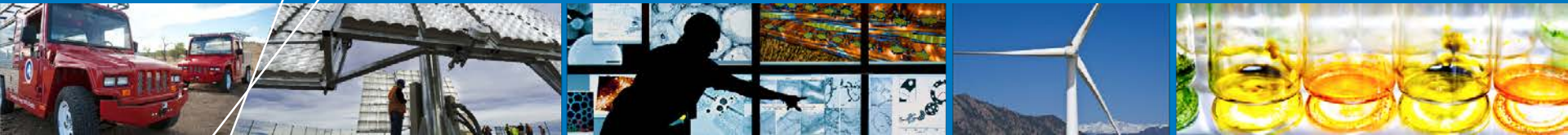


# Biomass to Hydrogen (B2H2)



**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

## Timeline

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

## Budget

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

## Barriers

Barriers addressed

- H<sub>2</sub> molar yield (AX)
- Feedstock cost (AY)
- System engineering (AZ)

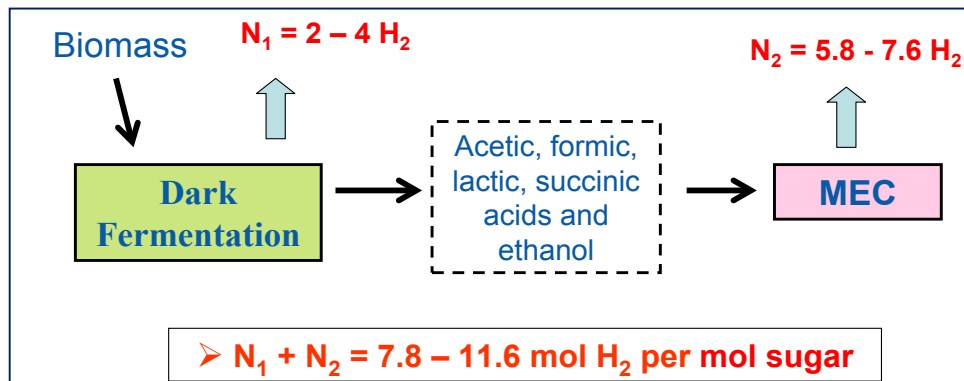
## Partners

- 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 <sup>a</sup>	Cents/lb sugar	13.5	10	8
Yield of $H_2$ production from glucose	Mol $H_2$ /mol glucose	<b>3.2<sup>b</sup></b>	4	6
MEC production rate	L- $H_2$ /L-reactor-day	-	1	4

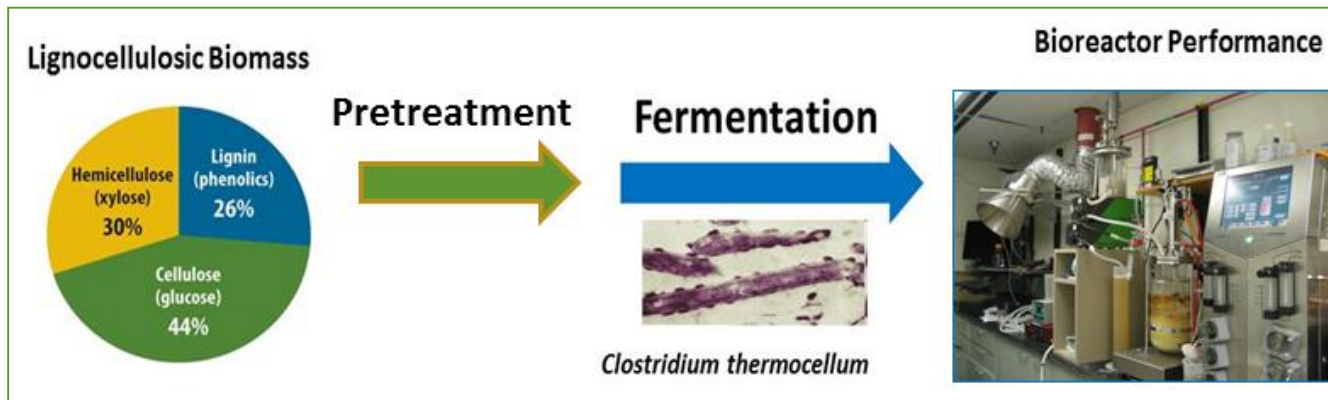
a. Status and target of the DOE Bioenergy Technology Office (BETO) – leverage BETO funding.

b. Low carbon substrate loading (1 g/L) led to high  $H_2$  molar yield.

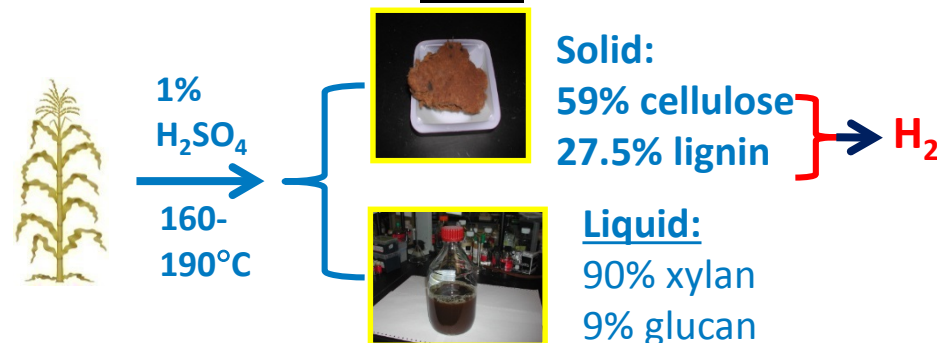
# Approach

## 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.

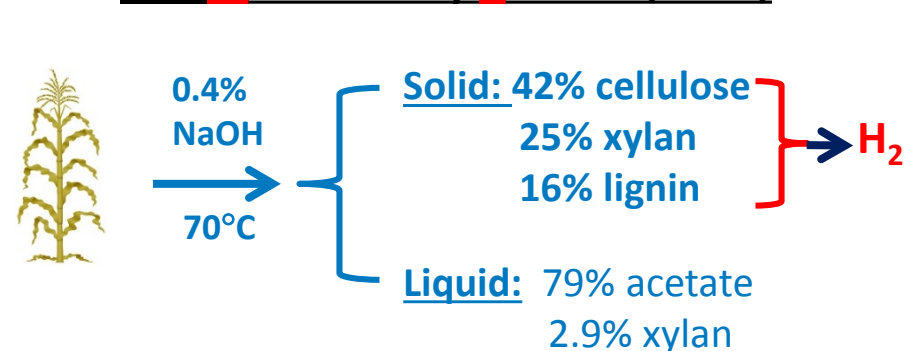


### Pretreated **C**orn **S**tover – Acid Hydrolysis (PCS)



More sugar loss, more inhibitors.

