

Fermentation and Electrohydrogenic Approaches to Hydrogen Production



Pin-Ching Maness, Shiv Thammannagowda, and Lauren Magnusson
National Renewable Energy Laboratory

Bruce Logan
Penn State University
(Subcontract)

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Overview

Timeline

- Project start date: FY05
- Project not funded in FY06
- Project end date: 2012
- Percent complete: N/A

Budget

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

Barriers

- Production barriers addressed
 - H₂ molar yield (AR)
 - Waste acid accumulation (AS)
 - Feedstock cost (AT)

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 H₂.
 - Determine effects of substrate loading on rates and yields (Task 1)
 - Develop genetic tools to improve H₂ molar yield (Task 2)
 - Develop continuous flow microbial electrolysis cell (MEC) reactor to improve H₂ molar yield (Task 3).
- **Relevance:** Address directly feedstock cost and H₂ molar yield barriers to improve techno-economic feasibility.

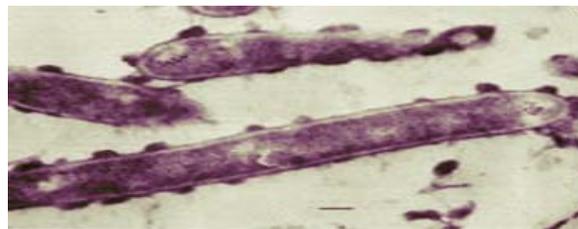
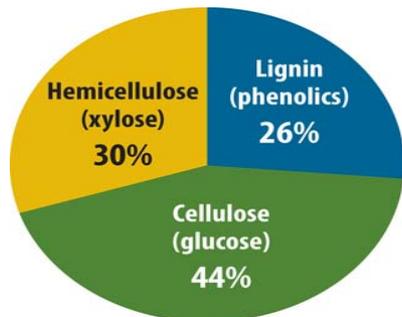
Characteristics	Units	2013 Target	2010 Status
Yield of H ₂ from glucose	Mole H ₂ /mol glucose	4	1.6 - 2.0
Feedstock cost	Cents/lb glucose	10	12

Objectives/Approach/Milestone

Task 1: Bioreactor Performance

- **Objective:** Address feedstock cost and optimize the performance of scaled-up bioreactors for H₂ via fermentation.
- **Approach:** Use corn-stover lignocellulose and cellulose-degrading bacteria to address feedstock cost.

