



DOE Hydrogen Program

# Fermentative and Electrohydrogenic Approaches to Hydrogen Production

2008 DOE Hydrogen Program Review

Pin-Ching Maness, NREL

Bruce Logan, Penn State Univ. (Subcontract)

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Project ID PDP27



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# Overview

## Timeline

- Project start date: FY05
- Not funded in FY06
- Project end date: continuing
- Percent complete: N/A

## Budget

- Total project funding
  - \$1,180K
- Funding received in FY07: \$500K (including \$130K subcontract to Penn State)
- Funding for FY08: \$680

## Barriers

- Production Barriers addressed
  - Barrier AR: H<sub>2</sub> molar yield
  - Barrier AT: glucose feedstock cost

## Partners

- Prof. Bruce Logan, Penn State Univ. (subcontract)

# Objectives

- The long-term objective is to develop direct fermentation technologies to convert renewable lignocellulosic biomass resources to H<sub>2</sub>
- The near-term objectives in FY08 are to
  - Optimize bioreactor performance for the cellulose-degrading bacterium *Clostridium thermocellum*
  - Identify key metabolic pathways to guide genetic engineering to improve H<sub>2</sub> molar yield
  - Integrate microbial electrolysis cell (MEC) (formerly BEAMR: bio-electrochemically assisted microbial reactor) process to improve H<sub>2</sub> molar yield

# Milestones

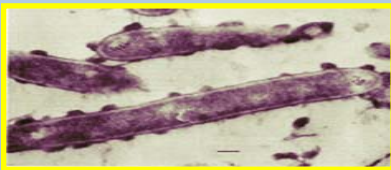


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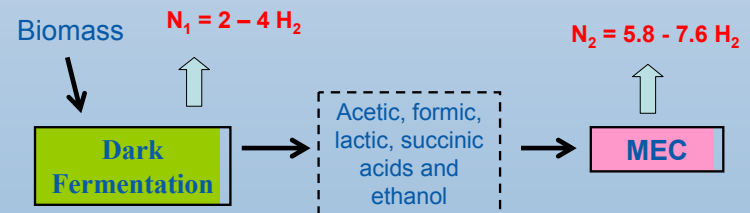
Month/year	Milestones
September - 07	Optimize growth conditions for <i>Clostridium thermocellum</i> 27405 (FY2007 project start date is April 2007) (NREL)
April - 08	Test H <sub>2</sub> production in a microbial electrolysis cells (MEC) using synthetic solution having the same composition as that produced from the NREL lignocellulose fermentation system (PSU)
June – 08	Test effects of metabolic pathway inhibitors on H <sub>2</sub> production (NREL)
August - 08	Determine H <sub>2</sub> molar yield and mass balance of fermentation using pretreated biomass as the feedstock (NREL)

# Approaches

- **Task 1: Bioreactor Performance**
  - Optimize cellulose-degrading bacterium *Clostridium thermocellum* 27405 to lower feedstock cost by converting cellulose to H<sub>2</sub> directly
- **Task 2: Metabolic Engineering**
  - Use genetic tools to improve the metabolic pathway of *C. thermocellum* (genome sequenced) to increase H<sub>2</sub> yield
- **Task 3: Microbial Electrolysis Cell (Penn State).**
  - Develop microbial electrolysis cells to produce H<sub>2</sub>, using waste generated from the NREL fermentation system



