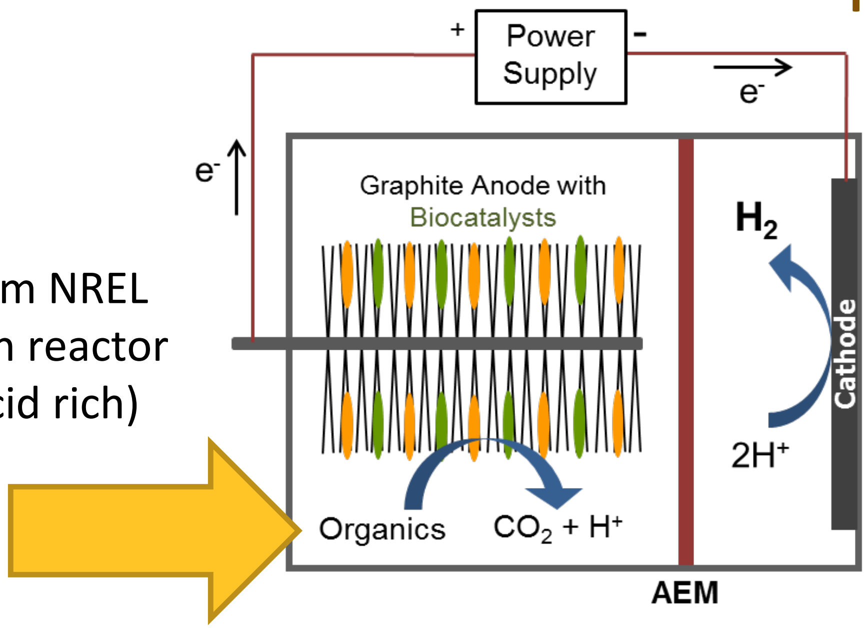
- **Goal:** Unravel metabolic changes responsible for increased H_2 production via ^{13}C-metabolic flux and growth analysis, using high-throughput GC-MS and data automation pipeline
- **Progress**: (**1**). Mapped the ^{13}C-flux of *Δhpt* as the baseline; (**2**). Analyzed the growth phenotype of H_2 production mutants.

Approach

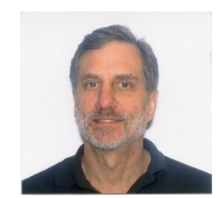
Task 4: Electrochemically Assisted Microbial Fermentation

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
FY19	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	Complete

Accomplishments and Progress : Alkaline Cathode Catalyst and Membrane Resistance

Goal: determine if alkaline pH could improve H_2 production

- Use activated carbon Ni (AC-Ni) cathodes with 8.8 mg/cm^2 Ni salt loading.

2 g/L acetate	H_2 Production
pH 7	0.31± 0.02 L/L-d
pH 12	0.4 ± 0.02L/L-d

31% increase

Goal: determine if anion-exchange membrane is the limiting factor

- Penn State has developed the "Electrode Potential Slope (EPS) to quantify internal resistance of various components including membrane.

Resistance	mΩ m^2
Total	120
Felt Anode	71 ± 5
Solution	25
Cathode	18 ± 2
Membrane	**6 ± 5**

Conclusion: With its low resistance, membrane is NOT a limiting factor, and will not test/select other membranes per the milestone.

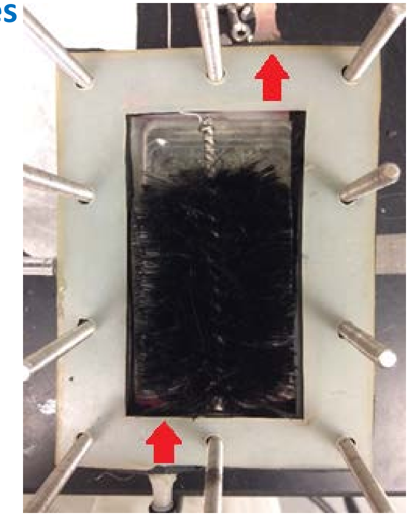
Accomplishment:
Achieve Stable Hydrogen Production of >2 L-H_2/L-d Over 90 Day Period

