NREL
NATIONAL RENEWABLE ENERGY LABORATORY

# Fermentation and Electrohydrogenic Approaches to Hydrogen Production

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**NREL/PR-560-48069**

Project ID #: PD038

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

# Overview

## Timeline

- Project start date: FY05
- Project not funded in FY06
- Project end date: 2018
- Percent complete: N/A

## Barriers

- Production barriers addressed
  - $H_2$ molar yield (AR)
  - Waste acid accumulation (AS)
  - Feedstock cost (AT)

## Budget

- Funding received in FY09: $400K (include $40K subcontract)
- Funding allocated for FY10: $230K (include $60K subcontract)

## Partners

- Dr. Bruce Logan, Penn State University
- Drs. David Levin and Richard Sparling, University of Manitoba, Canada (Genome Canada Program)

# Relevance

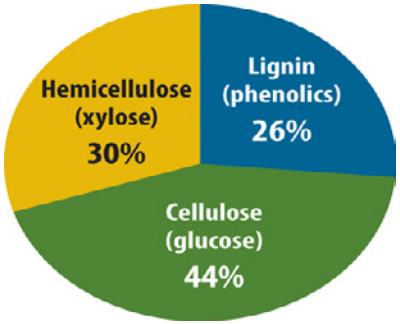
- **Objective:** Develop direct fermentation technologies to convert renewable, lignocellulosic biomass resources to $H_2$.
  - Determine effects of substrate loading on rates and yields (Task 1)
  - Develop genetic tools to improve $H_2$ molar yield (Task 2)
  - Develop continuous flow microbial electrolysis cell (MEC) reactor to improve $H_2$ molar yield (Task 3).
- **Relevance:** Address directly feedstock cost and $H_2$ molar yield barriers to improve techno-economic feasibility.

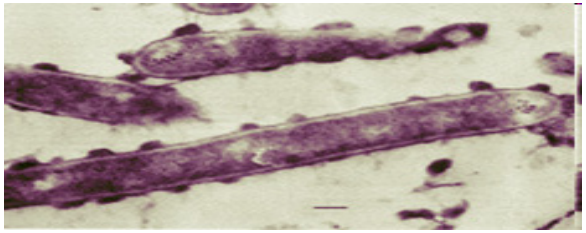
| Characteristics | Units | 2013 Target | 2010 Status |
|---|---|---|---|
| Yield of $H_2$ from glucose | Mole $H_2$/mol glucose | 4 | 1.6 - 2.0 |
| Feedstock cost | Cents/lb glucose | 10 | 12 |

# Objectives/Approach/Milestone
# Task 1: Bioreactor Performance

- **Objective:** Address feedstock cost and optimize the performance of scaled-up bioreactors for $H_2$ via fermentation.
- **Approach:** Use corn-stover lignocellulose and cellulose-degrading bacteria to address feedstock cost.

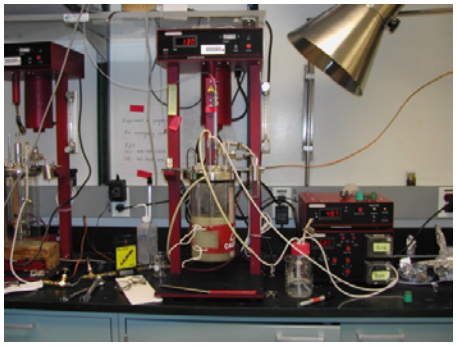
**Lignocellulosic Biomass**



***Clostridium thermocellum***

**Bioreactor Performance**



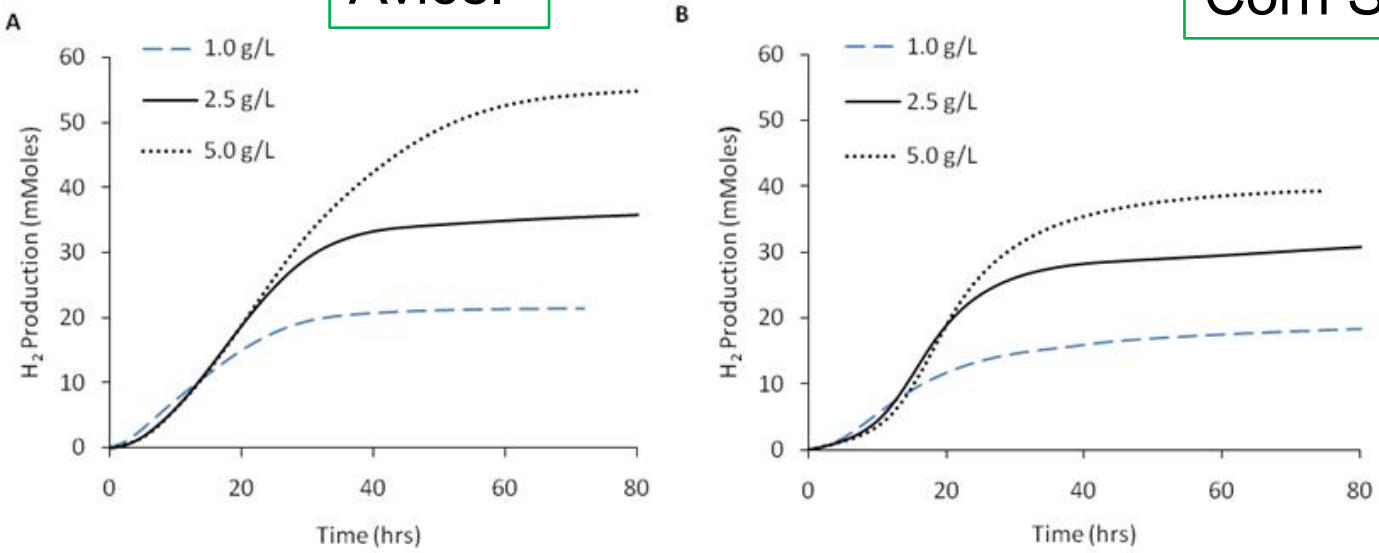
| | Milestone | Completion Date | Status |
|---|---|---|---|
| 3.2.1.1 | Determine effects of substrate loading on rates and yield of $H_2$ | 1/10 | Completed |
| 3.2.1.2 | Determine the optimal avicel solid retention time on rates and yield of $H_2$ in **fed-batch** reactor | 5/10 | In progress |