*Clostridium thermocellum*

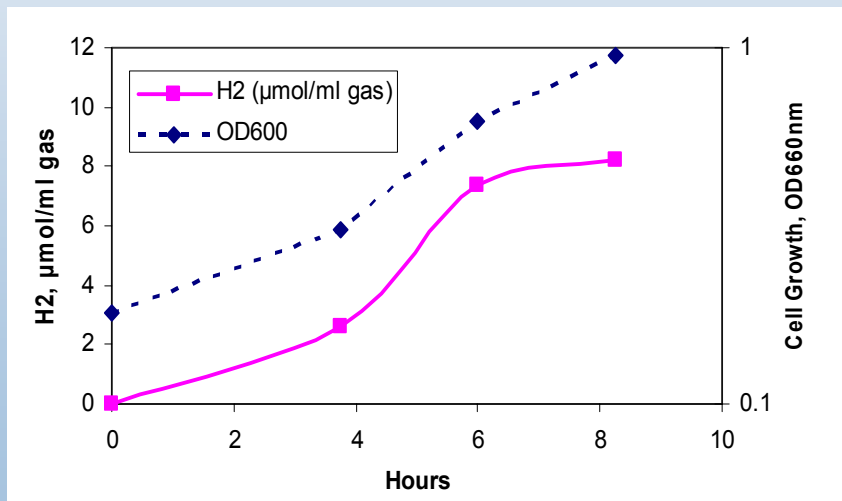


➤  $N_1 + N_2 = 7.8 - 11.6 \text{ mol H}_2 \text{ per mol sugar}$

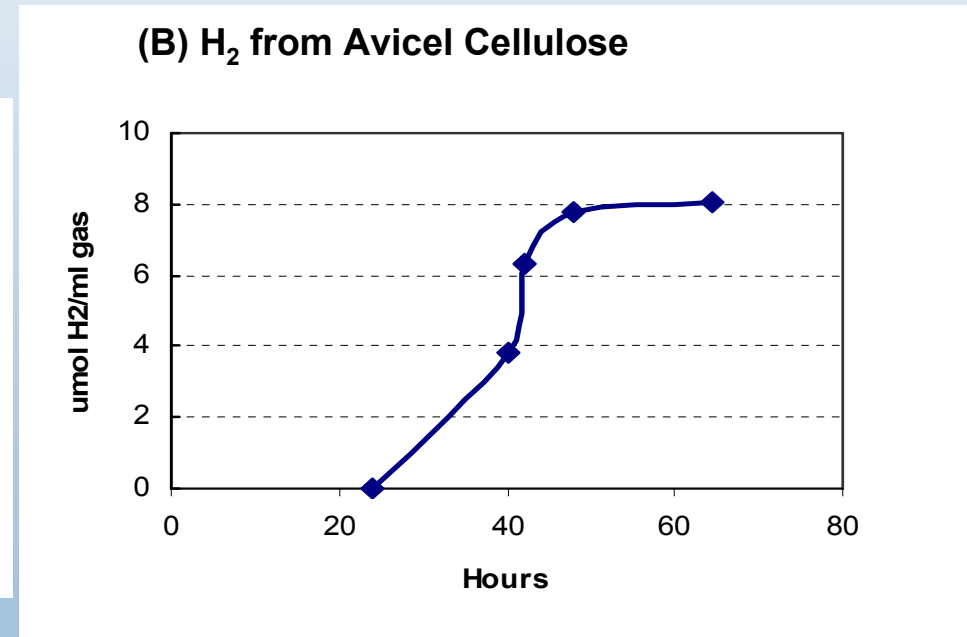
# Technical Accomplishments: Optimized Growth and H<sub>2</sub> Production

- Task 1:** Growth of *C. thermocellum* was optimized and it displayed a cell-doubling time of 2 hrs at 55 °C, while converting various cellulosic substrates to H<sub>2</sub>.

(A) cellobiose



(B) H<sub>2</sub> from Avicel Cellulose



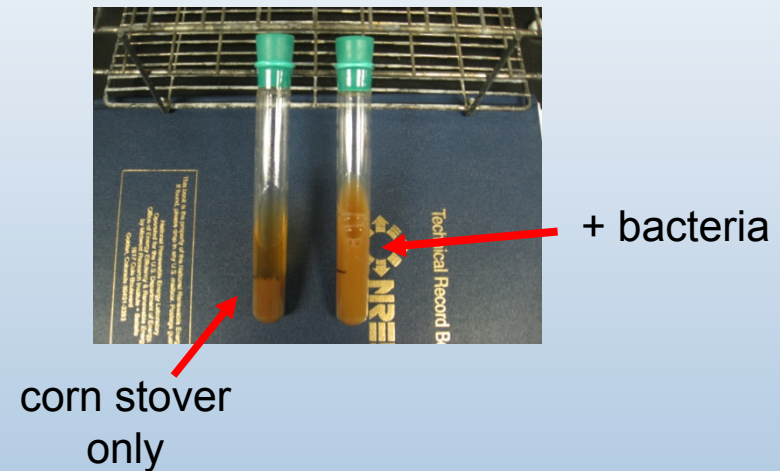
# Technical Accomplishments:

## Optimized Growth and H<sub>2</sub> Production

- Task 1:** *Clostridium thermocellum* converting various cellulosic substrates to H<sub>2</sub>.