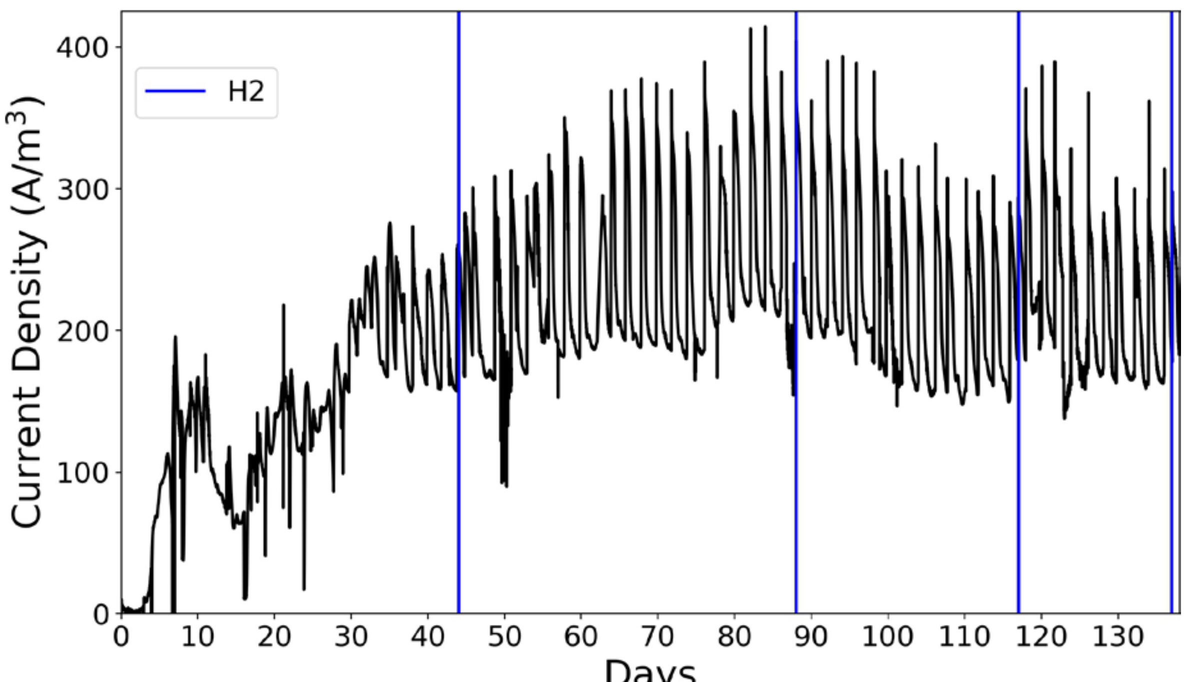
New MEC design: Reduce number of brush anodes from 7 to 1 to "fill chamber.

Anode: 1 brush anode (4.5cm diameter)
Cathode: 2 stainless steel wool cathodes
Electrolytes: both recirculated

Hydrogen production Average (90 d)= 2.62 L-H_2/L-d; Maximum = 3.76 L-H_2/L-d

Current density: Average (138 d) = 197 A/m^3 Maximum = 414 A/m^3





H_2 production average rate not yet increased by 30% over previous levels, but maximum rate was, and reactor stability was greatly improved

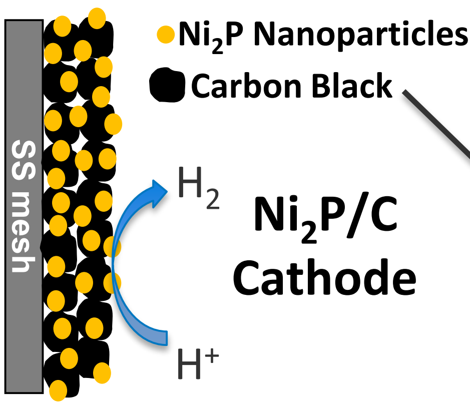
Future directions:

