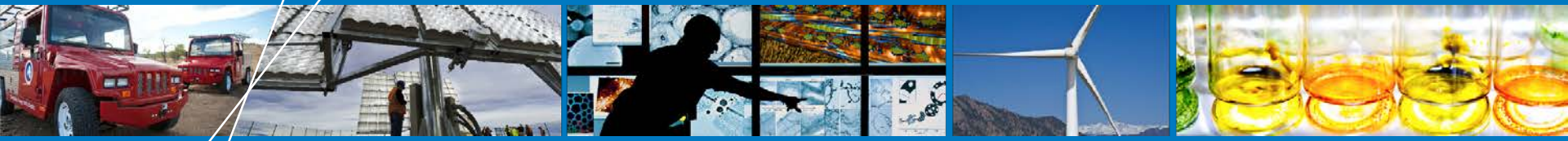
NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Biomass to Hydrogen (B2H2)



2016 Annual Merit Review and Peer Evaluation Meeting
June 7, 2016

Pin-Ching Maness (PI; Presenter); National Renewable Energy Laboratory

Bruce Logan (Presenter); Penn State University

Project ID #: PD038

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Overview

Timeline

- Project Start Date: FY16 (leveraging past NREL Fermentation and Electrohydrogenic Approaches project)
- Project End Date: 10/2016*

Barriers

Barriers addressed

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

Budget

- FY16 planned DOE Funding: $1M
- Total DOE funds received to date: $1M

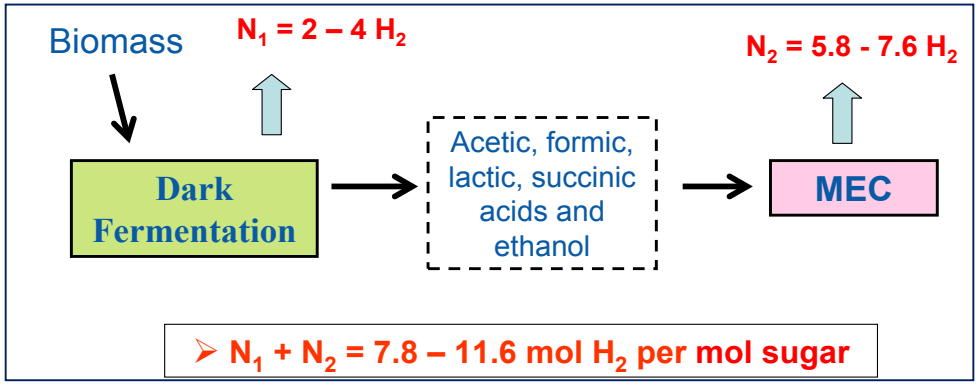
Partners

- Dr. Bruce Logan
  Pennsylvania State University
- Dr. Steven Singer, Lawrence Berkeley National Lab (LBNL)
- Drs. John Gladden and Ken Sale, Sandia National Lab (SNL)

*Project continuation and direction determined annually by DOE

Relevance

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



Directly Address Barriers

- Feedstock cost (AY): via bioreactor development using lignocellulose (Task 1), and biomass pretreatment via ionic liquid (Task 2).
- Hydrogen molar yield (AX) (N_1 & N_2: mol H_2/mol hexose): via genetic engineering (Task 3) and integration with Microbial Electrolysis Cell (MEC) (Task 4)

Address Key DOE Technical Targets

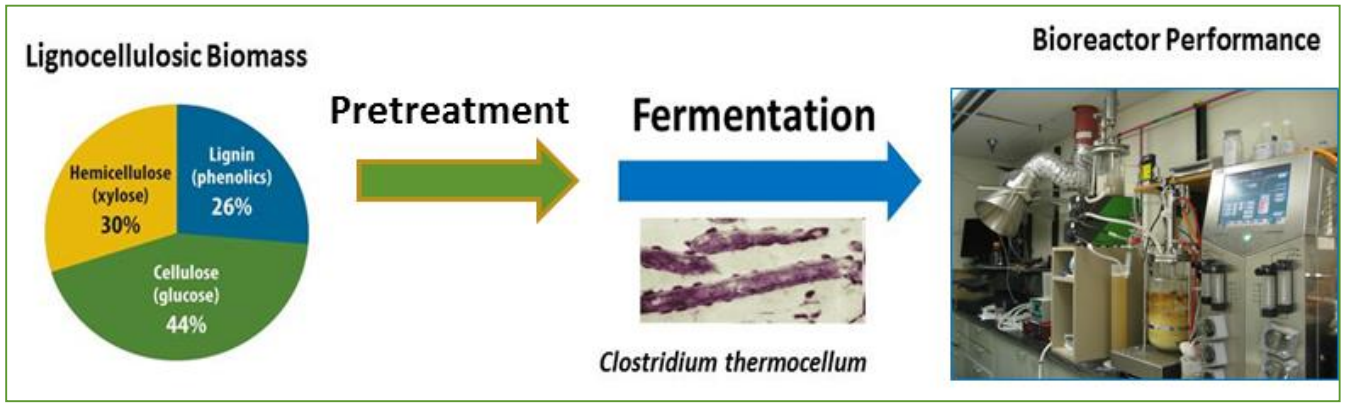
Characteristics	Units	2011 Status	2015 Target	2020 Target
Feedstock cost[a]	Cents/lb sugar	13.5	10	8
Yield of H_2 production from glucose	Mol H_2/mol glucose	**3.2**[b]	4	6
MEC production rate	L-H_2/L-reactor-day	-	1	4

a. Status and target of the DOE Bioenergy Technology Office (BETO) – leverage BETO funding.
b. Low carbon substrate loading (1 g/L) led to high H_2 molar yield.

Approach

Task 1: Bioreactor Performance

- **Approach:** Optimize bioreactor in batch and fed-batch modes by testing parameters such as corn stover lignocellulose loadings (PCS or DMR), hydraulic retention time (HRT), and liquid volume replacement and frequency, using the cellulose-degrading bacterium *Clostridium thermocellum*, one of the fastest cellulose-degraders.



Pretreated Corn Stover – Acid Hydrolysis (PCS)

1% H_2SO_4

160-190°C

Solid: 59% cellulose, 27.5% lignin → H_2

Liquid: 90% xylan, 9% glucan

More sugar loss, more inhibitors.

Pretreatment – De-acetylated and Mechanically Refined (DMR)

0.4% NaOH

70°C

Solid: 42% cellulose, 25% xylan, 16% lignin → H_2

Liquid: 79% acetate, 2.9% xylan

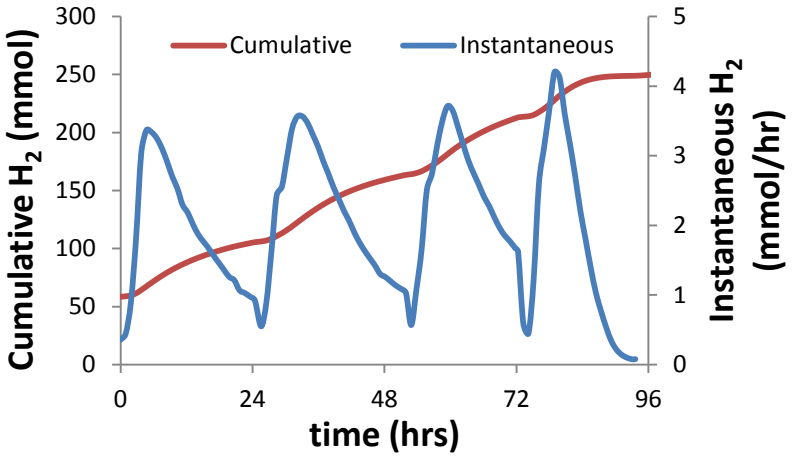
Less sugar loss, less inhibitors.