Lignocellulosic Biomass



Clostridium thermocellum

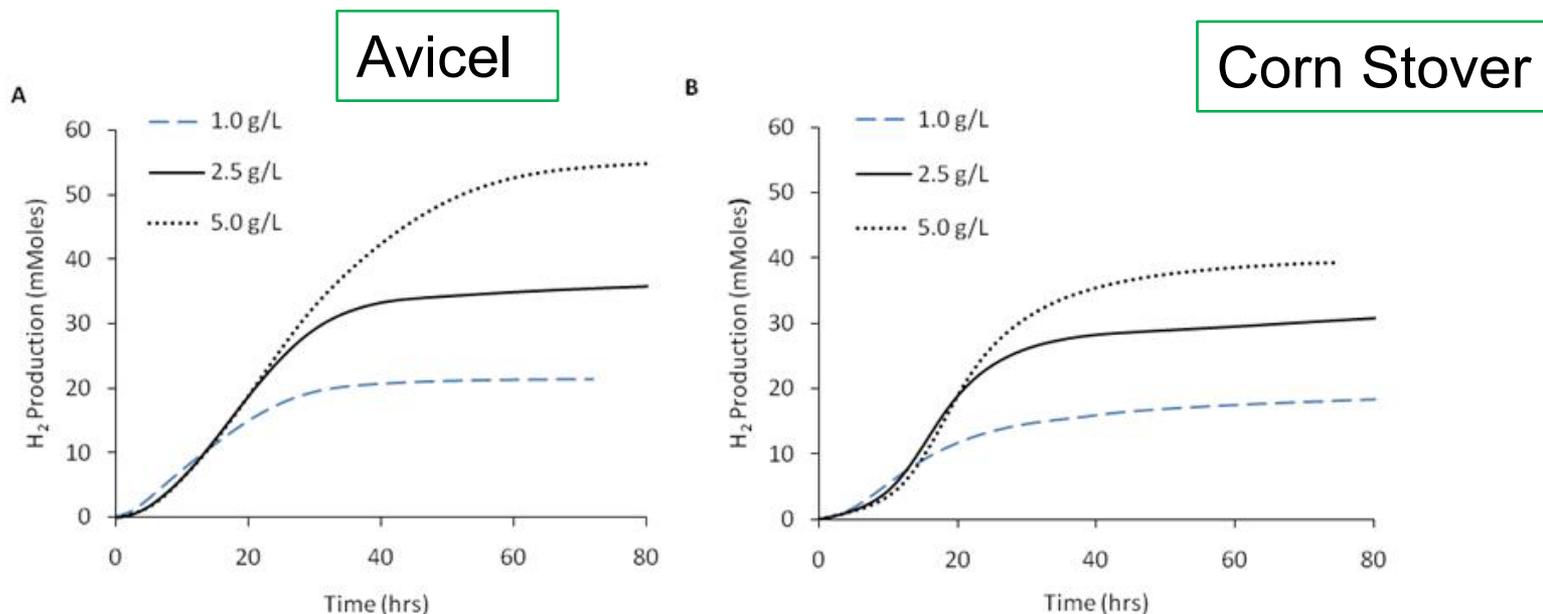
Bioreactor Performance



	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 - H₂ Production Profiles



- The residual cellulose contents were quantified via acid hydrolysis (H₂SO₄).
- Determined *C. thermocellum* cell formula of **C₅H₈O₂N**, consistent with published data in two different bacteria.

Cell formula enables more accurate determination of H₂ 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

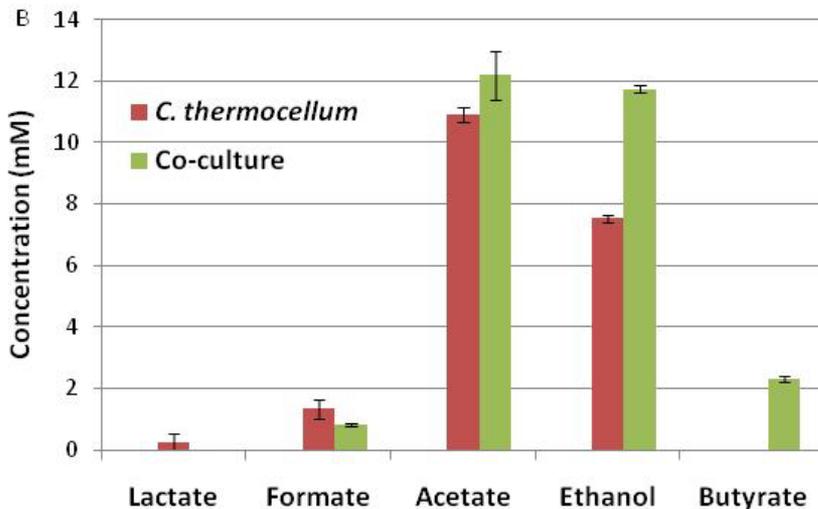
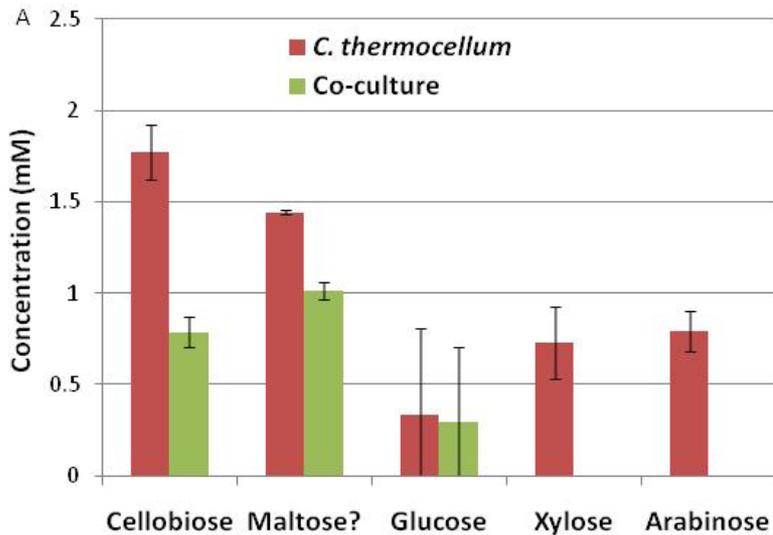
- H₂ production rates and molar yields varied with carbon loadings.
 - Higher carbon loading leads to faster rate of H₂ production
 - Lower carbon loading leads to higher H₂ molar yield.
 - The outcomes guide fed-batch bioreactor with daily feeding of 2.5 g/L.