Substrate*	μmol H <sub>2</sub> /ml culture/day
Corn stover	23.3
Avicel cellulose	15.7
Cellobiose	11.4
Filter paper	4.2

\*Added at 0.5% (w/v) except biomass at 1.4%

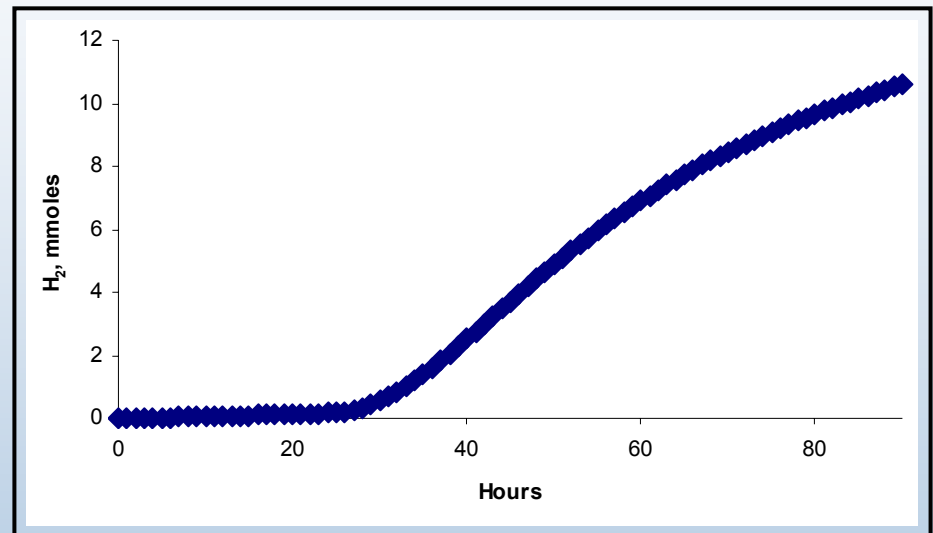
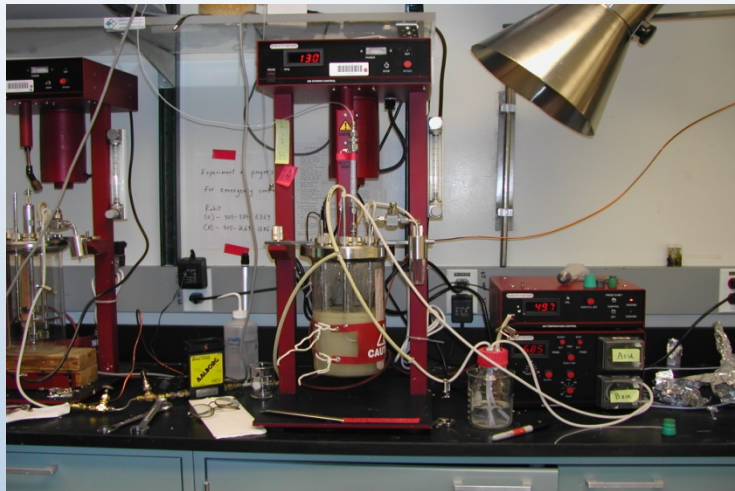


➤ Exceeding Milestone (09/07) in optimizing cell growth and cellulose utilization

# Technical Accomplishments:

## H<sub>2</sub> from Corn Stover in Bioreactor

### Task 1: Bioreactor performance using corn stover



- pH, temperature (50 °C), and pressure controls
- Continuous on-line measurements of H<sub>2</sub> and CO<sub>2</sub>