### Pretreatment – **D**e-acetylated and **M**echanically **R**efined (DMR)



Less sugar loss, less inhibitors.

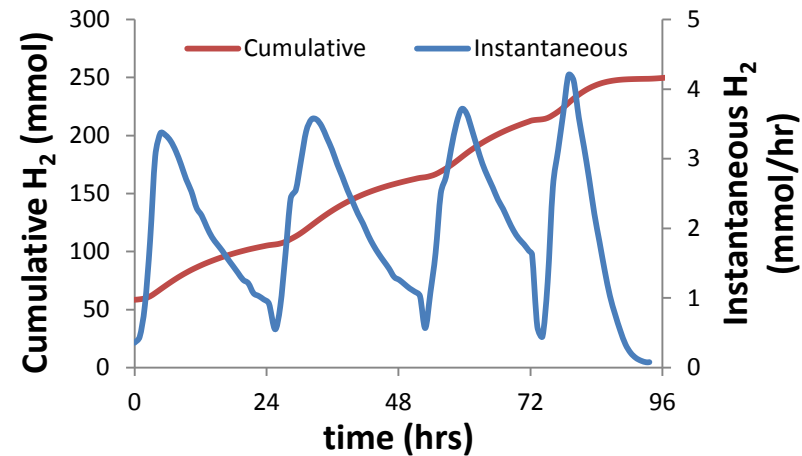
# Task 1 – Accomplishments/Progress

## H<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> also.



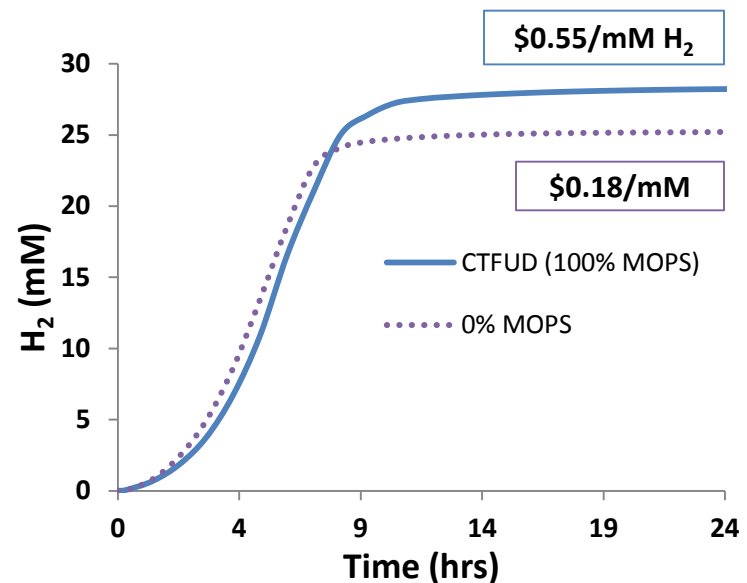
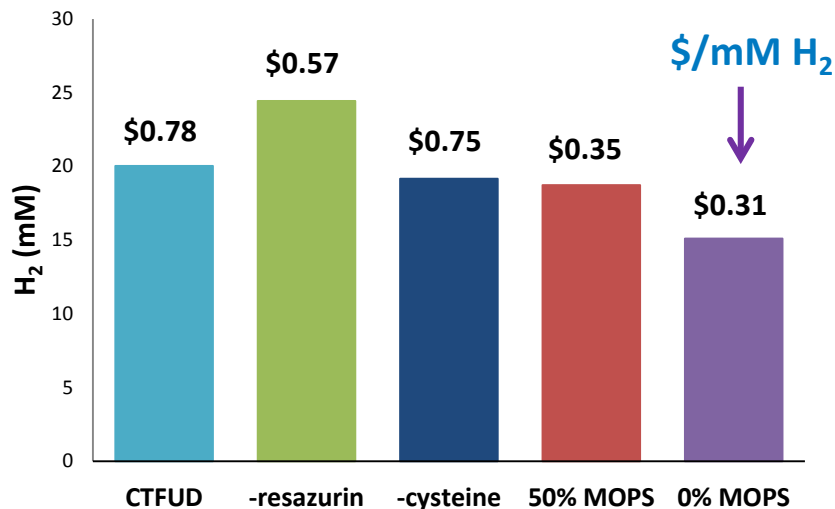
- Sequencing Fed-Batch fermentation of 5 g/L/d DMR produced an **average** H<sub>2</sub> production rate of **791 mL H<sub>2</sub>/L<sub>reactor</sub>/d** at a **48 h HRT** (max rate = **1.6 L H<sub>2</sub>/L<sub>reactor</sub>/d**).
- Higher substrate loading will meet toward Q4 milestone.

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

FY16 Milestone (regular) - NREL		
<b>Q4</b>	Optimize the hydraulic/solid retention time with respect to H <sub>2</sub> production and media utilization by testing HRT between 12 and 48 h in a sequencing fed-batch reactor, and obtain a continuous average H <sub>2</sub> production rate of 1L/L <sub>reactor</sub> /d using DMR.	9/2016
		On Track

# Task 1 – Accomplishments/Progress

## Reduce Medium Cost to Lower H<sub>2</sub> Selling Price



- The normal complex growth medium (CTFUD) has buffer (MOPS) and yeast extract (0.45%; w/v).
- Eliminating resazurin (\$1.57/L<sub>medium</sub>; redox-sensing dye) and cysteine (\$1.31/L<sub>medium</sub>; poisoning redox potential) have no effect on H<sub>2</sub> production, nor cell growth.
- MOPS buffer is costly (\$10.92/L<sub>medium</sub>) yet essential for pH control and cell fitness.
- Similar H<sub>2</sub> output in bioreactor with pH control, with or without MOPS, the latter lowers final cost of H<sub>2</sub> from \$0.55 to \$0.18/mM H<sub>2</sub>.