# Task 1 – Technical Accomplishments

## Substrate Loading - $H_2$ Production Profiles

Avicel

Corn Stover




- The residual cellulose contents were quantified via acid hydrolysis ($H_2SO_4$).
- Determined *C. thermocellum* cell formula of **$C_5H_8O_2N$**, consistent with published data in two different bacteria.

Cell formula enables more accurate determination of $H_2$ molar yield and carbon mass balance by accounting for carbons used toward cell growth.

# Task 1 – Technical Accomplishments

## Effect of Substrate Loadings on Rates and Yields

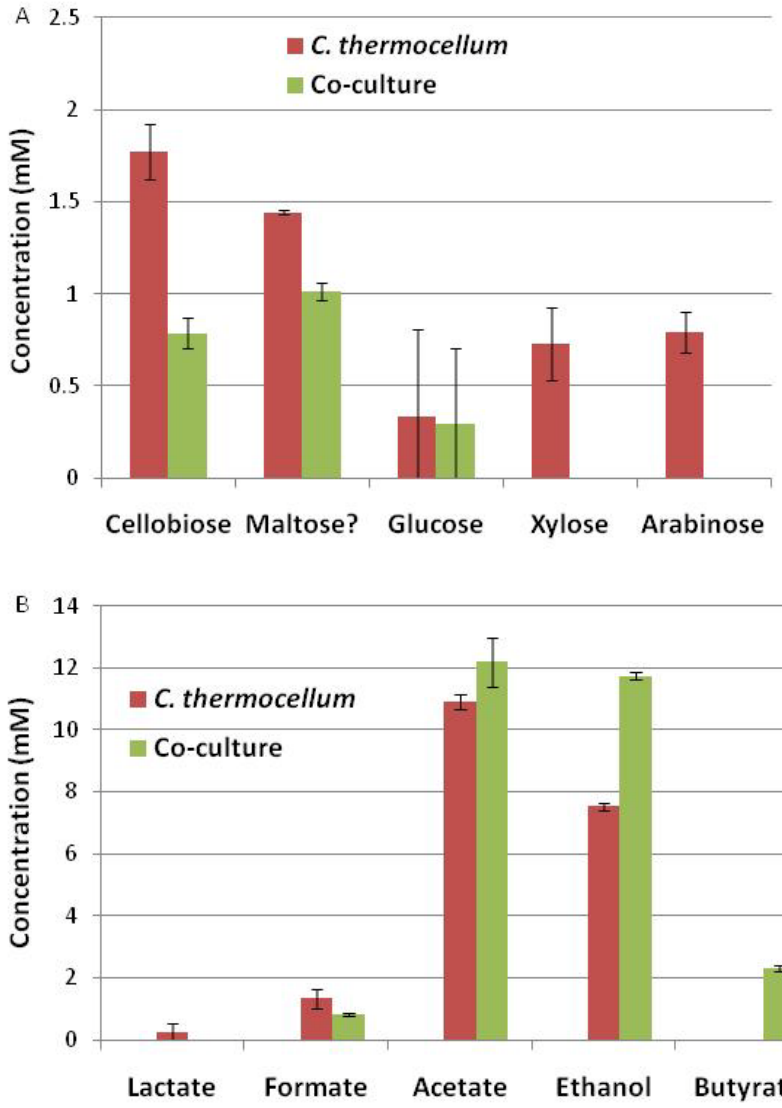
- $H_2$ production rates and molar yields varied with carbon loadings.
  - Higher carbon loading leads to faster rate of $H_2$ production
  - Lower carbon loading leads to higher $H_2$ molar yield.
  - The outcomes guide **fed-batch** bioreactor with daily feeding of **2.5 g/L.**

| Substrate | G/L | Rate (mmol $H_2$/L/hr) | $H_2$ Molar Yield | Carbon Balance (%) |
|---|---|---|---|---|
| Avicel | 1 | 0.58 | 3.2 | 74 |
| Avicel | 2.5 | 0.89 | 2.1 | 70 |
| Avicel | 5 | 0.98 | 1.6 | 70 |
| Corn stover | 1 | 0.51 | 2.8 | 70 |
| Corn stover | 2.5 | 1.06 | 2.0 | 94 |
| Corn stover | 5 | 1.21 | 1.2 | 51 |

**Completed Milestone "*Determine effect of substrate loading on rates and yields of $H_2$*" (1/10).**

# Task 1 – Technical Accomplishments

## $H_2$ from Milled, Untreated Corn Stover Using a Co-Culture




- Established a co-culture of *Clostridium thermocellum* and a *Clostridium* consortium (enriched from sewage sludge), the latter adapted to utilize xylose.
- *C. thermocellum* hydrolyzed cellulose to cellobiose and hemicellulose to xylose, the latter utilized by the consortium.