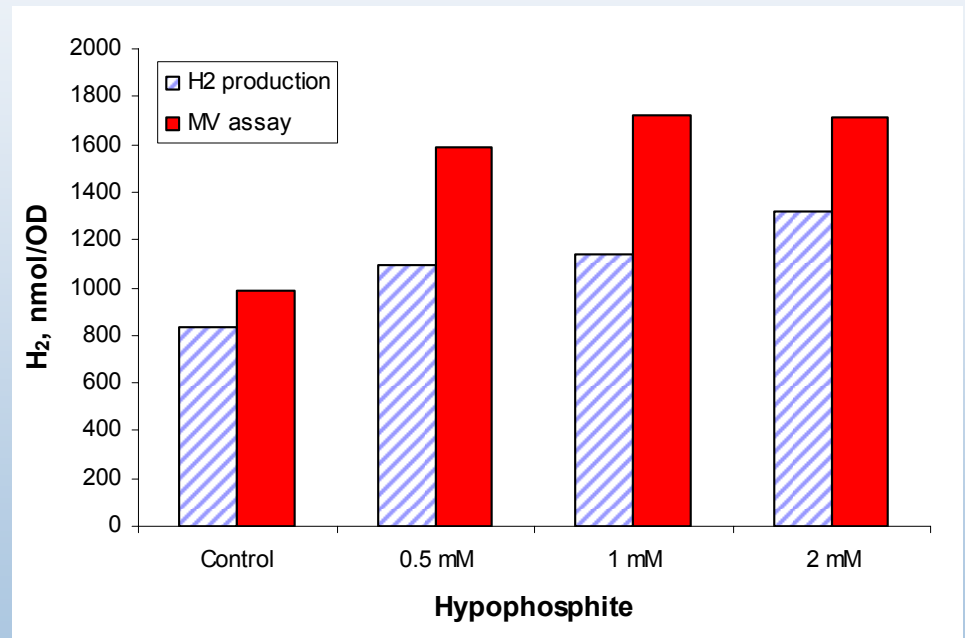
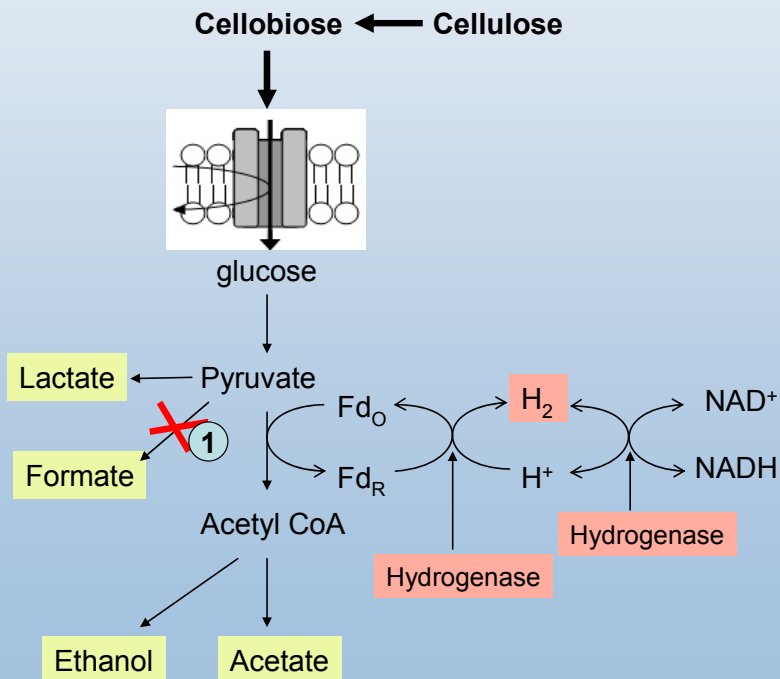
- Corn stover lignocellulose prepared by acid hydrolysis in 1.1% H<sub>2</sub>SO<sub>4</sub>
- 0.14% (w/v) corn stover was completely consumed in the end of fermentation
- Metabolite profiles and H<sub>2</sub> molar yield determinations underway

**Toward meeting Milestone (8/08)**



# Technical Accomplishments: Increased H<sub>2</sub> Yield

- **Task 2:** we studied effects of pathway inhibitors on H<sub>2</sub> production. The outcome will guide the most effective genetic engineering effort.
- Blocking the pyruvate-to-formate competing pathway by *hypophosphite* increased H<sub>2</sub> production.