Task 1 – Accomplishments/Progress

H_2 from DMR Corn Stover and Carbon Mass Balance

Lauren Magnusson

- DMR corn stover retains more intact biomass structure, hence more recalcitrant. Yet it can be fermented directly to H_2 by *C. thermocellum* without adding expensive enzyme cocktail.
- Mass analysis indicated (batch fermentation):
  - 94% total DMR solid was solubilized;
  - 98% cellulose solid was consumed by microbe;
  - 97% of xylan is solubilized, genetic engineering would to convert xylose to H_2 also.



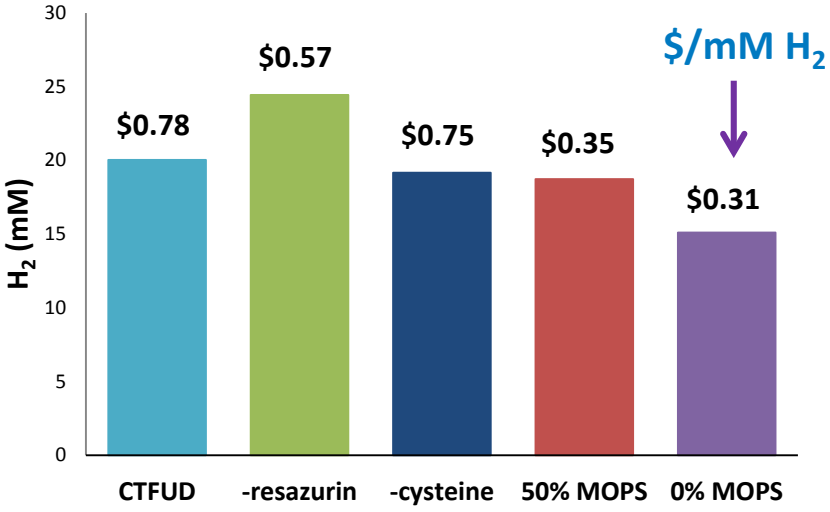
Cellulose 5 g/L	Dry Weight	Glucan (g/L)	Xylan	Lignin
Initial	11.9 +/- 0.37	5.3 +/- 0.38	3.7 +/- 0.02	1.7 +/- 0.08
Final	0.6 +/- 0.28	0.1 +/- 0.02	0.1 +/- 0.02	0.1 +/- 0.03
Solid Hydrolyzed (%)	94% +/- 0.01	98% +/- 0.00	97% +/- 0.01	91% +/- 0.02

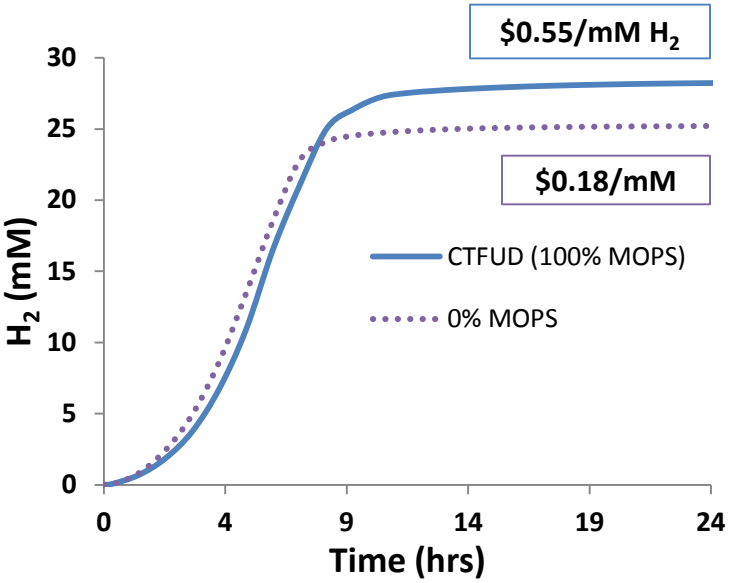
- Sequencing Fed-Batch fermentation of 5 g/L/d DMR produced an **average** H_2 production rate of **791 mL H_2/$L_{reactor}$/d at a 48 h HRT (max rate = 1.6 L H_2/$L_{reactor}$/d).**
- Higher substrate loading will meet toward Q4 milestone.

	FY16 Milestone (regular) - NREL		
Q4	Optimize the hydraulic/solid retention time with respect to H_2 production and media utilization by testing HRT between 12 and 48 h in a sequencing fed-batch reactor, and obtain a continuous average H_2 production rate of 1L/$L_{reactor}$/d using DMR.	9/2016	On Track

Task 1 – Accomplishments/Progress

Reduce Medium Cost to Lower H_2 Selling Price





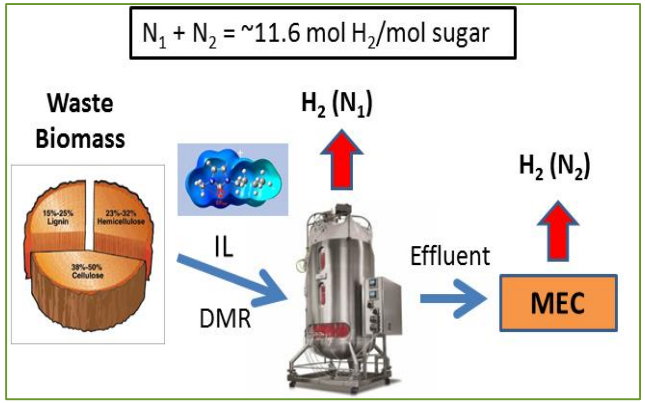
- The normal complex growth medium (CTFUD) has buffer (MOPS) and yeast extract (0.45%; w/v).
- Eliminating resazurin ($1.57/L $_{medium}$; redox-sensing dye) and cysteine ($1.31/L $_{medium}$; poising redox potential) have no effect on H_2 production, nor cell growth.
- MOPS buffer is costly ($10.92/L $_{medium}$) yet essential for pH control and cell fitness.
- Similar H_2 output in bioreactor with pH control, with or without MOPS, the latter lowers final cost of H_2 from $0.55 to $0.18/mM H_2.