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 H₂” (1/10).

Task 1 – Technical Accomplishments

H₂ 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.

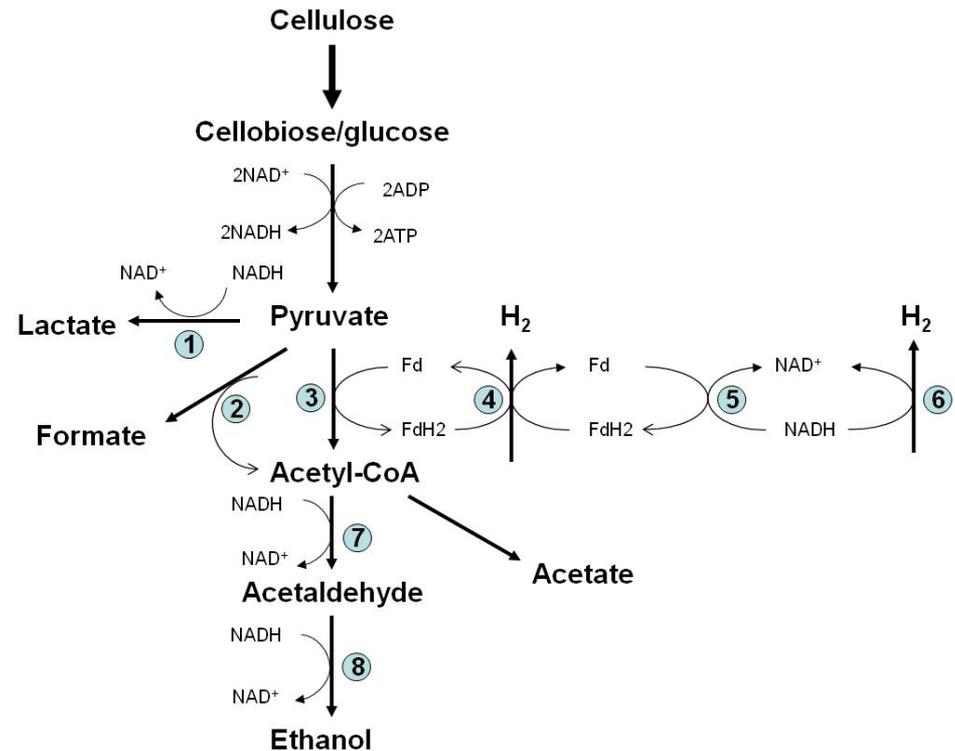
Culture	H ₂ (mM)
<i>C. thermocellum</i>	10.53 +/- 6.19
Co-culture	13.23 +/- 4.70

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.