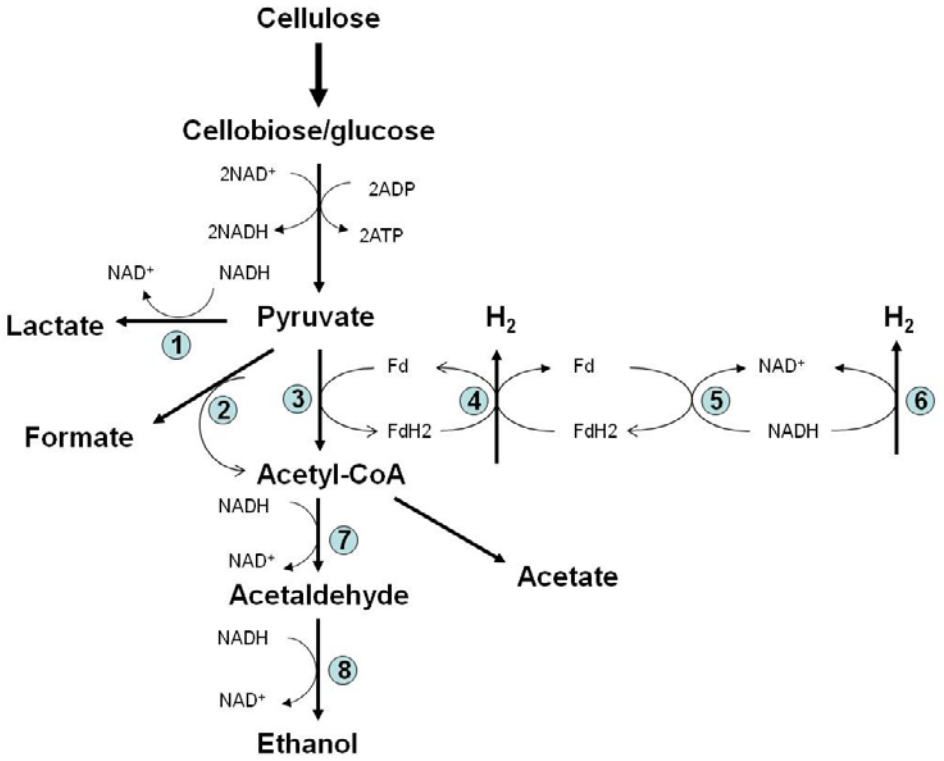
| Culture | $H_2$ (mM) |
|---|---|
| *C. thermocellum* | 10.53 +/- 6.19 |
| Co-culture | 13.23 +/- 4.70 |

**Address feedstock cost and direct biomass utilization of both cellulose and hemicellulose.**

# Objectives/Approach/Milestone

## Task 2 – Develop Genetic Methods for Metabolic Engineering

- **Objective:** Improve $H_2$ molar yield (mol $H_2$/mol hexose) via fermentation.
- **Approach:** Redirect metabolic pathways to maximize $H_2$ production via the development of genetic methods.

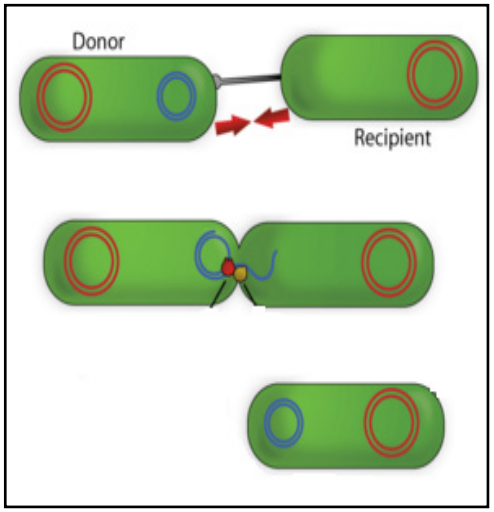



| | Milestone | Completion Date | Status |
|---|---|---|---|
| 3.2.2 | Elucidate role of hydrogenase in *C. thermocellum* | 6/10 | In progress |
| 3.2.5 | Produce one genetic transformant in *C. thermocellum* | 8/10 | In progress |