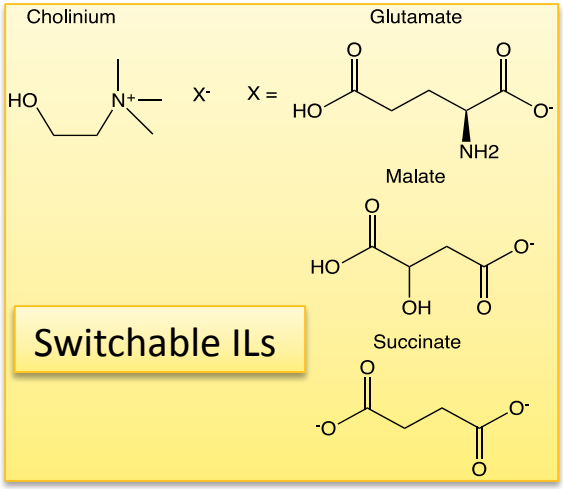
	FY16 Milestone (regular) - NREL		
Q2	Evaluate components in the *C. thermocellum* growth medium, eliminate, reduce, or replace one to two nutrients with minimal impact to cell fitness aimed to reduce medium nutrient cost.	3/2016	Complete

Approach

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

- Ionic liquids (IL) has been proposed as a method for biomass pretreatment, driven by the combination of electrostatic and hydrogen-bonding interactions between the IL and plant polymers.
- In a parallel and complimentary approach to NREL, LBNL/SNL will test three biocompatible ILs in biomass pretreatment followed by fermentation and compare with NREL DMR pretreatment in cost and H_2 output.





Task Leads

Steve Singer
LBNL

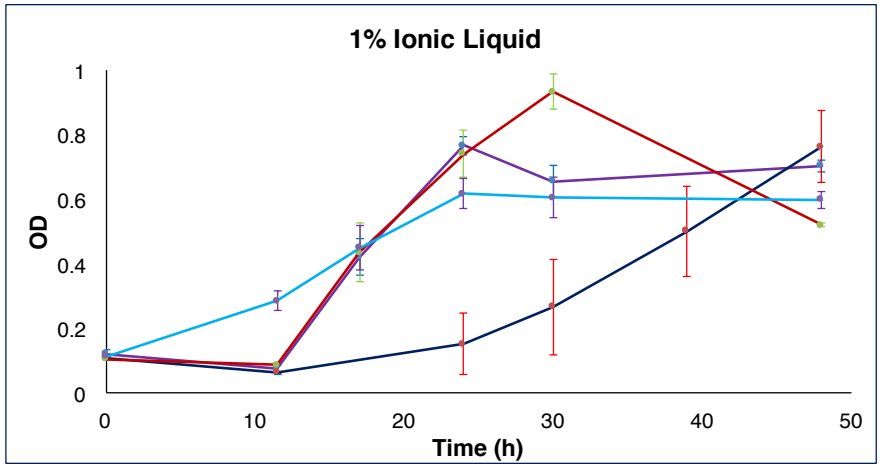
John Gladden
Ken Sale, SNL

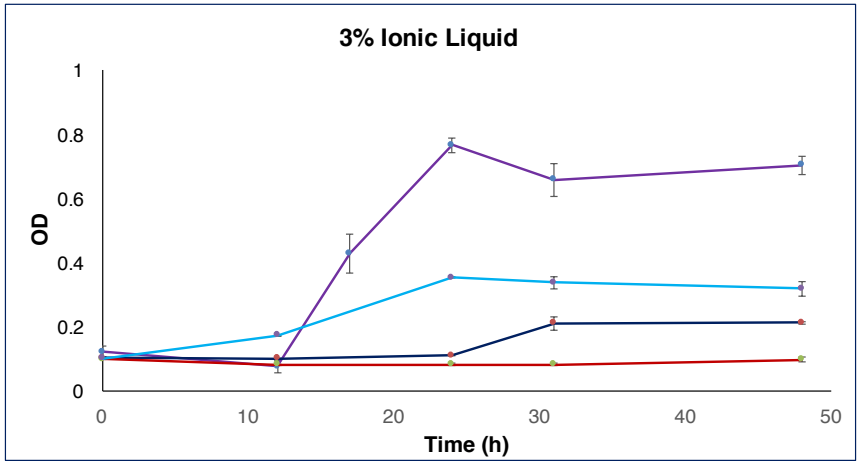
	FY16 Milestone (regular)	Completion Date	Status
Q1	Develop procedures at LBNL to grow *C. thermocellum* (LBNL)	12/2015	Complete
Q1	Perform corn stover pretreatment with three biocompatible ionic liquids and perform compositional analysis of the pretreated biomass (SNL)	12/2015	Complete

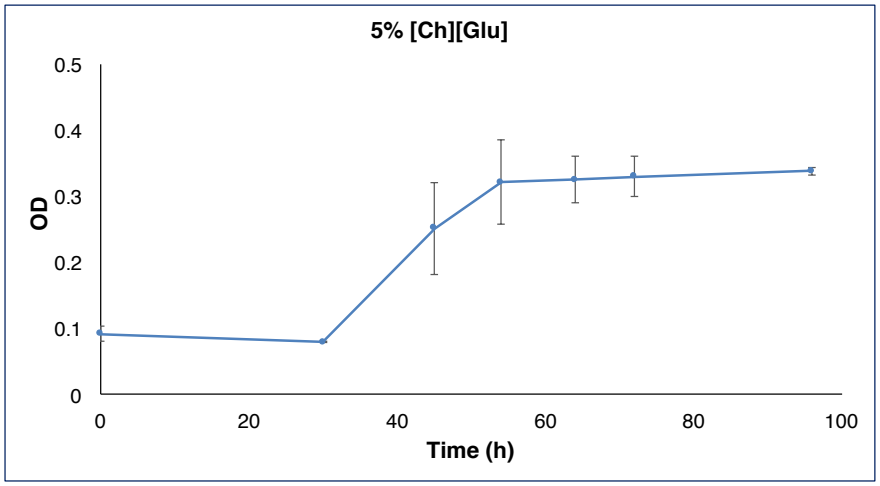
Task 2 – Accomplishments/Progress

Biocompatibility of Ionic Liquid Biomass for H_2 Production (LBNL)

- *C. thermocellum* showed no growth at ≥3% of ionic liquids.
- One culture with 5% cholinium glutamate grew after 9 days; growth in 5% [Ch][Glu] was maintained in successive cultures.







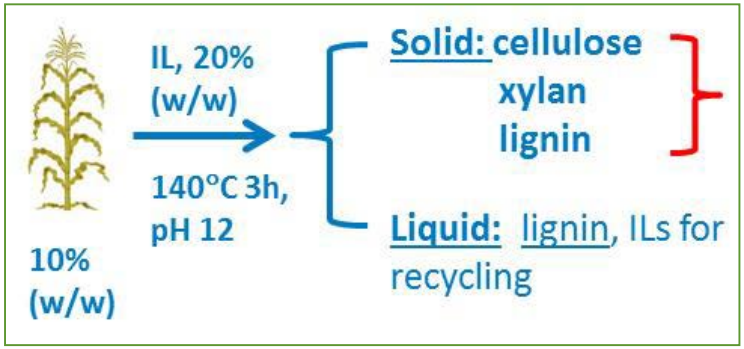
Control
[Ch][Suc]: Cholinium paired with succinate
[Ch][Mal]: Cholinium paired with malate
[Ch][Glu]: Cholinium paired with glutamate

- Culture grown in 5% [Ch][Glu] is being adapted to grow on 6-8% [Ch][Glu].
- Agar plating of *C. thermocellum* culture with tolerance to 6-8% [Ch][Glu] will isolate individual clones to test for tolerance to 10% [Ch][Glu] (Q2 milestone).