### 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<sub>2</sub> output.

### Task Leads

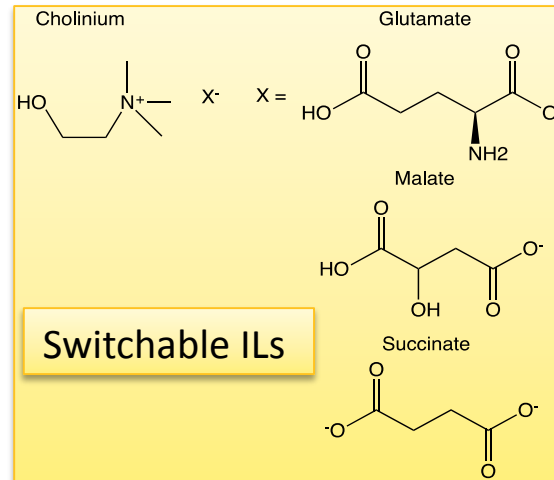
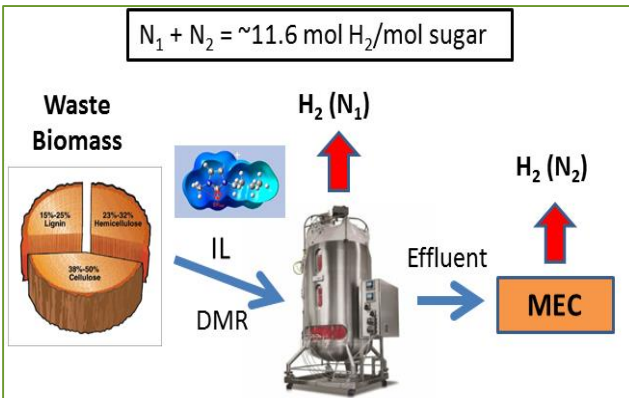
Steve Singer  
LBNL



John Gladden  
Ken Sale, SNL



$$N_1 + N_2 = \sim 11.6 \text{ mol H}_2/\text{mol sugar}$$



### FY16 Milestone (regular)

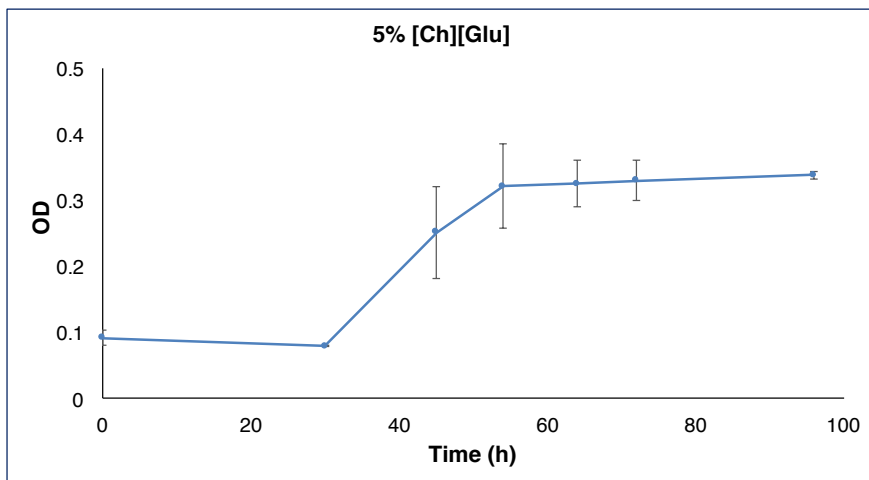
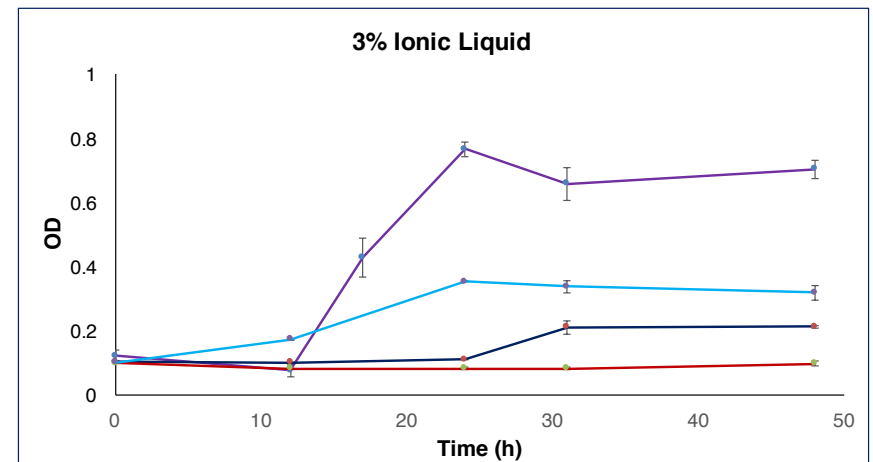
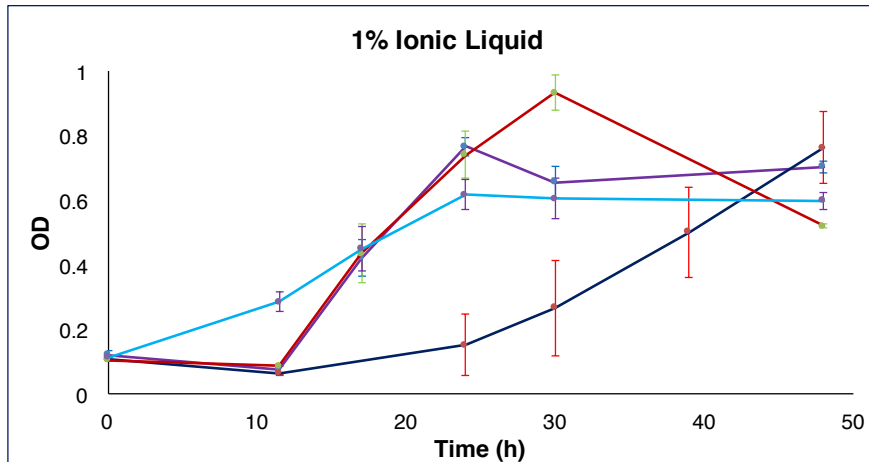
	FY16 Milestone (regular)	Completion Date	Status
Q1	Develop procedures at LBNL to grow <i>C. thermocellum</i> (LBNL)	12/2015	Complete
Q1	Perform corn stover pretreatment with three biocompatible ionic liquids and perform compositional analysis of the pretreated biomass (SNL)	12/2015	Complete

# Task 2 – Accomplishments/Progress



## Biocompatibility of Ionic Liquid Biomass for H<sub>2</sub> Production (LBNL)

- *C. thermocellum* showed no growth at  $\geq 3\%$  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

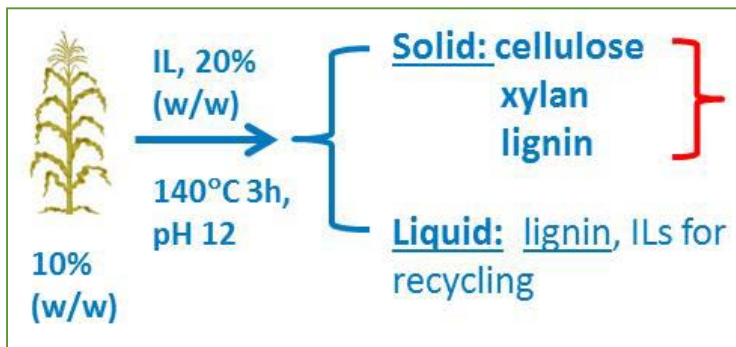
- Culture grown in 5% [Ch][Glu] is being adapted to grow on 6-8% [Ch][Glu].
- Agar plating of *C. thermocellum* culture with tolerance to 6-8% [Ch][Glu] will isolate individual clones to test for tolerance to 10% [Ch][Glu] (Q2 milestone).