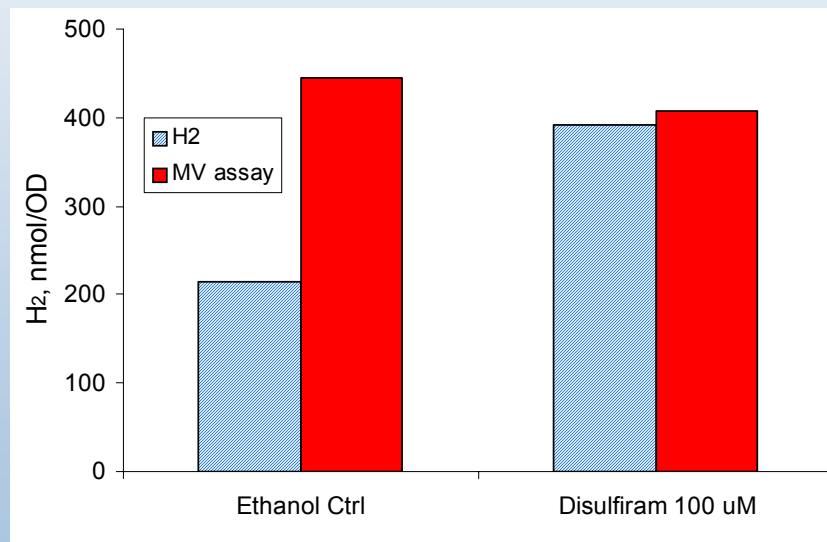
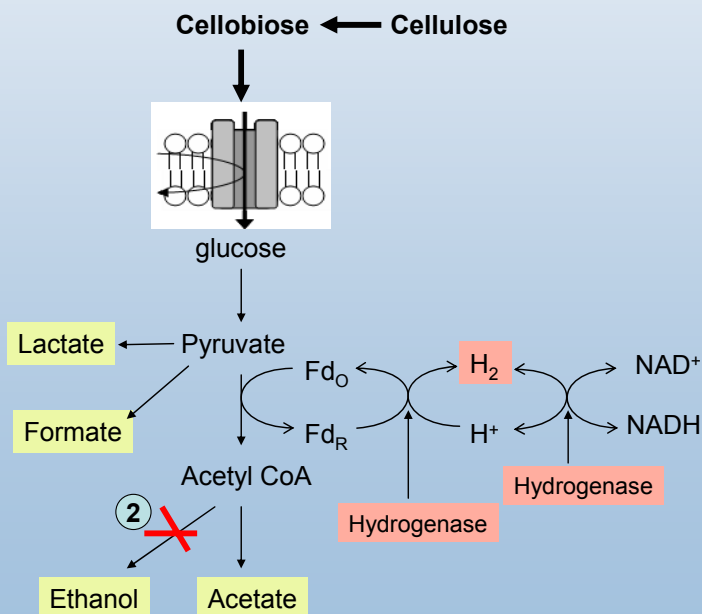


- **Increased H<sub>2</sub> yield by 31% to 58%**
- **Increased hydrogenase activity by 61-74%**