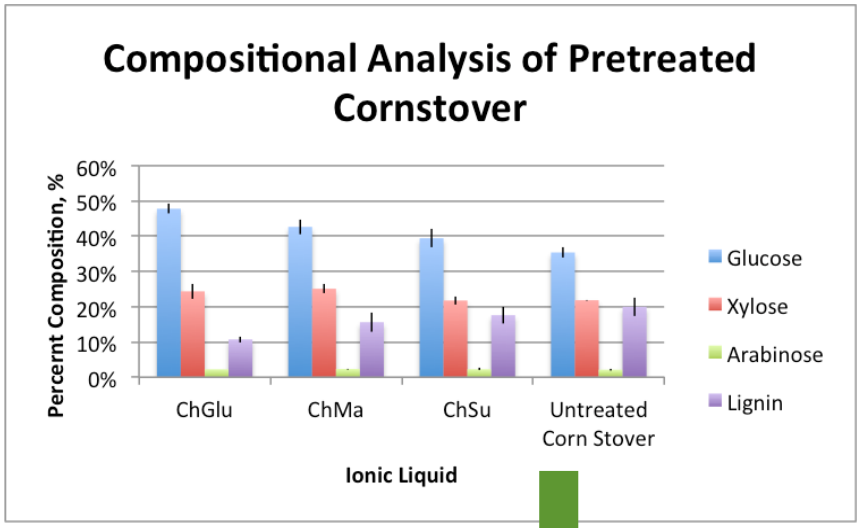
Task 2 – Accomplishments/Progress

Biomass Pretreatment via Ionic Liquid (SNL)

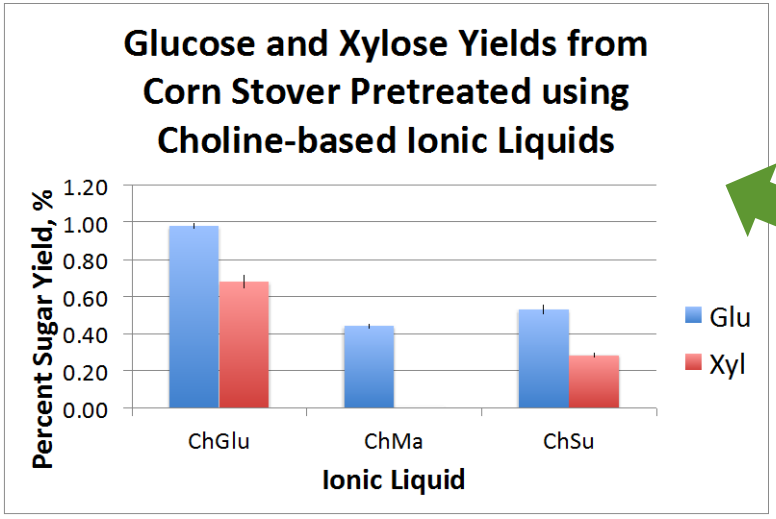
NREL sent untreated corn stover and SNL pretreated with three ionic liquids successfully: [Ch][Glu], [Ch][Mal], and [Ch][Su]



(A) Sugar Yields via Acid Hydrolysis



(B) Sugar Yields via Cellulase Enzyme Cocktail



Complete FY16/Q1 milestone

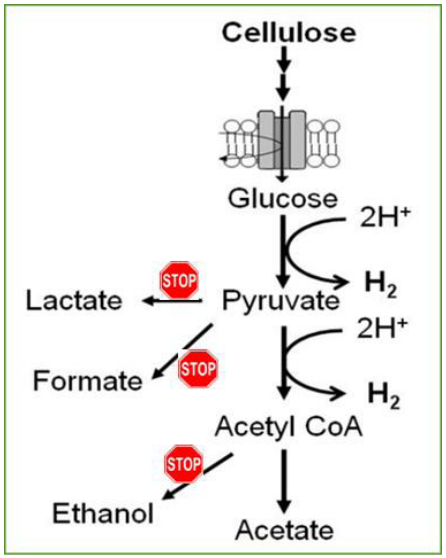
Complete FY16/Q2 milestone: Use cellulase enzyme cocktail to measure amounts of glucose and xylose release.

[Ch][Glu] has the best sugar recovery

Approach/Milestones

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

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



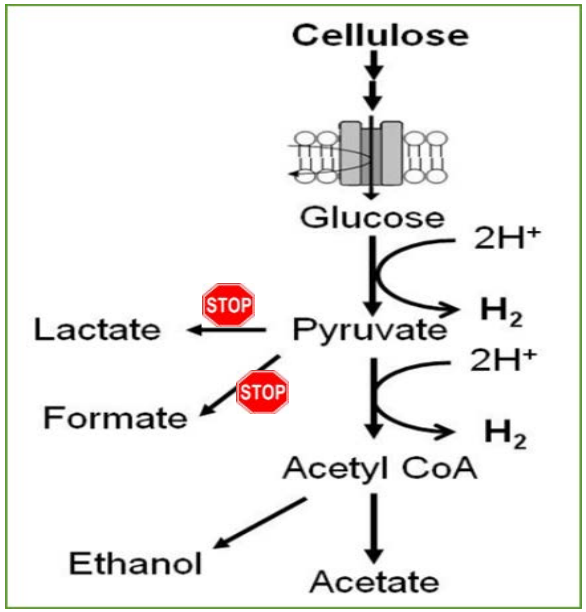
- NREL developed proprietary genetic tools in *C. thermocellum* that very few labs can rival (FY14 accomplishments).
- Single mutants lacking either lactate dehydrogenase (LDH, pyruvate-to-lactate step) or pyruvate-formate lyase (PFL; pyruvate-to-formate step) have been generated, but NOT in a <u>combined</u> strain (FY14/15 accomplishments).
- The goal in FY15/FY16 is to delete both competing pathways in the same strain and determine outcomes on H_2 production.

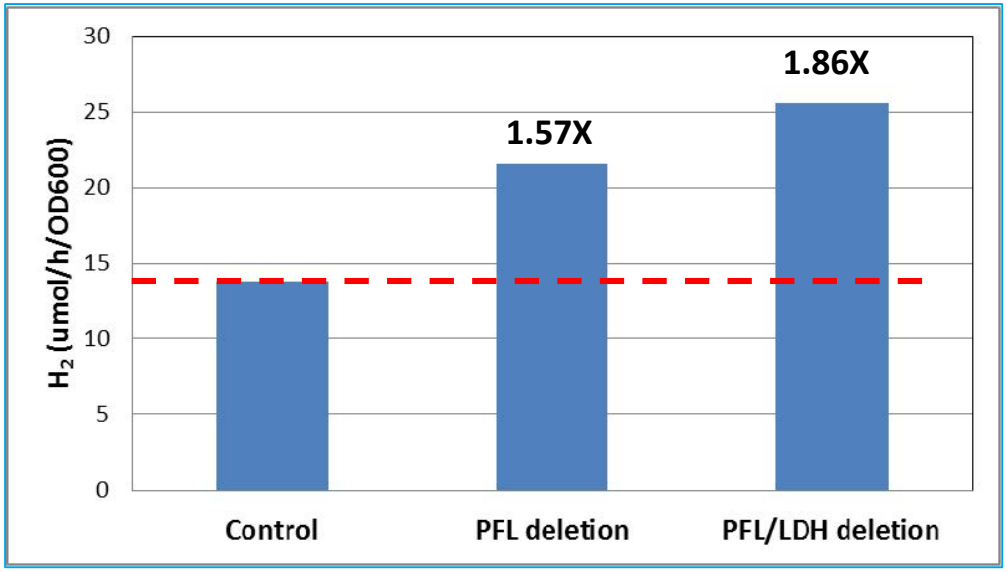
	FY16 Milestone – Regular	Com-pletion Date	Status
Q3	Measure transcriptional expression profiles of the three hydrogenases as well as H_2 production, the latter in rate and volume, at early-log, mid-log, and late-log phases of cell growth to best predict their role in either H_2 production or H_2 consumption, which will guide future genetic engineering strategy (NREL)	6/2016	Complete

Task 3 – Accomplishments/Progress

Delete Both Competing pathways to Make Formate and Lactate

- Generated double mutant in *C. thermocellum* lacking both the pyruvate-to-lactate step (conserves 2 electrons) and pyruvate-to-formate step (conserves more pyruvate) aimed to produce more H_2.
- The double mutant displayed **~90%** increase in specific rate of H_2 production.
- The double mutant did not produce formate and with negligible amount of lactate.