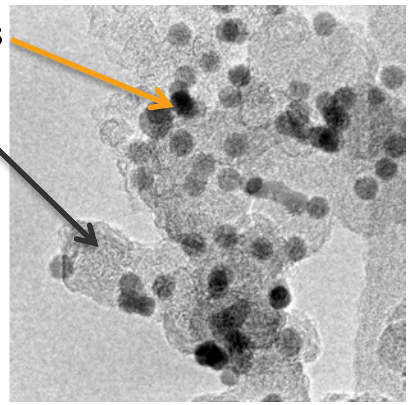
Increase anode brush diameter to 5.5 cm to completely fill the anode chamber

Emmanuel Fonseca (MS/PhD Student)

Task 4 Accomplishments and Progress:
Cathode Chamber Optimization: Replace Pt with alternative material

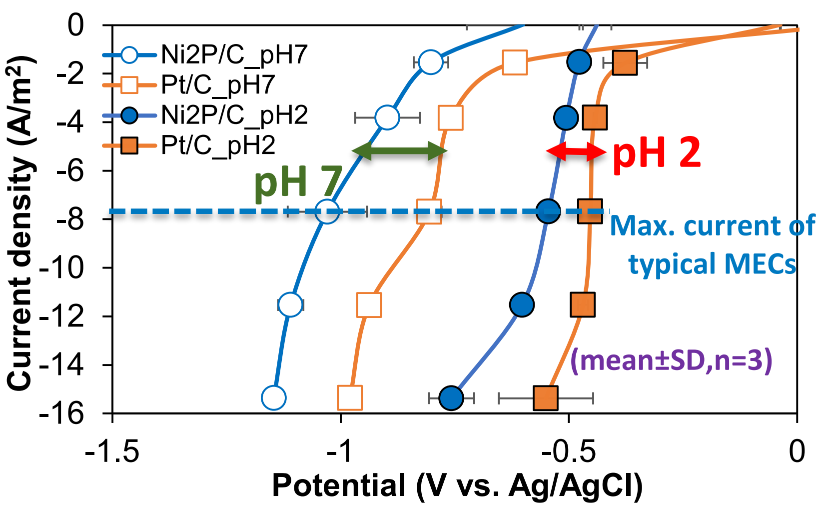
Nickel phosphide (Ni_2P) nanoparticles on carbon black (CB)

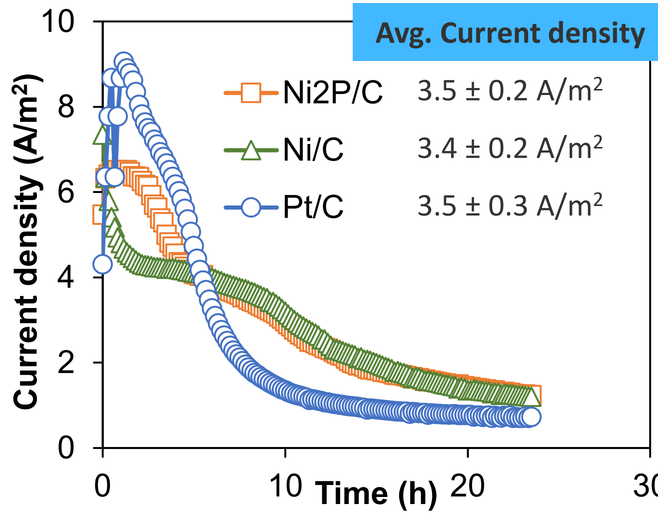




How does Ni_2P compare to Pt at pH=7 (vs pH=2) needed for MECs? (Abiotic electrochemical tests.)