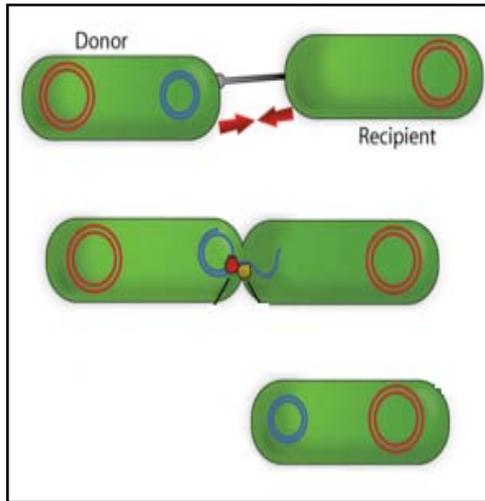


	Milestone	Completion Date	Status
3.2.2	Elucidate role of hydrogenase in <i>C. thermocellum</i>	6/10	In progress
3.2.5	Produce one genetic transformant in <i>C. thermocellum</i>	8/10	In progress

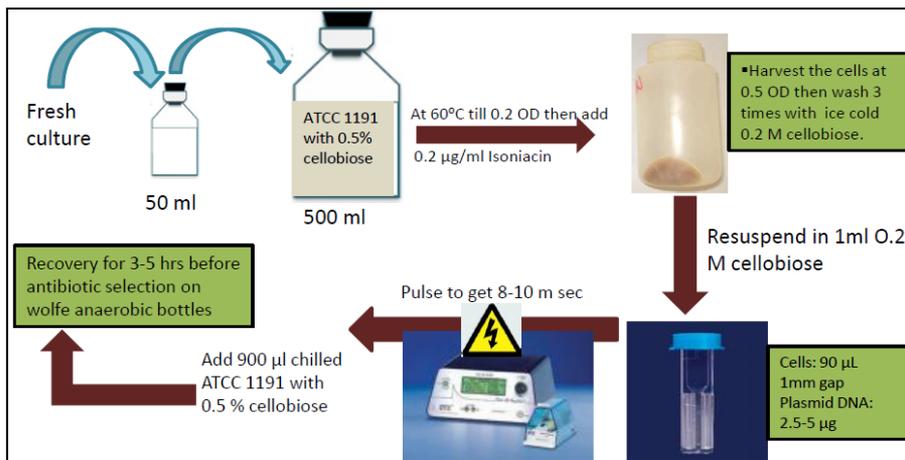
Task 2 – Technical Accomplishments

Developing Tools for Genetic Transformation

Conjugation



Electroporation



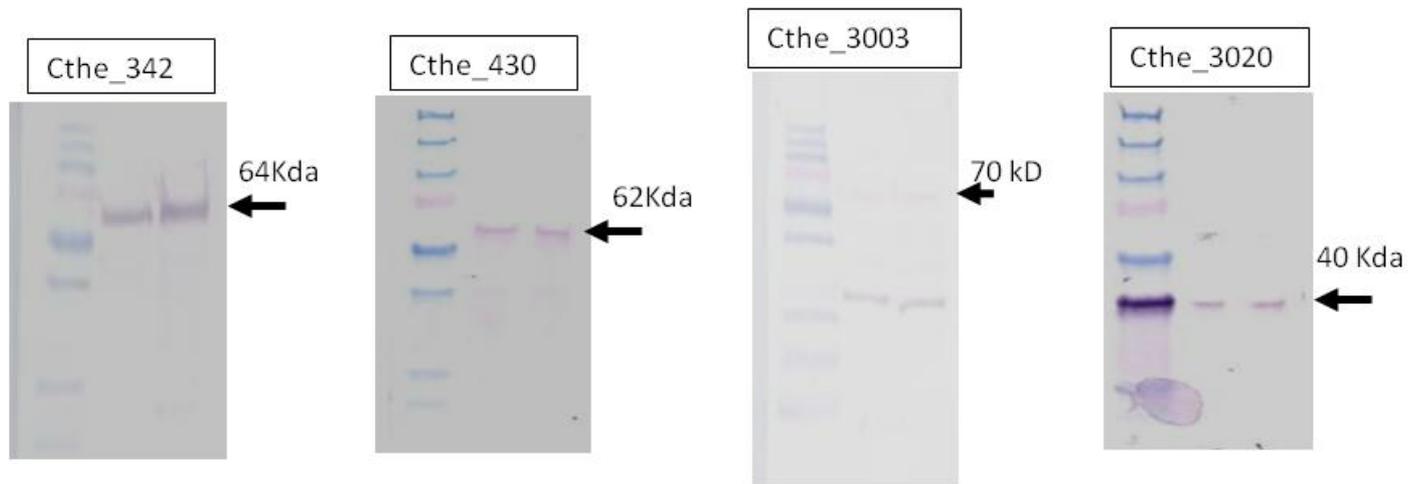
- We tested a proprietary protocol developed by the Oak Ridge National Lab using pKM1 and pHV33 plasmids; the results were not successful.
- We conducted transformation and tested various parameters using a new electroporator that delivers high voltage to the cells.
- Work is under way to prepare protoplast 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 H₂ production.