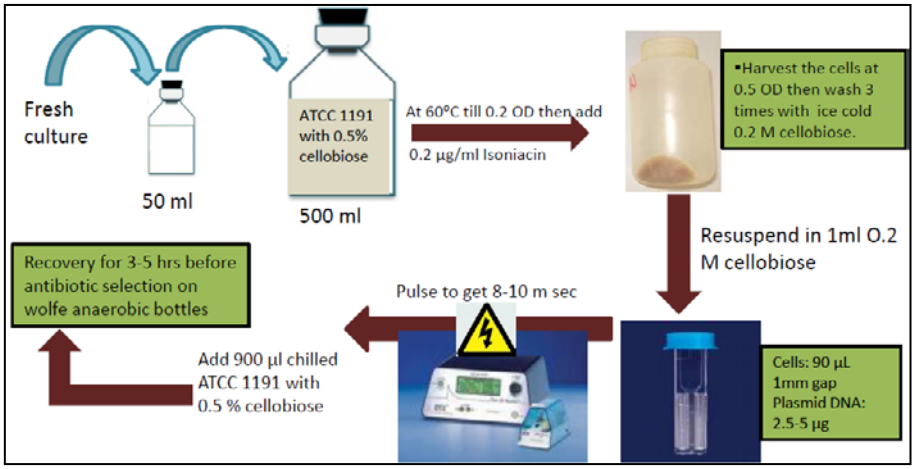
# Task 2 – Technical Accomplishments

## Developing Tools for Genetic Transformation

### Conjugation




### Electroporation



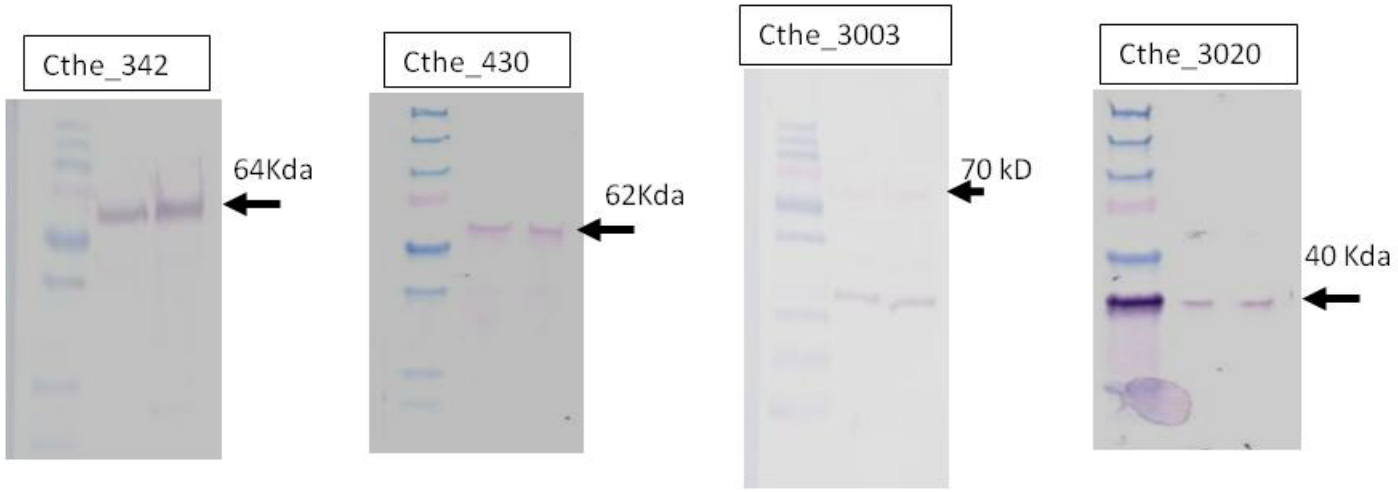

- We tested a proprietary protocol developed by the Oak Ridge National Lab using pIKM1 and pHV33 plasmids; the results were not successful.
- We conducted transformation and tested various parameters using a new electroporator that delivers high voltage to the cells.
- Work is under way to prepare protoplast and explore plasmid DNA methylations for both electroporation and conjugation.

Progressing toward Milestone "*Produce one genetic transformant in C. thermocellum*" (8/10).

# Task 2 – Technical Accomplishment

## Elucidate Roles of Hydrogenases

| Gene Locus | Enzyme | Putative Function |
|---|---|---|
| 342, 430, **3003 (HydA3)** | Three FeFe-hydrogenases | $H_2$ metabolism |
| 3020 | NiFe-hydrogenase | $H_2$ metabolism |



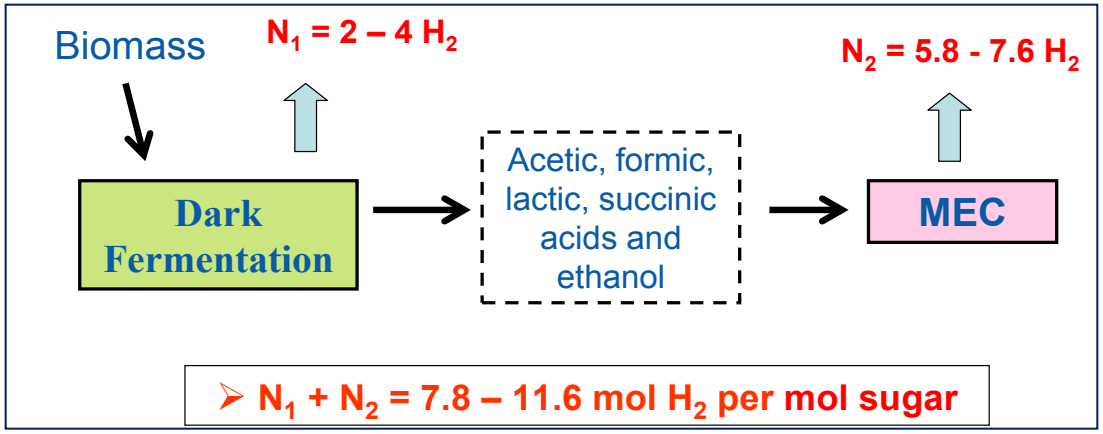

- Protein western blot revealed that HydA3 is not expressed amongst the four hydrogenases.
- Elucidating functions allows manipulations of growth conditions and/or hydrogenase genes to enhance $H_2$ production.

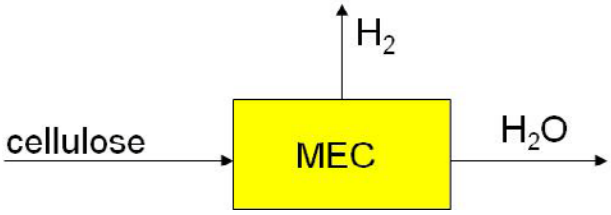
**Meeting toward Milestone "*Elucidate role of hydrogenase in C. thermocellum*" (6/10).**

# Objectives/Relevance

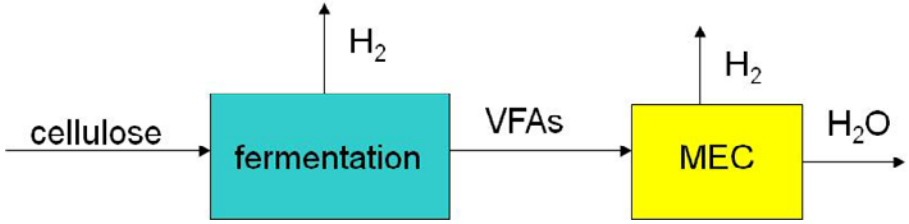
## Task 3 – Electrochemically Assisted Microbial Fermentation

- **Objective:** Improve $H_2$ molar yield (mol $H_2$/mol hexose) by integrating dark fermentation with microbial electrolysis cell (MEC) reactor to convert waste biomass to additional $H_2$.