Rate of H_2 production can be increased by deleting competing pathways.

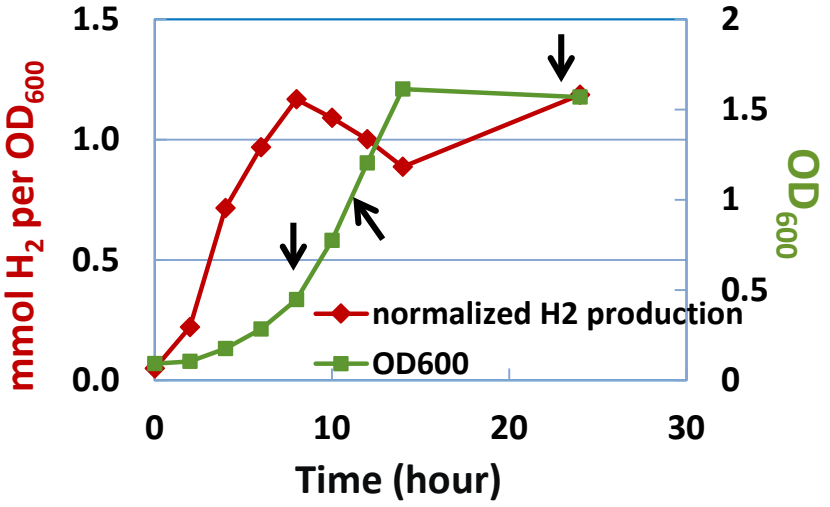
Katherine Chou

Task 3 – Accomplishments/Progress

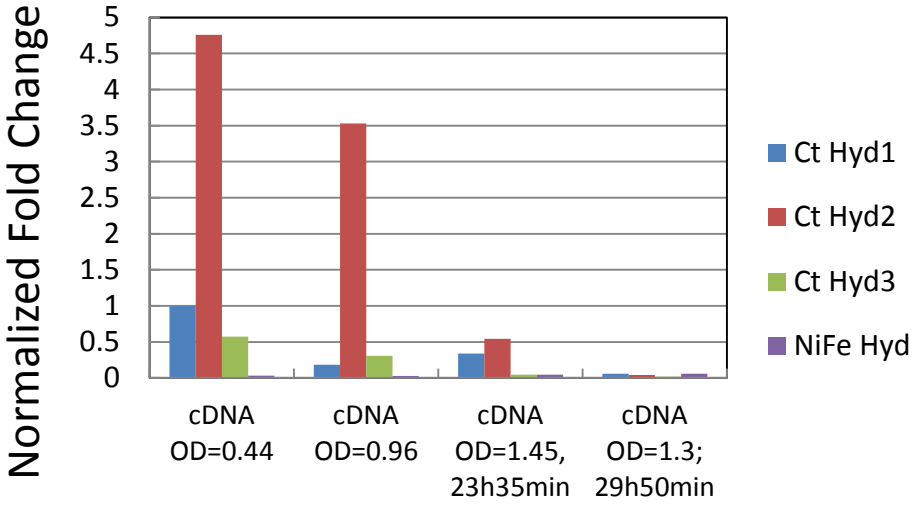
Hydrogenase Expression Profiles

- H_2 production (A) peaked at mid-log phase of growth (OD ~ 0.45), catalyzed by three FeFe-hydrogenases (Hyd1, 2, 3), and a NiFe-hydrogenase.
- Using real-time quantitative PCR (qRT-PCR) (B), **Hyd2** expression profile coincided with peak H_2 production, suggesting that Hyd2 plays a major role in H_2 production – meeting Q3 Milestone.

(A). Cell Growth and H_2 Production



(B). Hydrogenase Expression (qRT-PCR)



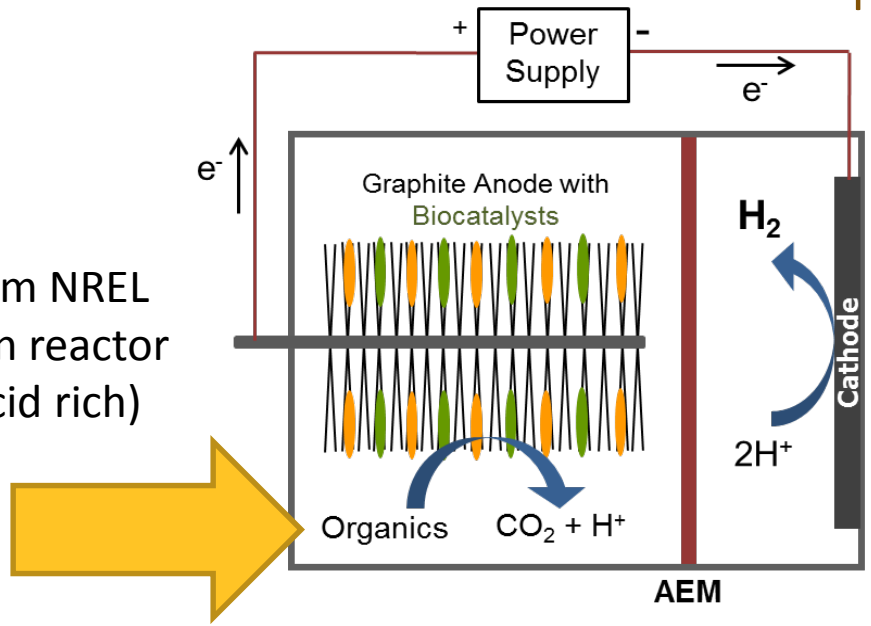
➢ **Hydrogenase expression profiles suggest that over-expression of Hyd2 most likely will boost H_2 production, which guides metabolic engineering strategy.**

Approach/Milestone

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 **1.2 L-H_2/L-reactor/d** over 3 HRT, using NREL fermentation effluent.