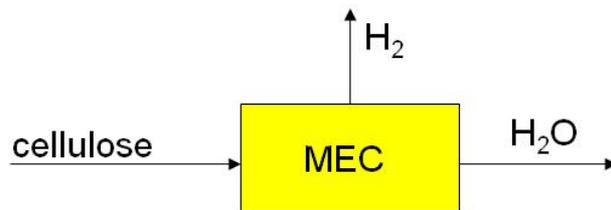
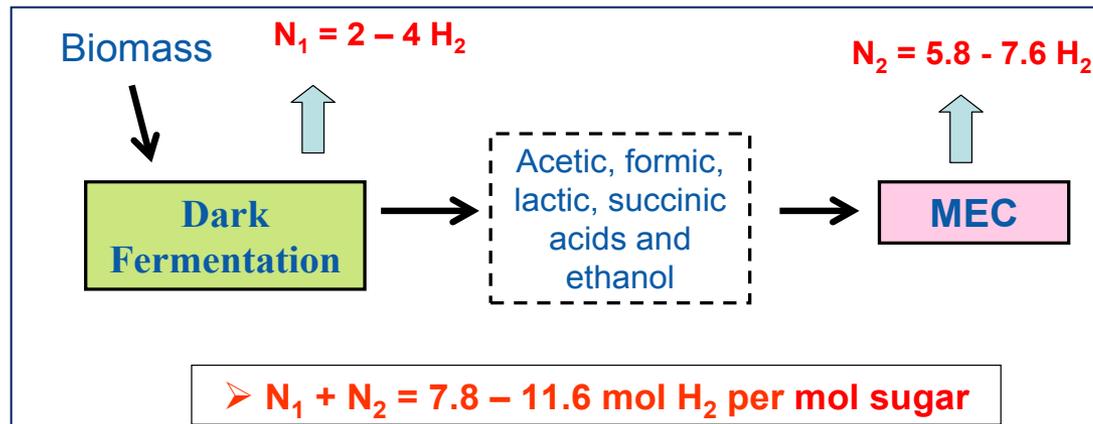
Meeting toward Milestone “Elucidate role of hydrogenase in *C. thermocellum*” (6/10).

Objectives/Relevance

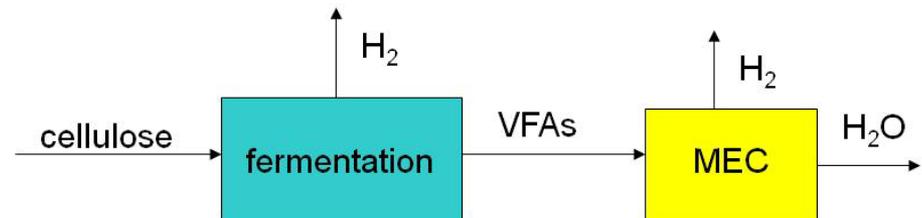


Task 3 – Electrochemically Assisted Microbial Fermentation

- 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 .