One-stage process: slow

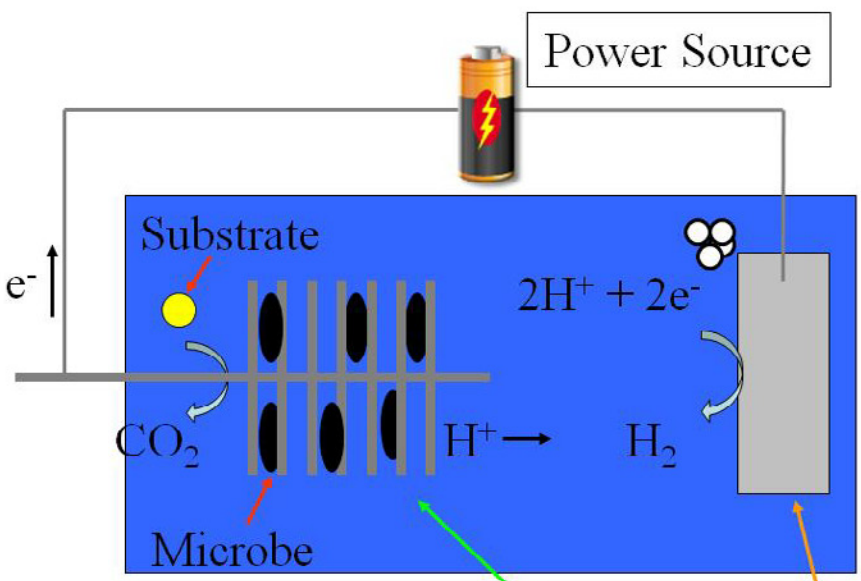



Two-stage process: fast

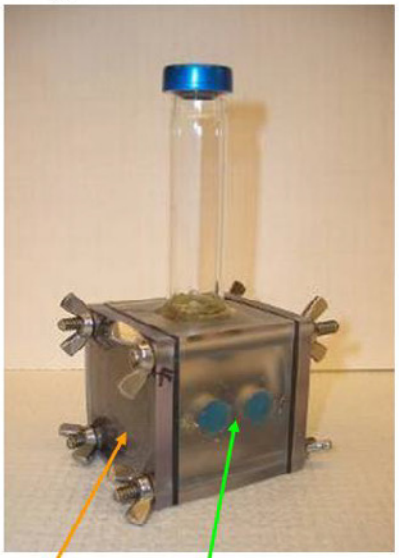
# Approach/Milestone

## Subtask 3: Electrochemically Assisted Microbial Fermentation

-Produce $H_2$ -Non-spontaneous (+ΔG) reaction




single-chamber MEC



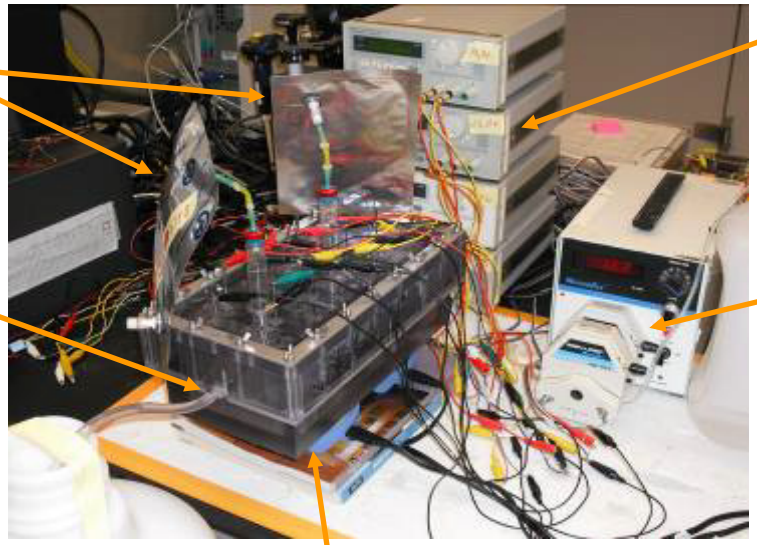
Cathode (anaerobic)

Brush Anode (anaerobic)

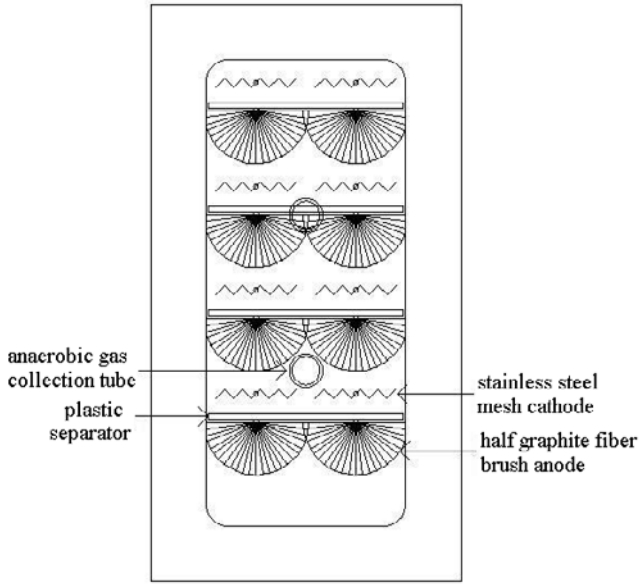
| | Milestone | Completion Date | Status |
|---|---|---|---|
| 3.2.3 | Perform hydraulic test of synthetic effluent | 4/10 | Completed |