# Technical Accomplishments:

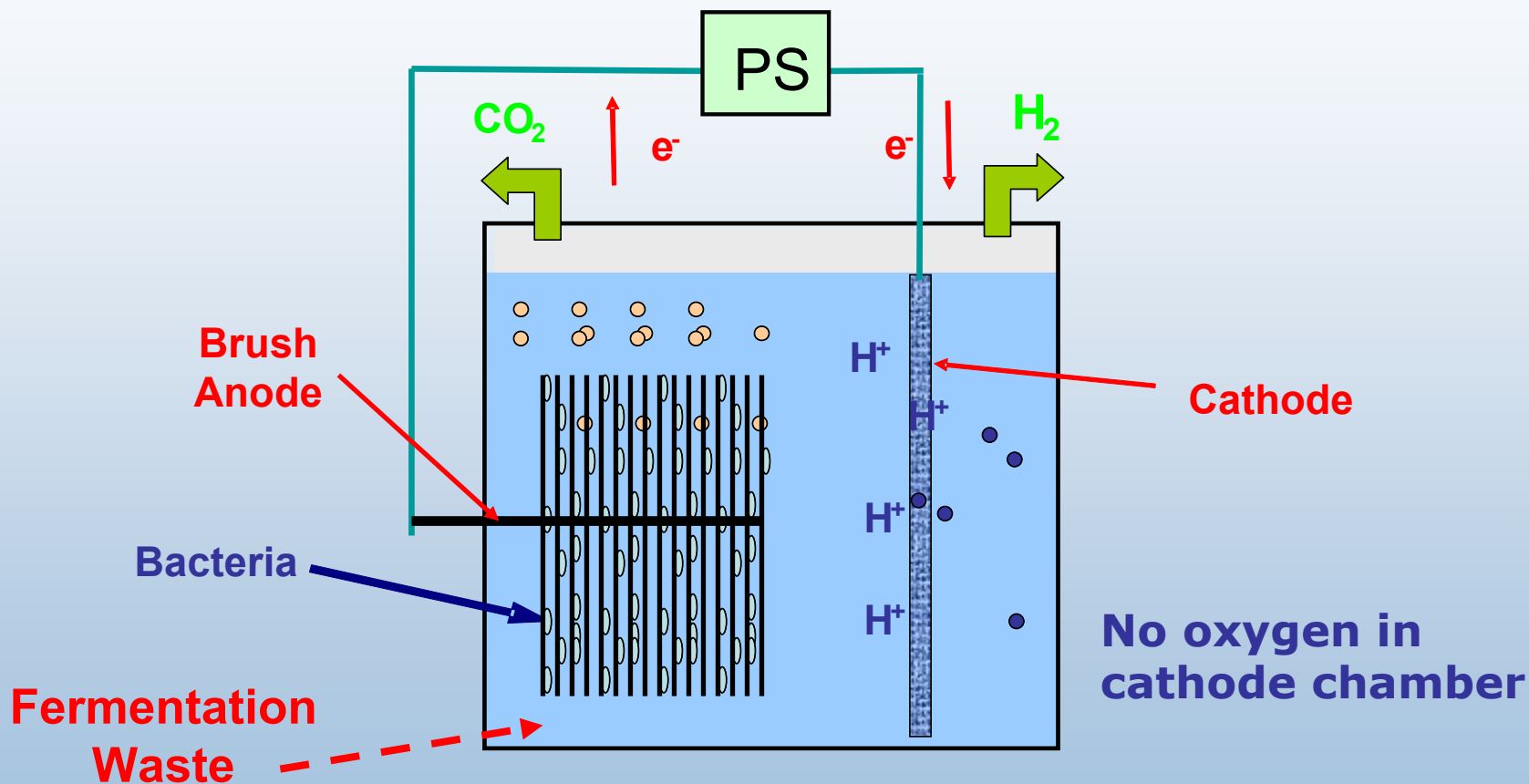
## Increased H<sub>2</sub> Yield

- **Cont'd Task 2:** Blocking the ethanol competing pathway by *disulfiram* increased overall H<sub>2</sub> production
- We demonstrated that blocking competing pathways is effective in increasing H<sub>2</sub> yield – **Milestone (6/08)**



- **Increased H<sub>2</sub> yield by 81%**
- No change in hydrogenase activity

# Task 3 Approach: Microbial Electrolysis Cell (MEC)



**0.25 V needed (vs 1.8 V  
for water electrolysis)**

Bruce Logan, Penn State University

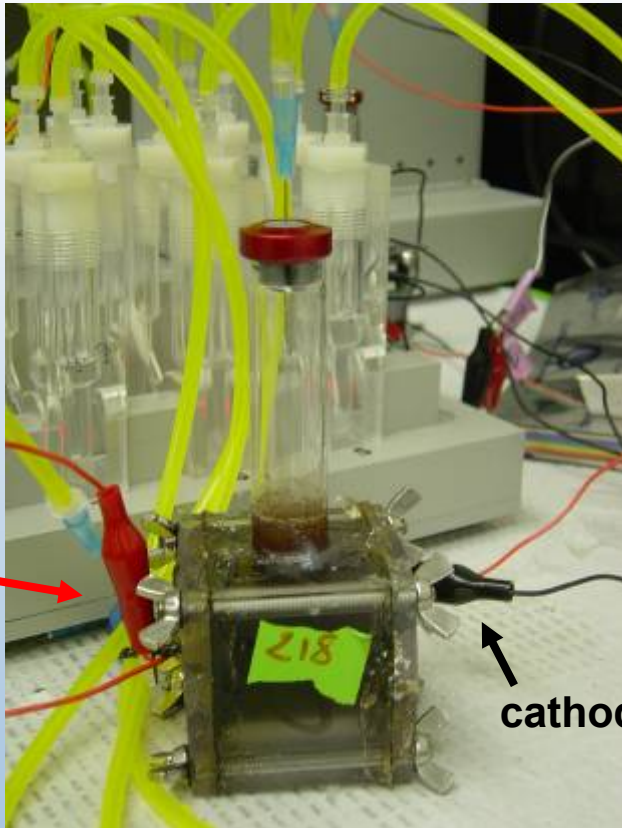
Ref: Liu, Grot and Logan, *Environ. Sci. Technol.* (2005)