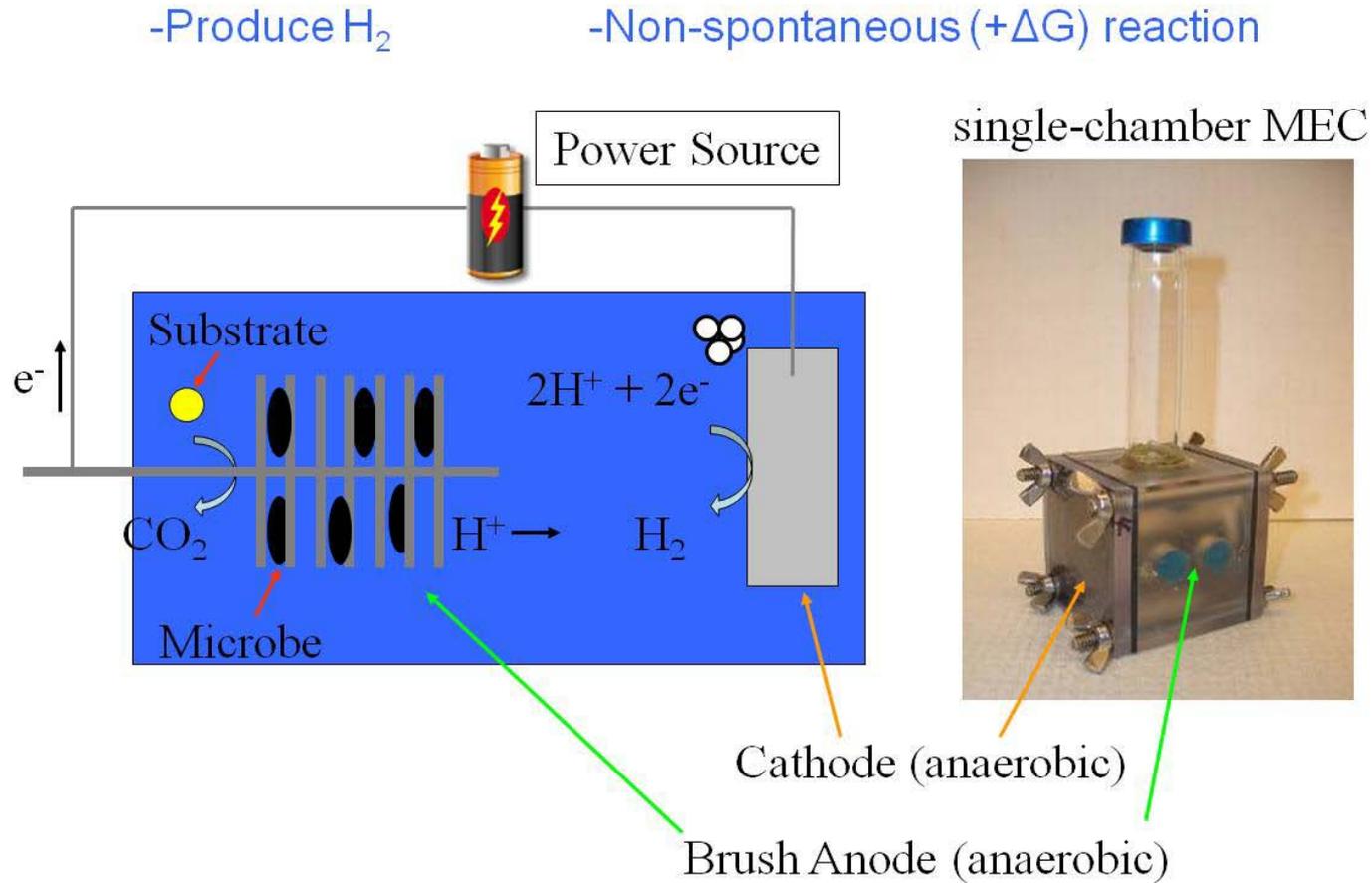
One-stage process: slow



Two-stage process: fast

Approach/Milestone

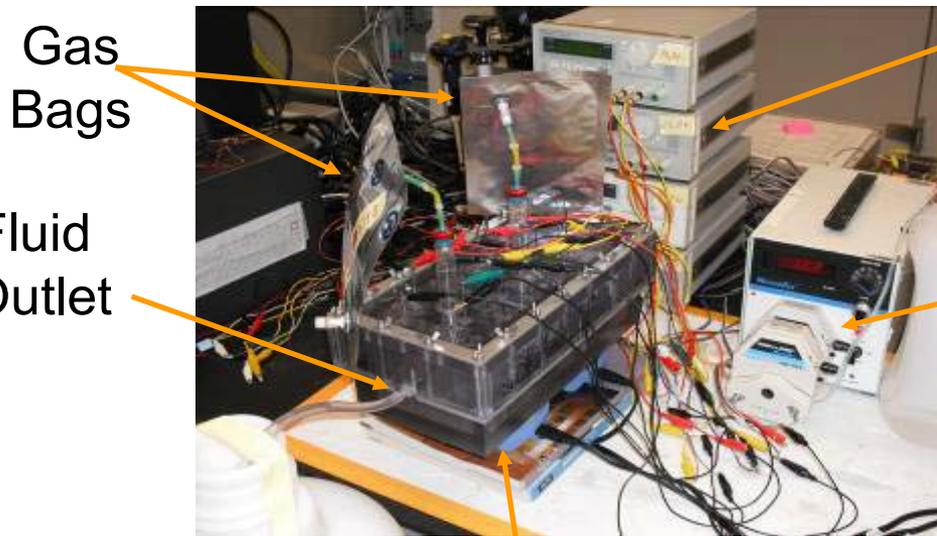
Subtask 3: Electrochemically Assisted Microbial Fermentation