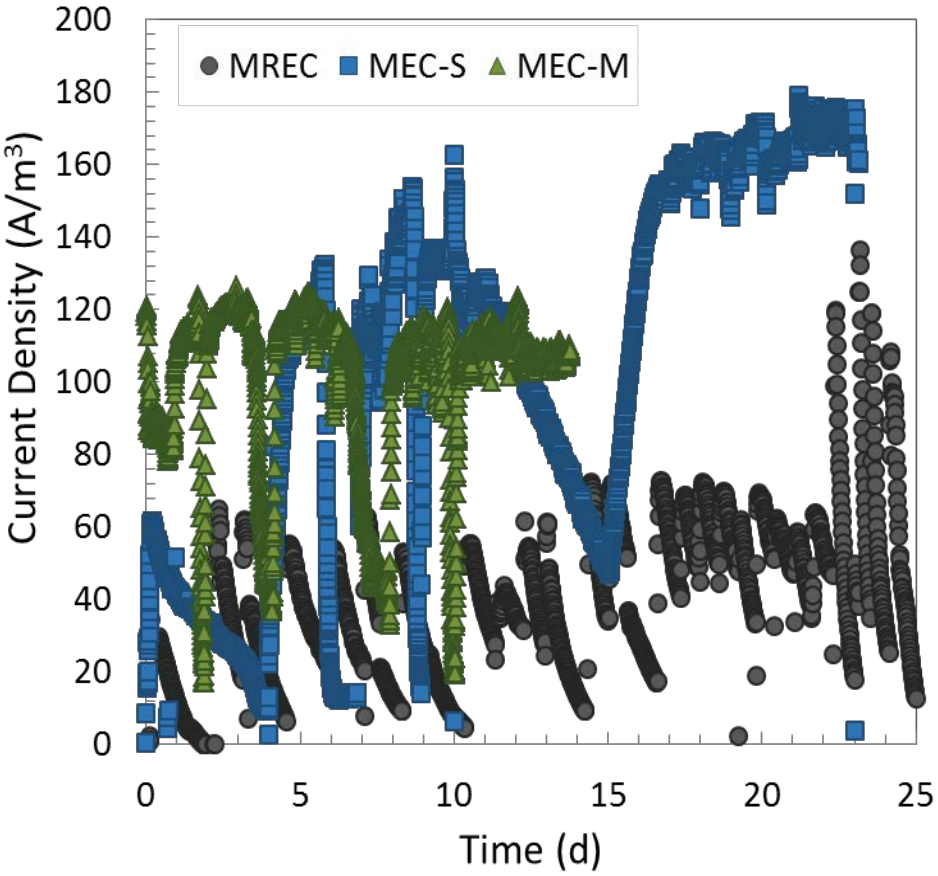
	Milestones	Completion Date	Status
FY15	Optimize the design of the cathode chamber to increase the volumetric hydrogen production rate to 1.2 L H_2/$L_{reactor}$/d (over 3 HRT, using synthetic effluent) in a continuous flow MEC, using Pt/C cathodes and improved configurations.	9/2015	Complete
FY16	Design MEC cathodes with reduced width to increase maximum H_2 production rate to 1.2 L/Lreactor/day based on overall reactor volume reduction.	9/2016	On Track

Task 4 – Accomplishments/Progress

Cathode Chamber Optimization: Reduction in Volume

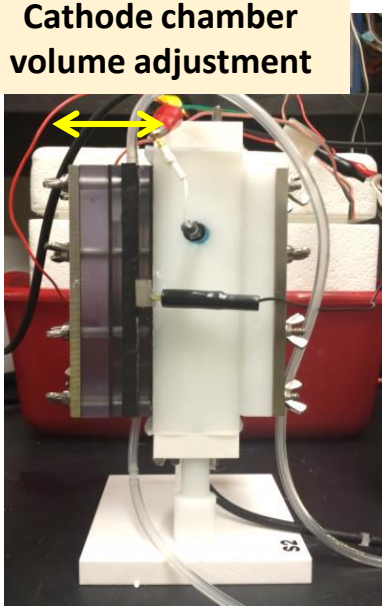
Hydrogen production in the MEC was increased to **1.4±0.2 L-H_2/L-reactor/d** by decreasing volume of the cathode chamber (76 mL → 28 mL). Rates comparable to MEC with a reverse electrodialysis (RED) stack.

These experiments met the 2015 milestone (1.2 L-H_2/L-reactor/d).



MEC-S
(S=small cathode chamber: 28 mL)
MEC-M
(M= modified cathode chamber: 76 mL)
MREC
(Result from previous MREC study)