# Task 3 Approach:

## Reactor and Solutions



anode

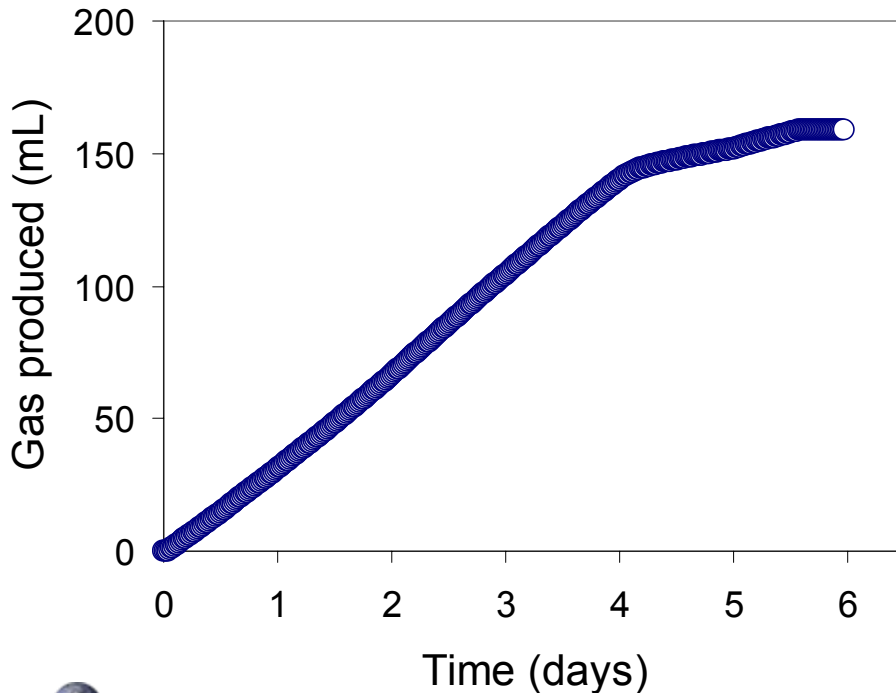
cathode

MEC used in tests (also called BEAMR)

- Reactor
  - Single chamber
  - Brush anode, carbon cathode with Pt
- Synthetic solution containing fermentation end products:
  - Substrates:
    - 26 mM acetic acid
    - 5.6 mM succinic acid
    - 1.8 mM lactic acid
    - 0.6 mM formic acid
    - 14 mM ethanol
  - 50 mM PBS + vitamins + minerals

# Technical Accomplishments: H<sub>2</sub> from Synthetic Fermentation End Products

**Task 3:** Successfully produced H<sub>2</sub> gas from synthetic solution of fermentation end products



- Gas production  
Total= 159 mL  
H<sub>2</sub>= 106 mL
- Conversion efficiency= 30 mL H<sub>2</sub>/gCOD\*
- COD removal=93% (3.6 g-COD)
- Time for cycle: 6 to 7 days
- Problems:
  - Methane gas production
  - Increased CH<sub>4</sub>, decreased H<sub>2</sub>

\*COD: chemical O<sub>2</sub> demand

Meeting Milestone (4/08)



# Technical Accomplishments: Adapted Culture in MEC

- **Task 3:** Developed acclimated cultures to individual compounds to improve yield and efficiency

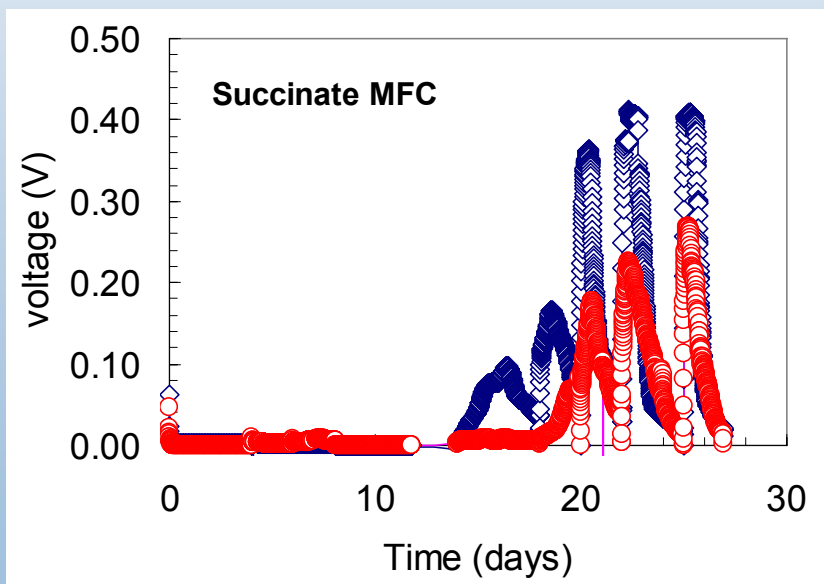
To increase H<sub>2</sub> yields, reduce methane production, reactors are being acclimated to individual compounds.