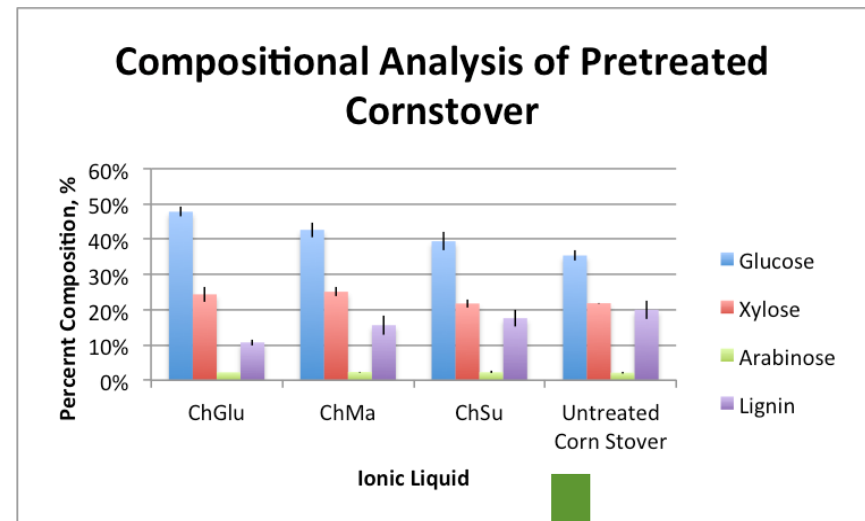
# Task 2 – Accomplishments/Progress

## Biomass Pretreatment via Ionic Liquid (SNL)

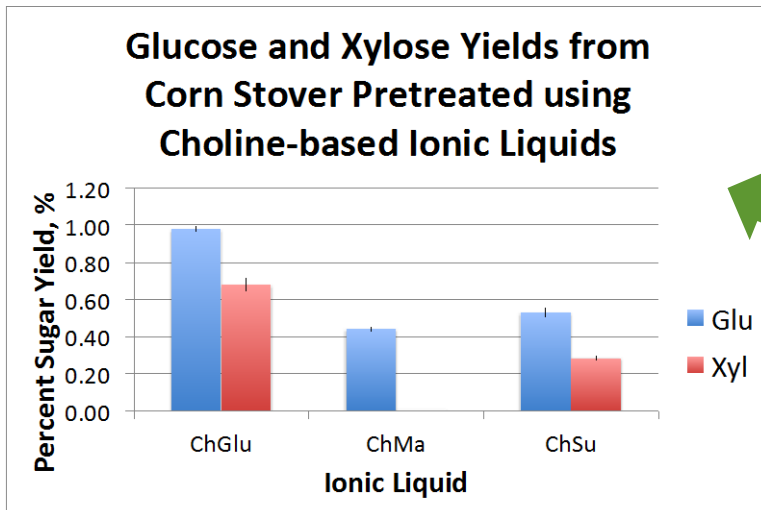
NREL sent untreated corn stover and SNL pretreated with three ionic liquids successfully: [Ch][Glu], [Ch][Mal], and [Ch][Su]



### (A) Sugar Yields via Acid Hydrolysis



### (B) Sugar Yields via Cellulase Enzyme Cocktail



Complete FY16/Q1 milestone

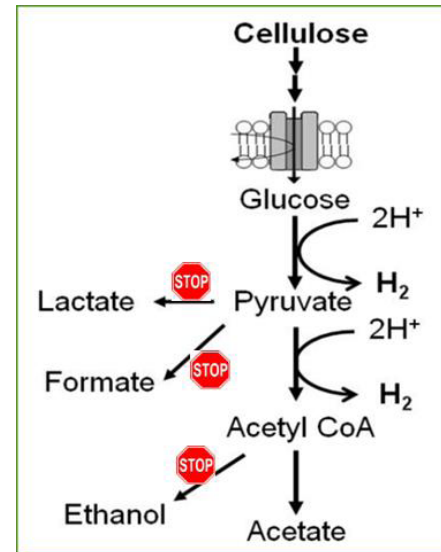
Complete FY16/Q2 milestone: Use cellulase enzyme cocktail to measure amounts of glucose and xylose release.

**[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<sub>2</sub> molar yield via developing genetic methods.



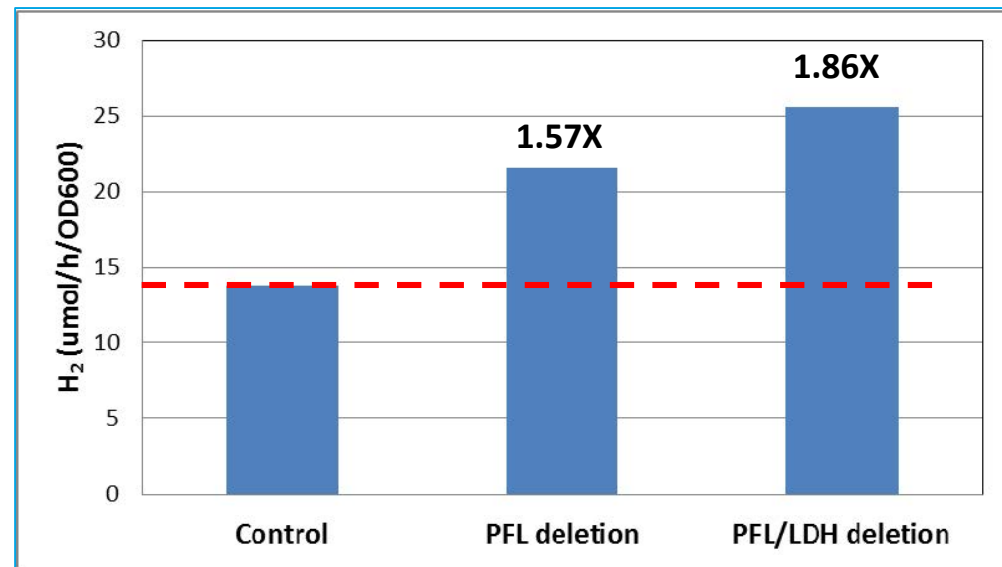
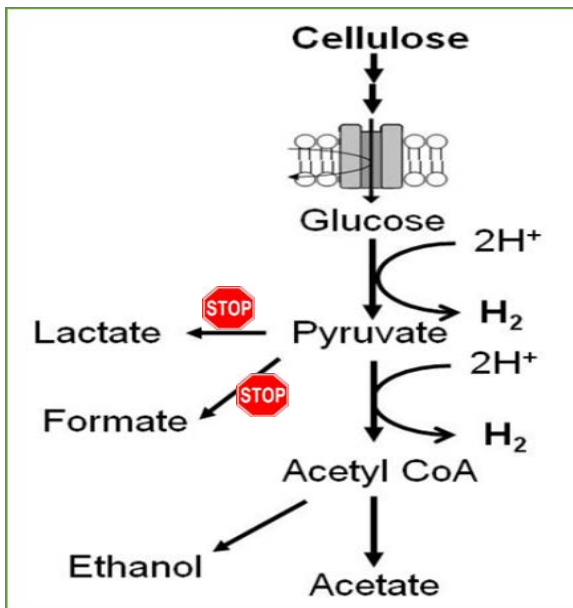
- NREL developed proprietary genetic tools in *C. thermocellum* that very few labs can rival (FY14 accomplishments).
- Single mutants lacking either lactate dehydrogenase (LDH, pyruvate-to-lactate step) or pyruvate-formate lyase (PFL; pyruvate-to-formate step) have been generated, but NOT in a combined strain (FY14/15 accomplishments).
- The goal in FY15/FY16 is to delete both competing pathways in the same strain and determine outcomes on H<sub>2</sub> production.