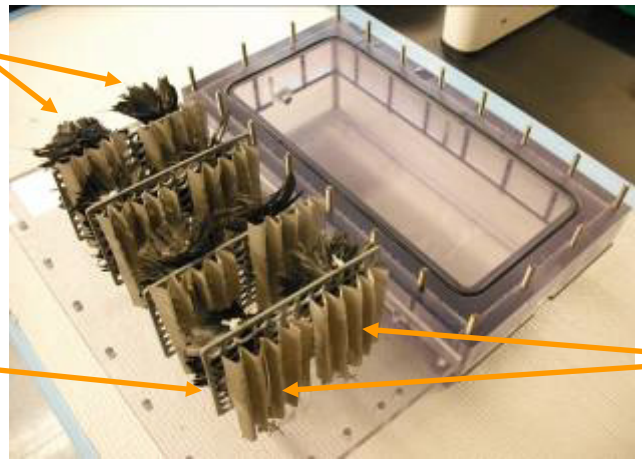
# Task 3 – Technical Accomplishments

## 2.5 L Continuous Flow MEC



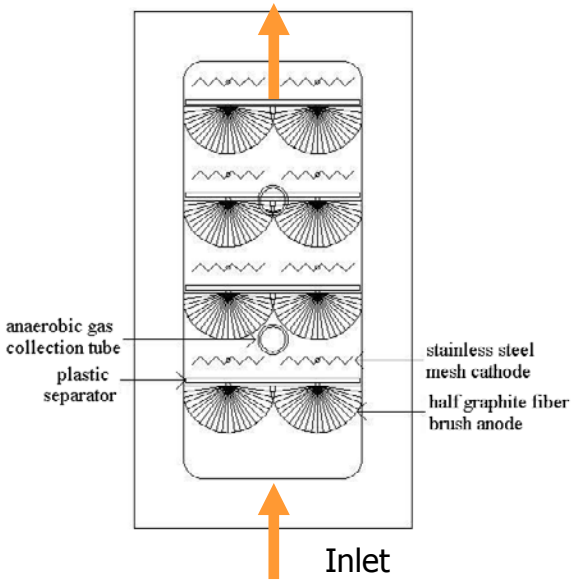

Schematic

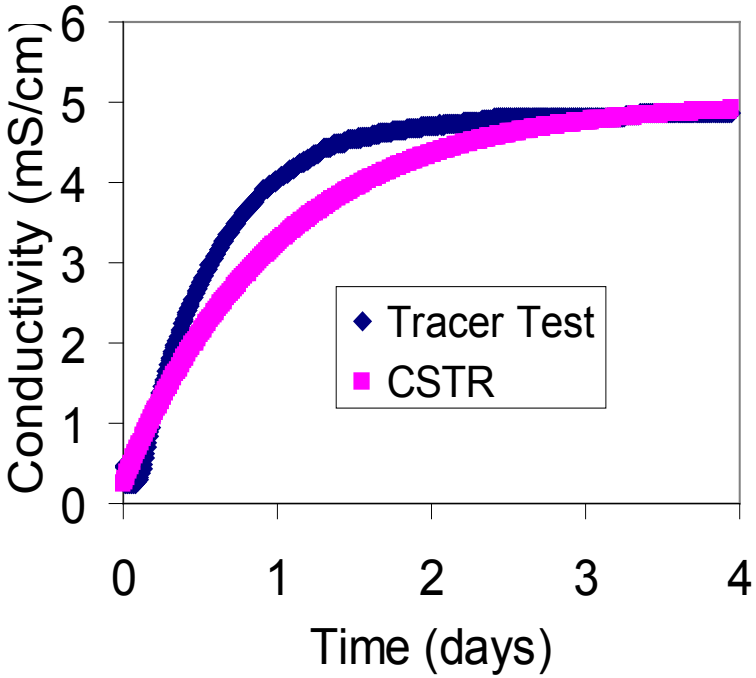


# Task 3 – Technical Accomplishments

## Hydrodynamics of MECs



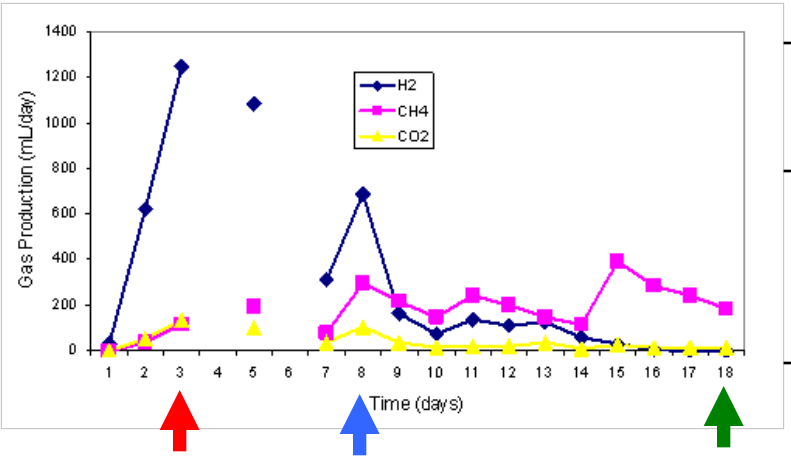

- Tracer conductivity increased more *quickly* than CSTR.
- Some short circuiting to outlet.
- May need to improve liquid flow using baffles.