Cathode chamber volume adjustment

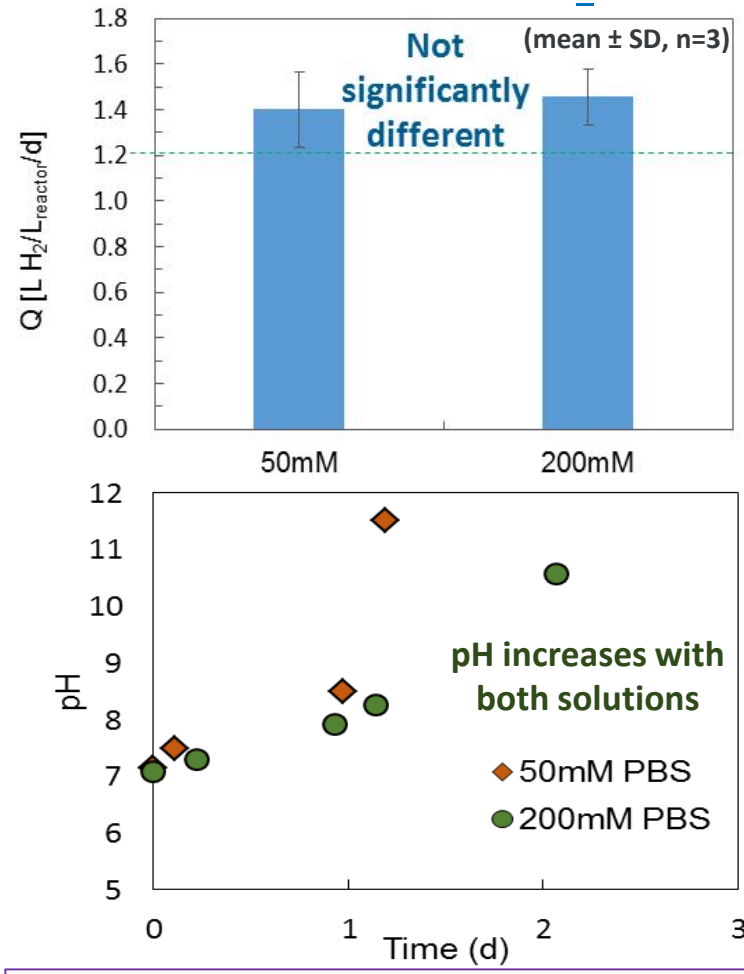


Kyoung Yeol Kim

Task 4 – Accomplishments/Progress

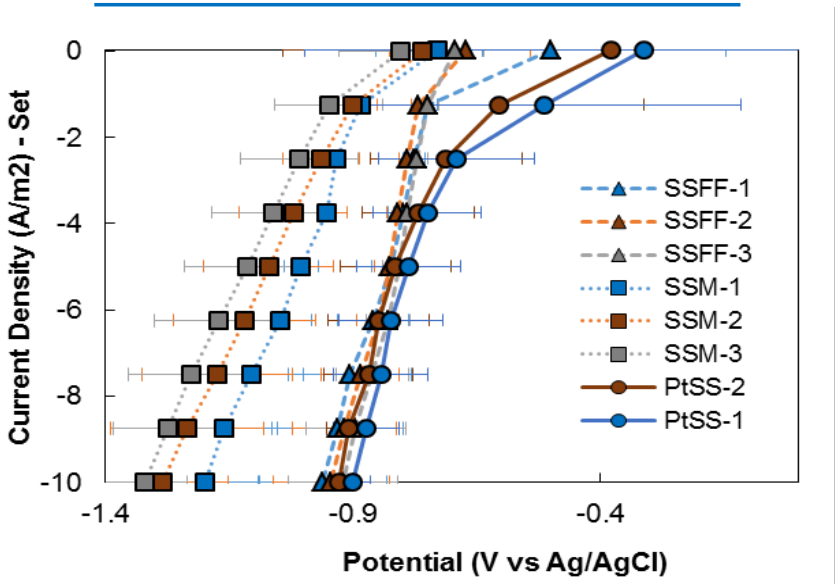
Cathode Chamber Optimization

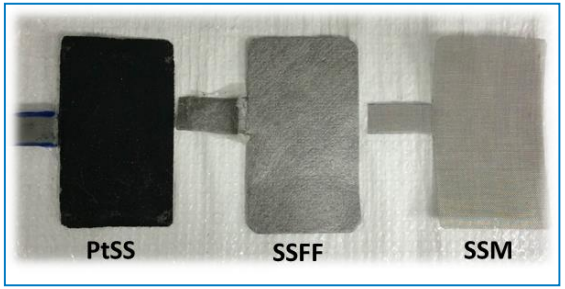
Impact of buffer/pH on H_2 production



- Change in pH did not adversely affect H_2 production with >1.2 L-H_2/L-reactor/d.
- Can achieve compact reactor design.

Electrochemical Performance





Cathodes tested:

PTSS: Pt/C on SS mesh

SSFF, non-Pt: SS Fiber Felt

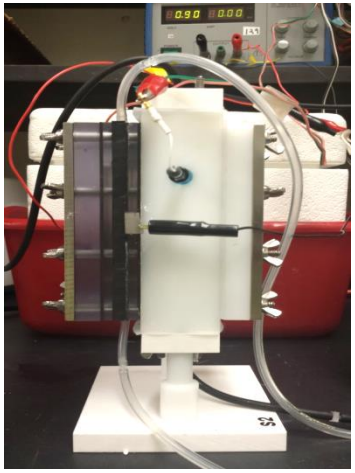
SSM: SS mesh only

Stainless steel (SS) mesh showing good promise of matching performance of Pt/C; further tests needed.

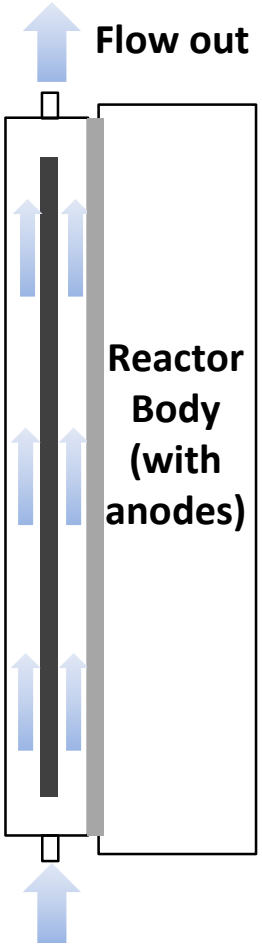
Task 4 – Accomplishments/Progress

MEC Cathode Flow Chamber Optimization

MEC with continuous flow adjustable volume cathode chamber

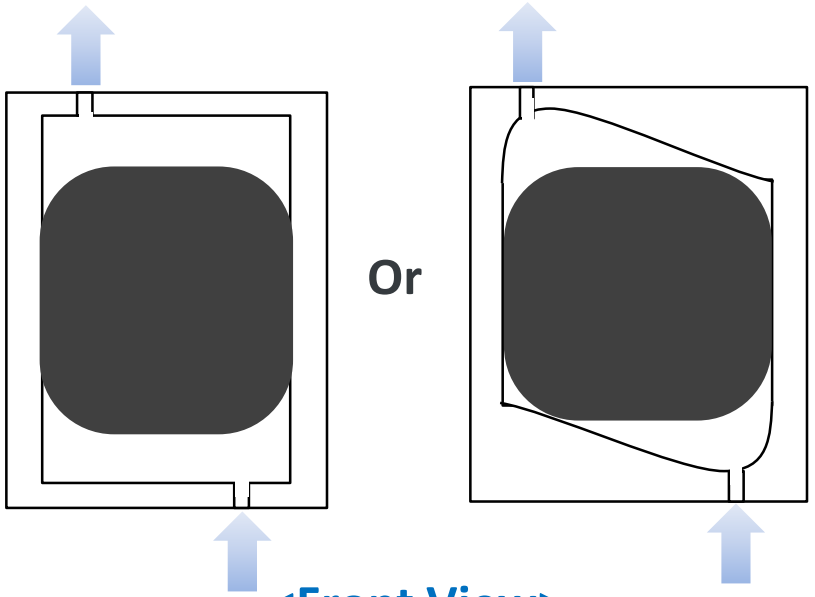


Flow chamber
MEC



Anion exchange membrane
Cathode

<Side View>



<Front View>

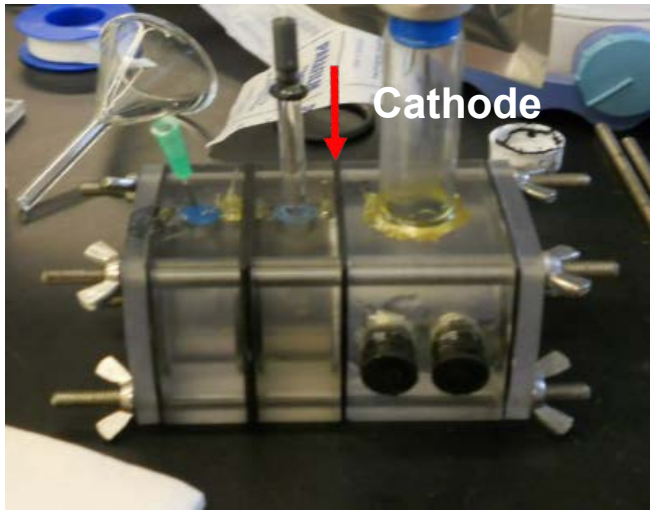
- Examine electrochemical performance of cathode by **improving hydrodynamics** of flow through thin chambers

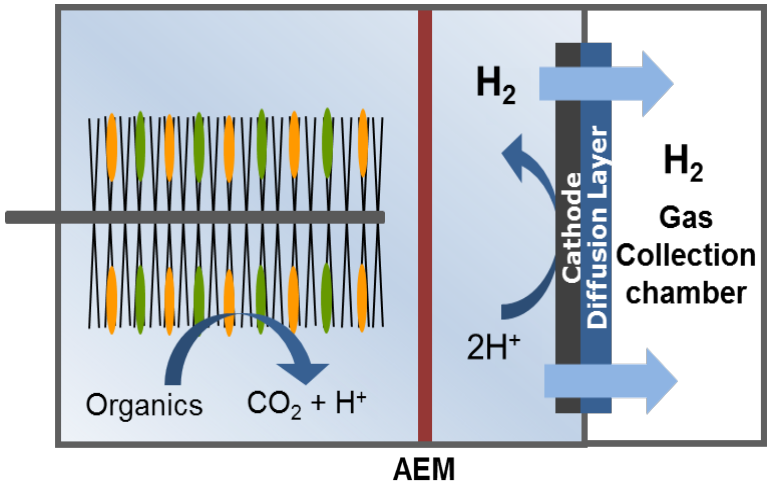
- Examine **alternative materials and catalysts** for the cathode.
→ Metal-loaded carbon fibers; and nitrogen, phosphorous, and sulfur-loaded activated carbon fibers.