	<b>FY16 Milestone – Regular</b>	<b>Completion Date</b>	<b>Status</b>
<b>Q3</b>	Measure transcriptional expression profiles of the three hydrogenases as well as H <sub>2</sub> 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 <sub>2</sub> production or H <sub>2</sub> 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<sub>2</sub>.
- The double mutant displayed ~**90%** increase in specific rate of H<sub>2</sub> production.
- The double mutant did not produce formate and with negligible amount of lactate.



Rate of H<sub>2</sub> production can be increased by deleting competing pathways.



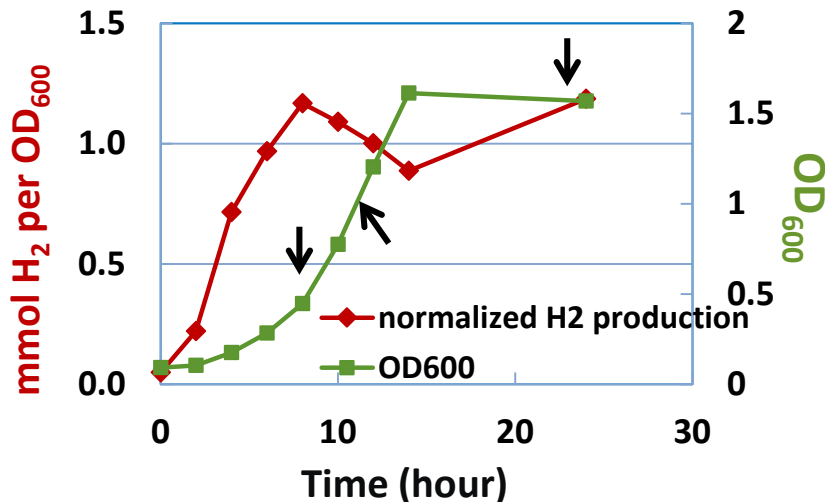
Katherine Chou

# Task 3 – Accomplishments/Progress

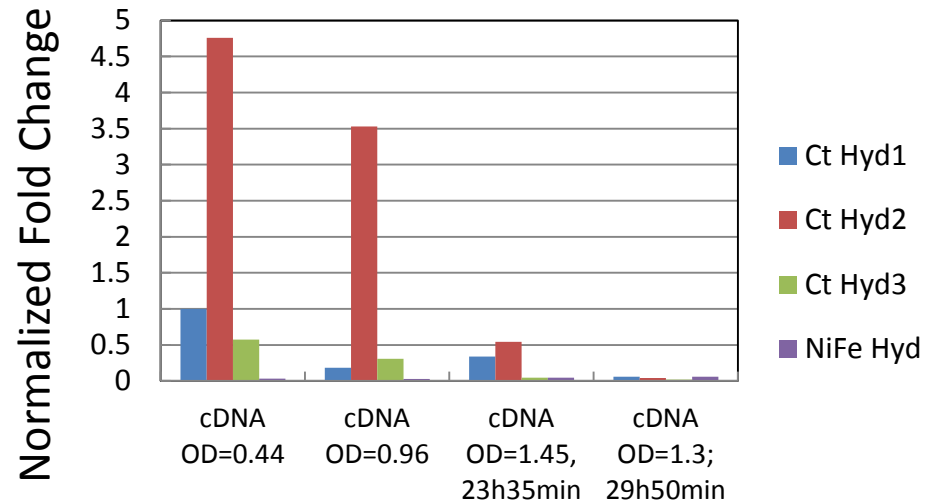
## Hydrogenase Expression Profiles

- H<sub>2</sub> 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<sub>2</sub> production, suggesting that Hyd2 plays a major role in H<sub>2</sub> production – meeting Q3 Milestone.

**(A). Cell Growth and H<sub>2</sub> Production**



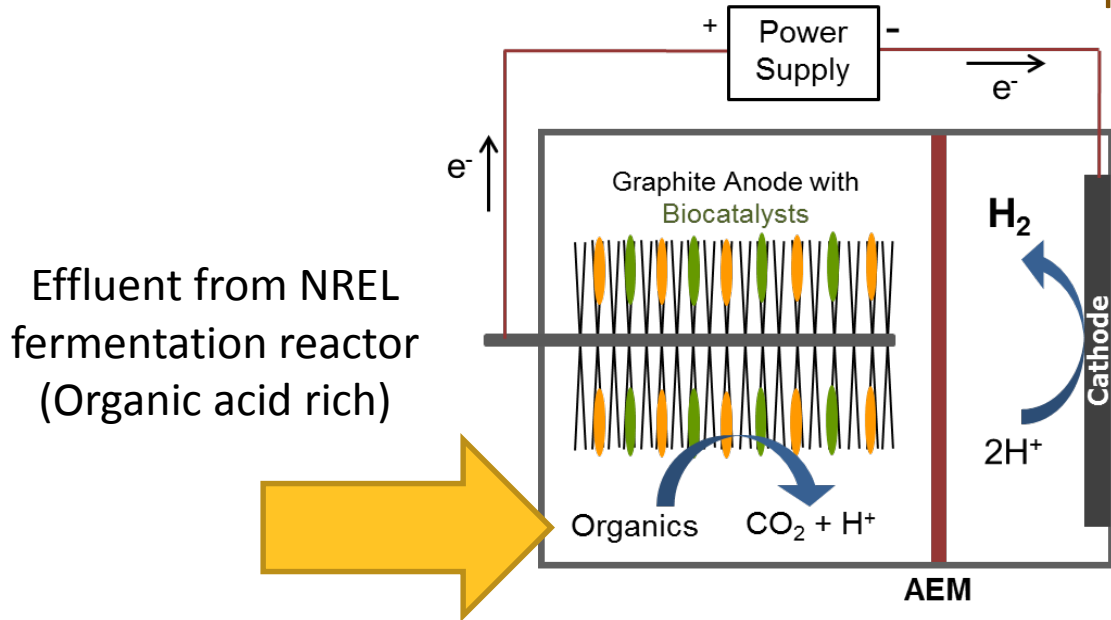
**(B). Hydrogenase Expression (qRT-PCR)**



➤ Hydrogenase expression profiles suggest that over-expression of Hyd2 most likely will boost H<sub>2</sub> production, which guides metabolic engineering strategy.