**Completed Milestone "*Perform hydraulic test of synthetic effluent*" (4/10)**

# Task 3 – Technical Accomplishments

## MEC Performance

Overall Performance:




### Energy Recovery Considering Only $H_2$:

| Q ($m^3/m^3/d$) | Day | $\eta_E$ (%) | $\eta_S$ (%) | $\eta_{E+S}$ (%) |
|---|---|---|---|---|
| 0.53 | Day 3 | 140 | 130 | 68 |
| 0.30 | Day 8 | 80 | 49 | 30 |
| 0.0001 | Day 18 | 0.004 | 0.03 | 0.016 |

Current density: ~72 $A/m^3$

### Energy Recovery Considering $H_2$ and $CH_4$:

| Day | $W_{H2}$ (kJ) | $W_{CH4}$ (kJ) | $W_{H2+CH4}$ (kJ) | $\eta_E$ (%) | $\eta_S$ (%) | $\eta_{E+S}$ (%) |
|---|---|---|---|---|---|---|
| Day 3 | 15 | 4.3 | 19 | 190 | 170 | 87 |
| Day 8 | **8.0*** | **10.8*** | 19 | 190 | 120 | 71 |
| Day 18 | 0.004 | 6.7 | 6.7 | 67 | 56 | 30 |

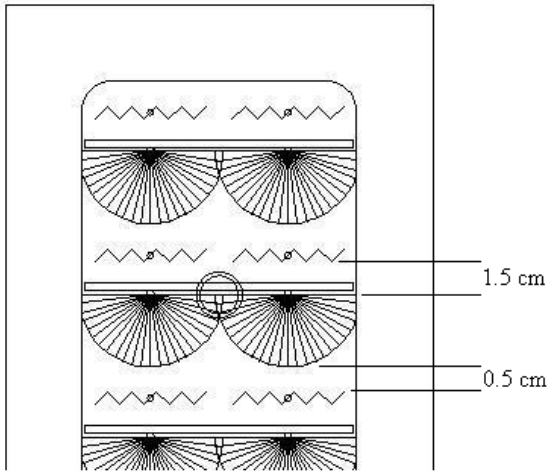
*Higher heat of combustion for $CH_4$ (891 kJ/mol vs. 286 kJ/mol for $H_2$) allows for more energy recovery from a smaller volume

# Task 3 - Technical Accomplishments

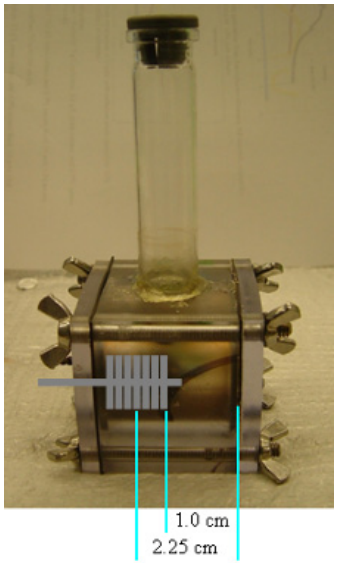
## Scalability: Comparison Based on Cathode Current

| | Appl. Voltage (volts) | Electrode Spacing (cm) | Maximum Current (A) | Cathode Surface Area ($m^2$) | Current Density ($A/m^2$) | Current Density ($A/m^3$) |
|---|---|---|---|---|---|---|
| This Study | 0.9 | 1.5 | 0.18 | 0.15 | 1.18 | 74 |
| Selembo et al. | 0.9 | 1 | 0.0032 | 0.0018 | 1.83 | 100 ±4 |
| Call et al. | 0.6 | 0.5 | 0.0054 | 0.023 | 0.24 | 194 ±1 |

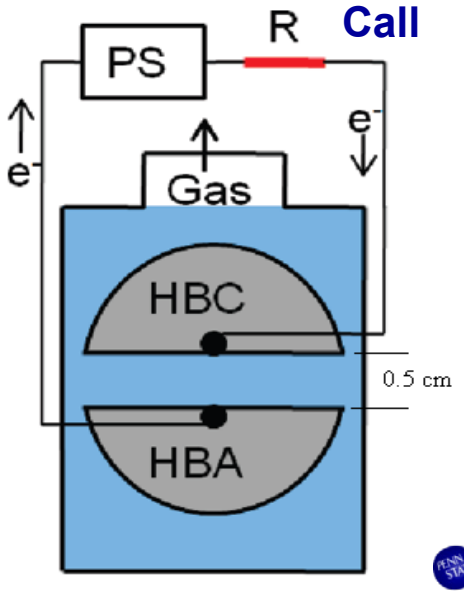
This Study




Selembo




Call




Call et al.

# Collaborations

- **Task 1 (Bioreactor):**

  Drs. Ali Mohagheghi, Melvin Tucker, and Nick Nagle, National Bioenergy Center at NREL (Biomass pretreatment and characterization).

- **Task 2 (Genetic Methods):**
  - Dr. David Yang at ORNL
  - Drs. Mike Himmel and Shiyou Ding at NREL
  - Drs. David Levin and Richard Sparling at the University of Manitoba, Canada (funded by Genome Canada Program). NREL is an international collaborator in the Genome Canada Grant award to co-develop genetic tools for pathway engineering in *C. thermocellum*.

- **Task 3 (MEC):**

  Dr. Bruce Logan, Penn State University (microbial electrolysis cells to improve $H_2$ molar yield).

# Proposed Future Work

## Task 1:

- Repeat 1 and 5 g/L substrate experiments (both avicel and corn stover) for carbon consumption and $H_2$ molar yield (FY10).
- Begin fed-batch bioreactor with daily feeding of avicel at 2.5 g/L (FY10 /11).
- Scale up and optimize fermentation using co-culture and untreated biomass (FY10 /11).

## Task 2:

- Continue to optimize transformation protocols in house and via collaboration (FY10 /11).
- Investigate the effects of plasmid DNA methylations and protoplast formation on *C. thermocellum* transformation (FY10/11).
- Test different sources of *C. thermocellum* for the presence of HydA3 hydrogenase and its role on $H_2$ production (FY10).

## Task 3:

- Design new tubular cathodes for MECs that allow for recirculation of liquid in the tubes (FY10).
- Build the reactor with the tubular cathode (FY10).
- Conduct tests first on performance with respect to gas retention, internal resistance, and liquid separation of the anode and cathode chamber, and $H_2$ production (FY10/11).

# Summary

## Task 1:

- Determined effects of substrate loading on $H_2$ molar yield and rates.
- Low carbon loading leads to high molar yield, whereas high carbon loading leads to faster rate.
- Established a co-culture (*C. thermocellum* and a *Clostridium* consortium) and improved substrate utilization (both hemicellulose and cellulose).