(Duplicate reactors shown below)

- Reactors first run in microbial fuel cell mode (MFC); and switch to MEC mode later.

- Successful acclimation, with maximum voltage of:

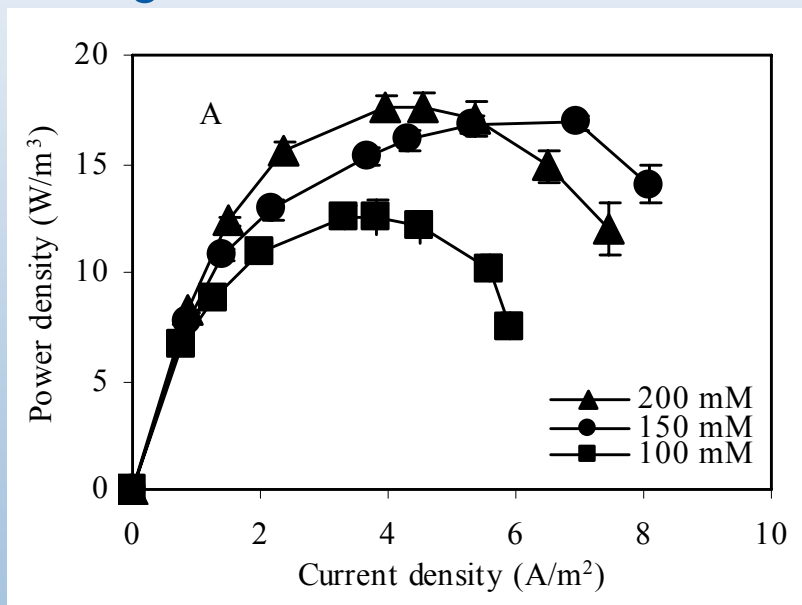
- Acetate: 556 mV
- Lactate: 543 mV
- Ethanol: 523 mV
- Succinate: 412 mV
- Formate: 228mV



# Technical Accomplishments: Testing Xylose Feedstock

**Task 3:** Examined electricity production using **xylose** (major sugar of hemicellulose) at different concentrations and solution ionic strength

- Work primarily supported by visiting researcher at Penn State
- Provided an opportunity to examine implications of bioenergy production using an alternative feedstock, and effects of scale-up
- Produced  $13 \text{ W/m}^3$  ( $673 \text{ mW/m}^2$ ) at Coulombic efficiencies of 61-85% in a medium-scale reactor (0.8 L) at 100 mM ionic strength, with slightly higher power in other solutions.



**These results will be useful in considering scale up of MEC systems using hemicellulose**

# Future Work: Task 1 (NREL)



- Optimize bioreactor performance for scale-up fermentation of corn stover
- Determine H<sub>2</sub> molar yield, carbon mass balance, and profiles of metabolites (milestone 8/08)
- Provide above fermentation waste products for Penn State MEC process for additional H<sub>2</sub> production
- Test other pretreated feedstock, i. e., switch grass
- Develop continuous (vs batch) fermentation with cellulosic substrates



# Future Work: Task 2 (NREL)

- Test other metabolic pathway inhibitors in improving H<sub>2</sub> yield (Milestone 6/08)
- Combine inhibitors for cumulative improvement
- Optimize growth of *C. thermocellum* on agar plates
- Develop genetic methods for pathway engineering
  - collaborate with Univ. Manitoba to accelerate progress and leverage DOE funding)
- Test scale-up fermentation using metabolic pathway inhibitors for improving H<sub>2</sub> molar yield

# Future Work: Task 3 (Penn State)

- Acclimate cultures to all components in the synthetic fermentation product in MEC to improve yield and efficiency
- Test adapted culture using all components
- Use actual biomass fermentation waste products provided by NREL



# Summary

- Growth conditions have been optimized for *Clostridium thermocellum* using various cellulosic substrates (cellulose, corn stover)
- Identified key metabolic pathways to block to improve H<sub>2</sub> yield
  - Improved H<sub>2</sub> yield up to 81%
  - Provide a knowledge-based approach to guide metabolic pathway engineering
- H<sub>2</sub> has been successfully produced in MEC using synthetic fermentation waste products