## Task 4 – Electrochemically Assisted Microbial Fermentation

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



**Goal:**  
Achieving **1.2 L-H<sub>2</sub>/L-reactor/d** over 3 HRT, using NREL fermentation effluent.

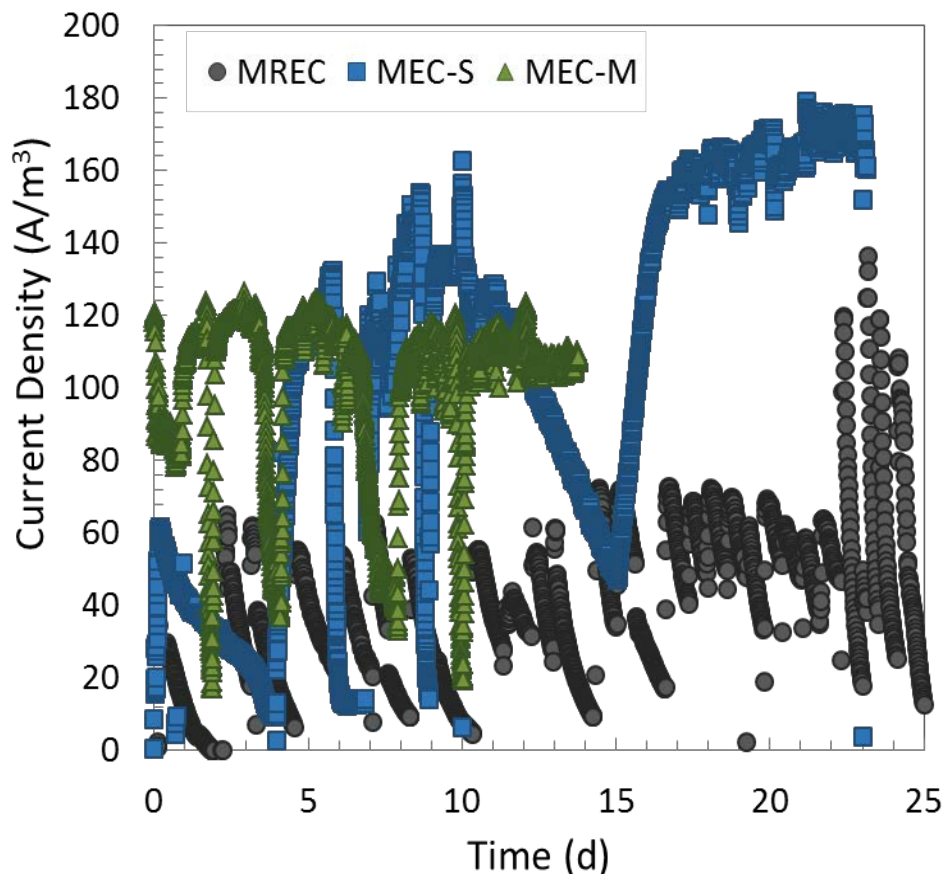
	Milestones	Completion Date	Status
<b>FY15</b>	Optimize the design of the cathode chamber to increase the volumetric hydrogen production rate to 1.2 L H <sub>2</sub> /L <sub>reactor</sub> /d (over 3 HRT, using synthetic effluent) in a continuous flow MEC, using Pt/C cathodes and improved configurations.	9/2015	Complete
<b>FY16</b>	Design MEC cathodes with reduced width to increase maximum H <sub>2</sub> 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  $1.4 \pm 0.2$  L-H<sub>2</sub>/L-reactor/d by decreasing volume of the cathode chamber (76 mL → 28 mL). Rates comparable to MEC with a reverse electro dialysis (RED) stack.

These experiments met the 2015 milestone (1.2 L-H<sub>2</sub>/L-reactor/d).



**MEC-S**

(S=small cathode chamber: 28 mL)

**MEC-M**

(M= modified cathode chamber: 76 mL)

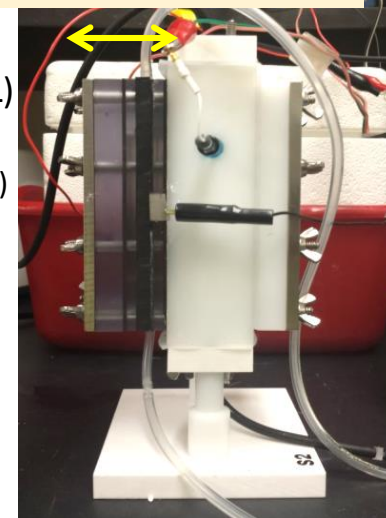
**MREC**

(Result from previous MREC study)