## Task 2:

- Obtained plasmid tools and tested a proprietary protocol developed by ORNL, albeit not successful.
- Continue to optimize protocols (both electroporation and conjugation) to develop genetic methods and broaden collaboration with others in the field.
- In probing functionality, we discovered that one of the FeFe-hydrogenases (HydA3) is mutated in *C. thermocellum.*

## Task 3:

- Performed hydraulic test and achieved steady $H_2$ performance in the reactor using a continuous flow system.
- Achieved up to 0.53 $m^3/m^3$-d at a cathode surface area of 0.15 $m^2/m^3$.
- Current slightly lower than expected based on cathode surface area; this could be improved by reducing electrode spacing.

# Supplemental Slides

# Response to Reviewers' Comments

- The $H_2$ production rate in this project is very slow and needs to be increased dramatically in order for this project to be viable.
  - *The production needs to be economical. It is a goal that production rate is increased, but this must be done in a cost-effective manner. The fermentation will be tested in a fed-batch mode so that microbes are adapted to degrade cellulose, with the intent to increase $H_2$ production rate.*
- This project requires a relatively expensive feedstock.
  - *An ultimate goal is to use high-energy crop in lieu of corn stover. Techno-economic analysis (conducted by DTI) based on corn stover (including feedstock cost) projects a final $H_2$ selling price of \$4.33/kg $H_2$, or \$2.09/kg $H_2$ if co-product sale is included. Both prices are within the economic range for renewable $H_2$. Moreover, NREL has had initial success fermenting untreated yet finely milled corn stover to bypass pretreatment, thus decreasing feedstock cost.*
- Hydrogen gas produced is not pure; therefore purification technologies will be required…the compression cost will be high.
  - *Gas separation and compression is not unique to this work. $H_2$ production via electrolysis or steam methane reforming requires gas separation and compression; the former yields a mixture of $H_2/O_2$ and the latter $H_2/CO_2$. Moreover, $H_2$-$CO_2$ gas separation is a well-proven commercial process.*
- Over the past year, the team presented on 11 occasions, which would consume a significant amount of time. It is recommended that they limit their conference attendance to the most prestigious conferences in order to better utilize their funds and time.
  - *Subcontract (Logan) did not use DOE funds for travel to report NREL-PSU collaboration. Whenever DOE work is reported, DOE was acknowledged. The NREL travel included postdoc, not just the PI. Moreover, several presentations are either local or paid for by the organizer .*

# Publications


Lalaurette, E., S. Thammannagowda, A. Mohagheghi, P. C. Maness, and B. E. Logan 2009. “Hydrogen production from cellulose in a two-stage process combining fermentation with electrohydrogenesis.” *Intl. J. Hydrogen Energy* 34: 6201-6210.

Magrini-Bair, K. A., S. Czernik, H. M. Pilath, R. J. Evans, P. C. Maness, and J. Leventhal. 2009. “Biomass-derived, carbon sequestering, designed fertilizers.” *Annals Environ. Sci.* 3: 217-225.

Ghirardi, M. L., S. N. Kosourov, P. C. Maness, S. Smolinski, and M. Seibert. 2009. “Hydrogen Production, algal.” *Wiley Encyclopedia of Industrial Biotechnol.* In print.

Thammannagowda, S., L. Magnusson, J. H. Jo, P. C. Maness, and M. Seibert. 2010. “Renewable hydrogen from biomass.” Accepted for publication in *Encyclopedia of Biol. Chem.*

Logan, B.E. 2010. Scaling up microbial fuel cells and other bioelectrochemical systems. *Appl. Microbiol. Biotechnol.* 85(6):1665-1671.

Kiely, P.D., G.K. Rader, J.M. Regan, and B.E. Logan. 2010. Long-term cathode performance and the microbial communities that develop in microbial fuel cells fed different fermentation endproducts. *Biores. Technol.* Submitted .

Kiely, P.D., D.F. Call, M.D. Yates, J.R. Regan, and B.E. Logan. 2010. Anodic biofilms in microbial fuel cells harbor low numbers of higher-power producing bacteria than abundant genera. *Appl. Microbiol. Biotechnol.* Submitted.

# Presentations

"Hydrogen production via the fermentation of lignocellulosic biomass in *Clostridium thermocellum,"* presented at the Renewable and Sustainable Energy Institute (RASEI) at the Univ. of Colorado, Boulder, CO October 21, 2009 (S. Thammannagowda).

"An overview of the NREL hydrogen fermentation research," Invited presentation at the kick-off meeting of the Genome Canada Program. Maness is an international collaborator with all expenses paid for by Genome Canada, Winnipeg, Canada, October 22-25, 2010 (P. C. Maness).

Kiely, P.D., E. Lalaurette, G. Radar, and B.E. Logan. "The conversion of cellulose fermentation end products to hydrogen using a defined microbial consortium and a microbial electrolysis cell." International Microbial Fuel Cell Symposium, Gwanju, Korea, June 10-12, 2009 (P. D. Kiely).

# Critical Assumptions and Issues

**The feedstock cost of lignocellulosic biomass will be reduced significantly to improve the selling price of $H_2$.** The DOE Biomass Program is funding research to improve yields of high-energy crops, increasing cellulose contents, reducing recalcitrance, and improving pretreatment technologies. NREL has yielded preliminary data supporting the fermentation of untreated corn stover.

**Genetic toolbox will be developed to improve $H_2$ production via pathway engineering.** Both *Clostridium acetobutylicum* and *Clostridium cellulolyticum* can be genetically engineered, which provides the proof of concept while increasing the likelihood of success for our approach.

**Microbial electrolysis cells can be scaled up with reduced cost in anode and cathode materials.**