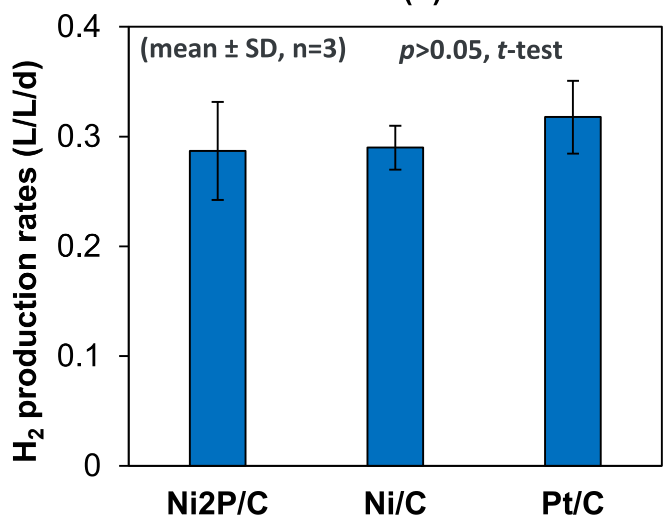
Pt/C works better at pH=7 (but also true at pH=2)





MEC tests: How does Ni_2P compare to Ni or Pt over a 24 h cycle?

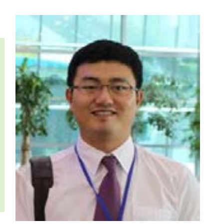
Result: Initially Ni_2P > Ni
(but not as good as Pt)



MEC tests: Final results in H_2 produced over a 24 h cycle?

Result: Insignificant different between the materials in averaged H_2 production (p>0.05)

Conclusion: Ni_2P/C cathode produced 0.29±0.04 L-H_2/L-d, comparable to a Pt cathode



Kyoung-Yeol Kim

Accomplishments and Progress: Responses to Previous Year Reviewers' Comments

This project was presented as a poster but was not reviewed during the 2018 AMR

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.
  - This challenge will be addressed by research in a separate project BioHydrogen Consortium, carried out by Lawrence Berkeley National Lab.

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 – addressed by research in a separate project “BioHydrogen Consortium”
  - Continue with adapted 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)

- The Penn State subcontract ended in January 17th, 2019.

Proposed Future Work: project is scheduled to close out in FY19/Q4

Task 1 (NREL)

- Subject 19-9 strain to laboratory adapted evolution by continuously evolving it in avicel (earlier evolution using cellobiose) and xylan and test H_2 production in bioreactors.

Task 2 (LBNL/SNL)

- None. Task 2 was discontinued in FY17/Q1.

Task 3 (NREL)

- Adapt the various *C. thermocellum* competing-pathway mutants to grow in cellobiose for comparison of carbon flux channeling through the different metabolic pathways leading to increased H_2 production. The outcome will guide metabolic engineering strategies to further improve H_2 production (FY19 Q3 Milestone).

Task 4 (Penn State):

- None. The Penn State subcontract ended on January 17th, 2019.

Closeout Report. A project closeout report will be submitted to DOE in FY19/Q4, documenting progress in the performance period of FY16-FY19 (FY19 Q4 Milestone).

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

- Using a laboratory-evolved strain we observed a **15%** increase in rate of H_2 production and **16%** in total H_2 production by co-fermenting both cellulose and xylan, benchmarking the progress.
- The evolved strain utilized **22%** more xylan, which accounts for the above increases.
- The outcomes lower the feedstock cost by converting more biomass sugars to H_2.

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

Task 3

- The genotype of the various mutants were verified via PCR.
- Using a 24-well plate Microplate Reader, we performed semi high-throughput growth of the various *C. thermocellum* mutants in cellobiose which will be used for ^{13}C-metabolic flux analysis.

Task 4

- Alkaline pH improved H_2 production by 31%.
- Achieve stable H_2 production of >2.6 L-H_2/L-d over 90 day period by increasing anode brush size.
- Anion exchange membrane is not a rate-limiting step in H_2 production.
- Ni_2P/C cathode produced 0.29±0.04 L-H_2/L-d, comparable to a Pt cathode.

Thank You

www.nrel.gov

Publication Number

Technical Back-Up Slides

(Include this “divider” slide if you are including back-up technical slides [maximum of five]. These back-up technical slides will be available for your presentation and will be included in Web PDF files released to the public.)