Kyoung Yeol Kim

Cathode chamber volume adjustment

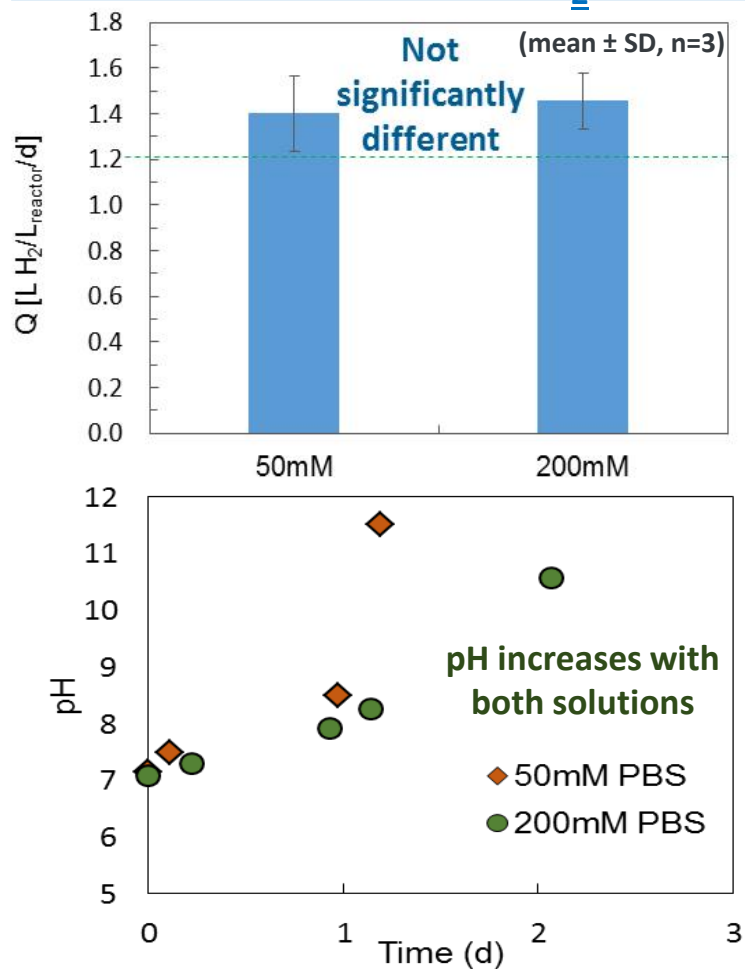


# Task 4 – Accomplishments/Progress

## Cathode Chamber Optimization

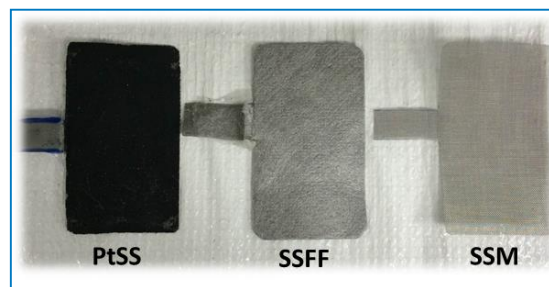
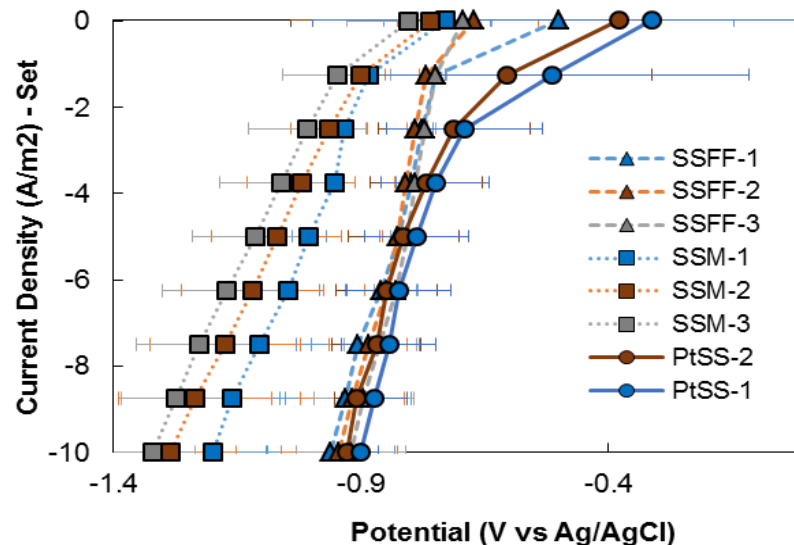


### Impact of buffer/pH on H<sub>2</sub> production



- Change in pH did not adversely affect H<sub>2</sub> production with >1.2 L-H<sub>2</sub>/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

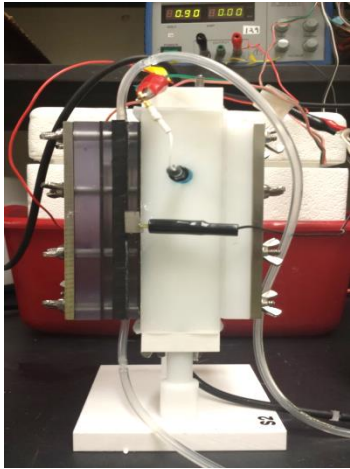
**Stainless steel (SS) mesh showing good promise of matching performance of Pt/C; further tests needed.**

# Task 4 – Accomplishments/Progress

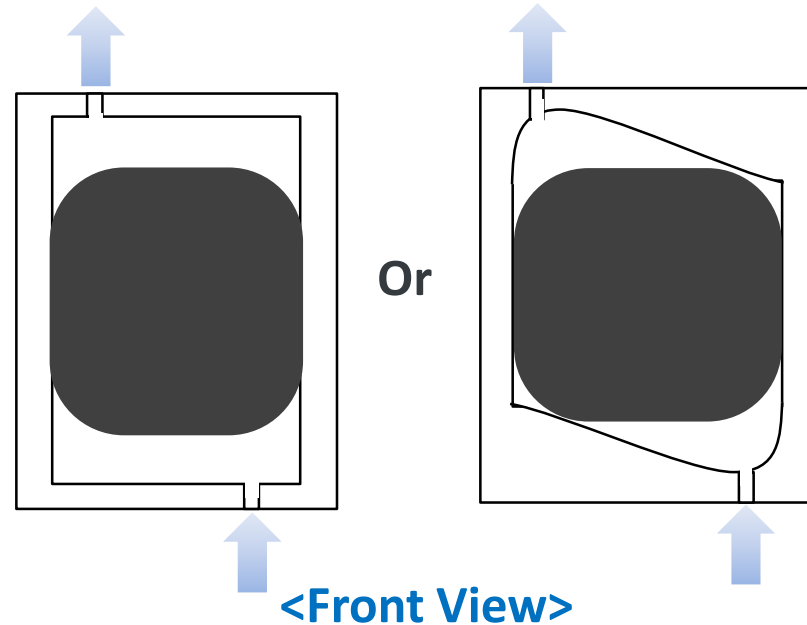
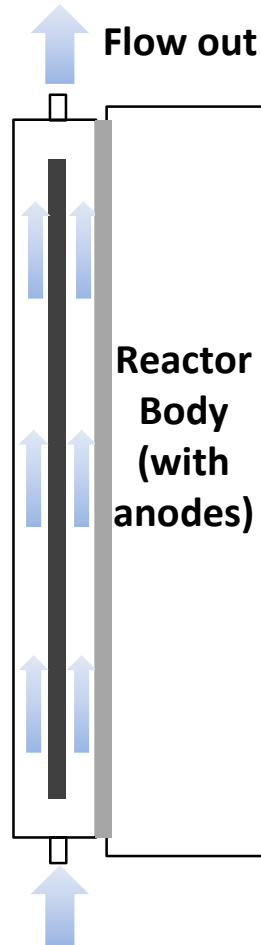
## MEC Cathode Flow Chamber Optimization



### MEC with continuous flow adjustable volume cathode chamber



Flow chamber  
MEC



- 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.