Task 4 – Accomplishments/Progress

Gas Diffusion Chamber: Abiotic tests

Gas Diffusion Cathode (3 chamber-setup). **Goal is to eliminate liquid catholyte**

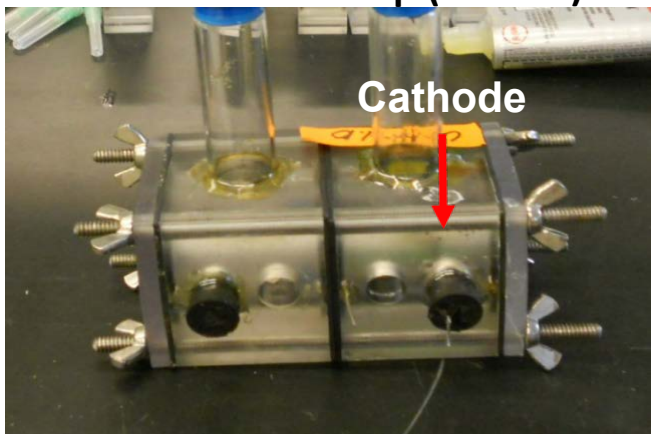


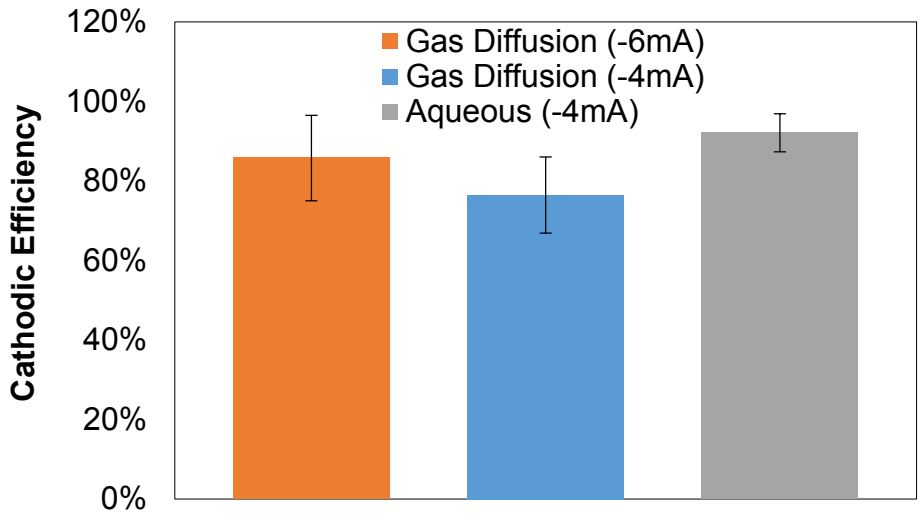


Catalyst layer:
Carbon black (CB) 10%
Platinum with PDMS binder

Gas diffusion layer:
Activated carbon (AC) with PVDF binder.

Aqueous Cathode-
2 Chamber-Setup (Control)





Preliminary test results: Cathodic efficiencies were significantly different ($p<0.05$) according to the type of cathode chamber.

Response to Previous Year Reviewers' Comments

- **No Reviewers' Comments - the previous fermentation project was presented but *not reviewed* in the 2015 AMR.**

- **This new fermentation project was started in FY2016 responding to a FCTO Lab Call via collaboration with Penn State, LBNL, and SNL.**

Collaborations

- **Task 1 (Bioreactor)**

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

- **Task 2 (Ionic Liquid)**

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

- **Task 3 (Genetic Methods)**

  Drs. David Levin and Richard Sparling at the University of Manitoba, Canada: NREL is an international collaborator of the Genome Canada Grant award to pathway engineering in *C. thermocellum* - leveraging Canadian 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 challenge to ensure sufficient mixing.
  - Impeller design with high power/low torque will address this challenge.

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

- Overcoming potential toxicity of high concentrations of ionic liquid on microbes.
  - Acclimate microbes to tolerate high levels of ionic liquid.

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

- Deleting competing pathways to increase H_2 molar yield might cause a redox imbalance (excess NADH) and hinder mutant generation.
  - Over-express hydrogenase-encoding genes to maintain redox balance.

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

- Current designs use precious metal catalysts, which must be avoided to make the process economical, but further improvements in performance are needed.
  - Demonstrate equal or improved performance using non-precious metal alternatives to Pt.
  - Further reduce reactor size by improving hydrodynamics or eliminating aqueous flow.

Proposed Future Work

Task 1 (NREL)

- Optimize sequencing-fed batch reactor using DMR corn stover to obtain average rate of 1 L $H_2/L_{reactor}/d$ (FY16 Q4 Milestone).
- Test "pretreatment" of de-acetylated biomass with *C. thermocellum's* cellulosomes (cellulase enzyme cocktail) to accelerate the initial fermentation kinetics (FY16/17).

Task 2 (LBNL/SNL)

- Adapt *C. thermocellum* to grow robustly in 10% cholinium glutamate and test H_2 production (FY16).

Task 3 (NREL)

- Repeat hydrogenase expression profile experiment and verify the role of hydrogenase 2 in catalyzing H_2 production, aimed to meet Q3 milestone (FY16).
- Over-express hydrogenase 2 to balance electrons and increase H_2 production (FY16/17).

Task 4 (Penn State)

- Improve electrochemical performance of cathode by achieving better hydrodynamics of flow through thin cathode chambers (FY16).
- Examine alternative materials and catalysts for the cathode (FY16/17).
- Further examine gas diffusion cathode for improving reactor operation (FY16/17).

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

- 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

- Eliminate MOPS as a costly nutrient and lower overall cost of H_2 production in bioreactor.
- Achieved an average H_2 production rate of 791 mL/L/d fermenting DMR cellulose directly to H_2. 97% of the xylan was also solubilized, genetic engineering will convert xylose to H_2 also.

Task 2

- Pretreated corn stover with three ionic liquids. Obtained highest glucose and xylose yields with the [Ch][Glu] after saccharification using cellulase enzyme cocktail.
- Tested three ionic liquid and improved tolerance of *C. thermocellum* to 5% of [Ch][Glu] ionic liquid via adaptation strategy.

Task 3

- Generated double mutant lacking both lactate- and formate-competing pathways leading to ~90% increase in specific rate of H_2 production.
- Hydrogenase expression profiles reveal Hyd2 is most active in H_2 production.

Task 4

- A more compact reactor design was successful since pH changes did not adversely affect H_2 production. PSU achieved the 2015 milestone of > 1.2 L-H_2/L-reactor/d.
- SS fiber felt (SSFF) cathode showed good promise of matching performance of cathodes containing Pt in electrochemical tests. Further MEC tests are ongoing.
- Preliminary tests show aqueous cathode still better than gas diffusion cathode.