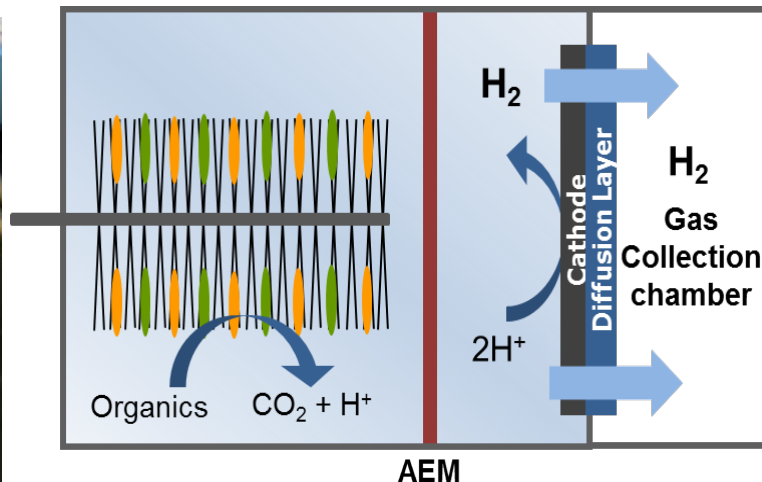
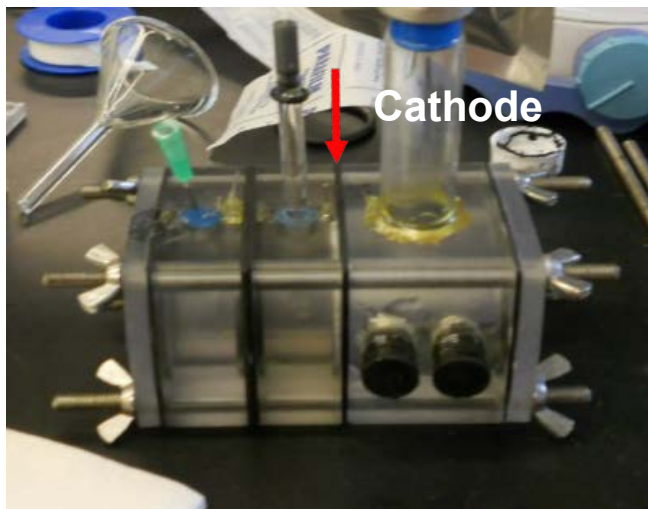


# Task 4 – Accomplishments/Progress

## Gas Diffusion Chamber: Abiotic tests



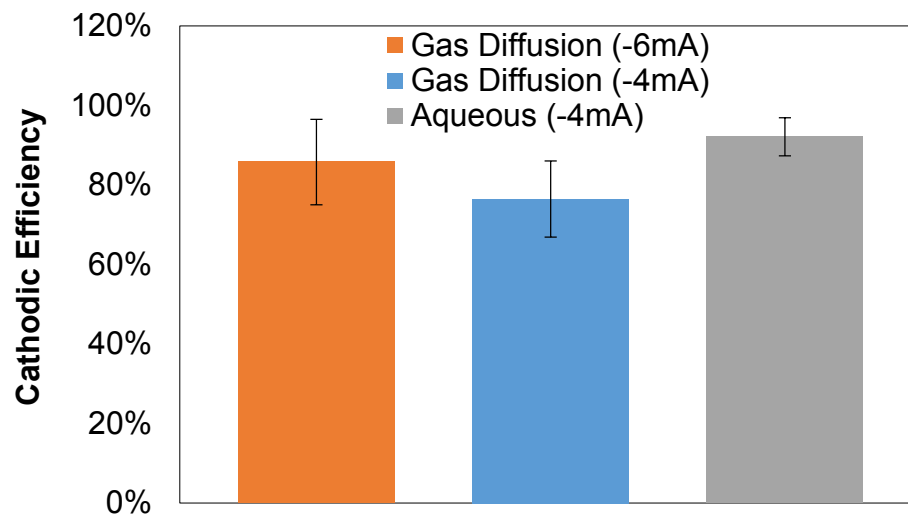
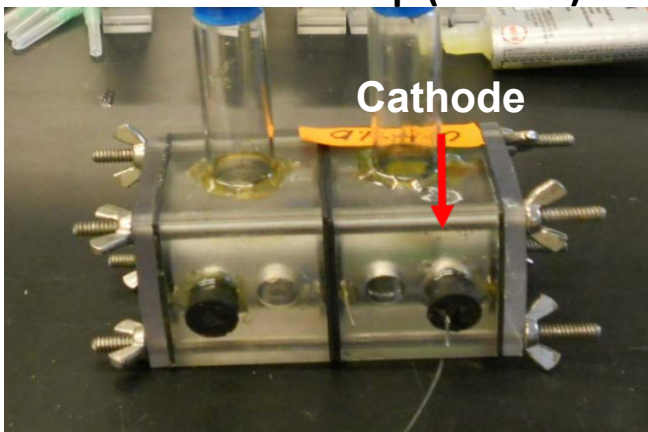
Gas Diffusion Cathode (3 chamber-setup). Goal is to eliminate liquid catholyte



**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

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- **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.**

- **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<sub>2</sub> molar yield.

# Remaining Challenges and Barriers

## Task 1. Bioreactor Performance

- High solid-substrate loading (175 g/L) is needed to lower H<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>/L<sub>reactor</sub>/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<sub>2</sub> production (FY16).

## Task 3 (NREL)

- Repeat hydrogenase expression profile experiment and verify the role of hydrogenase 2 in catalyzing H<sub>2</sub> production, aimed to meet Q3 milestone (FY16).
- Over-express hydrogenase 2 to balance electrons and increase H<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> from alternative sources (natural gas).

## Plans for future funding

- Network with biofuels industry to expand the use of H<sub>2</sub>.
- Advocate the advantages of “green” H<sub>2</sub> rather than fossil-fuel derived H<sub>2</sub>

## 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<sub>2</sub> production in bioreactor.
- Achieved an average H<sub>2</sub> production rate of 791 mL/L/d fermenting DMR cellulose directly to H<sub>2</sub>. 97% of the xylan was also solubilized, genetic engineering will convert xylose to H<sub>2</sub> 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<sub>2</sub> production.
- Hydrogenase expression profiles reveal Hyd2 is most active in H<sub>2</sub> production.

## Task 4

- A more compact reactor design was successful since pH changes did not adversely affect H<sub>2</sub> production. PSU achieved the 2015 milestone of > 1.2 L-H<sub>2</sub>/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.