	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

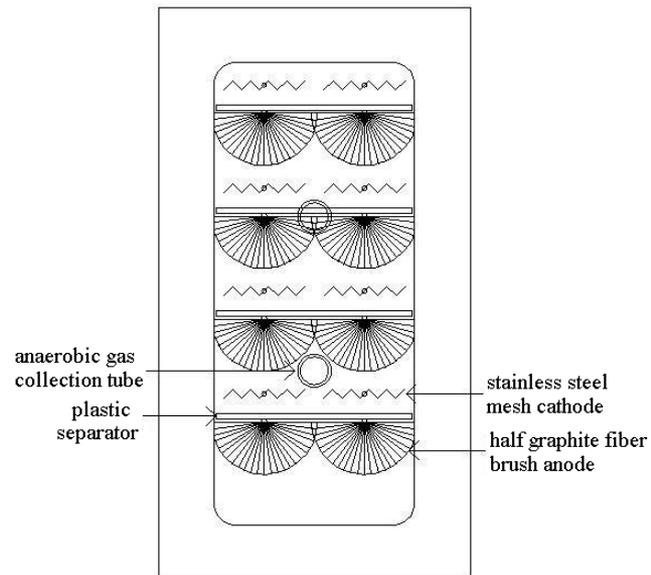


Power Sources

Fluid Pump

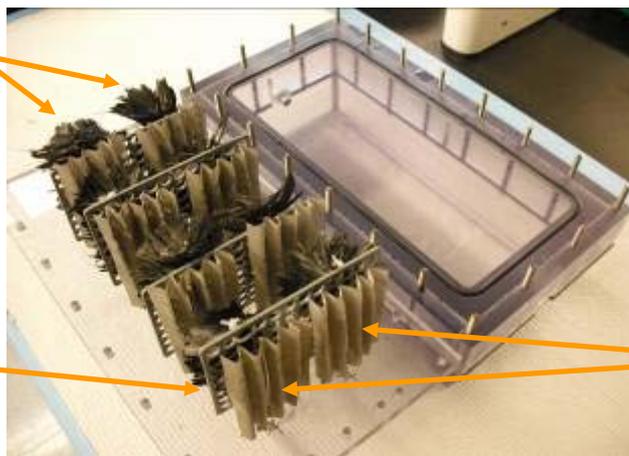
Reactor

Schematic



Half Graphite Fiber Brush Anodes

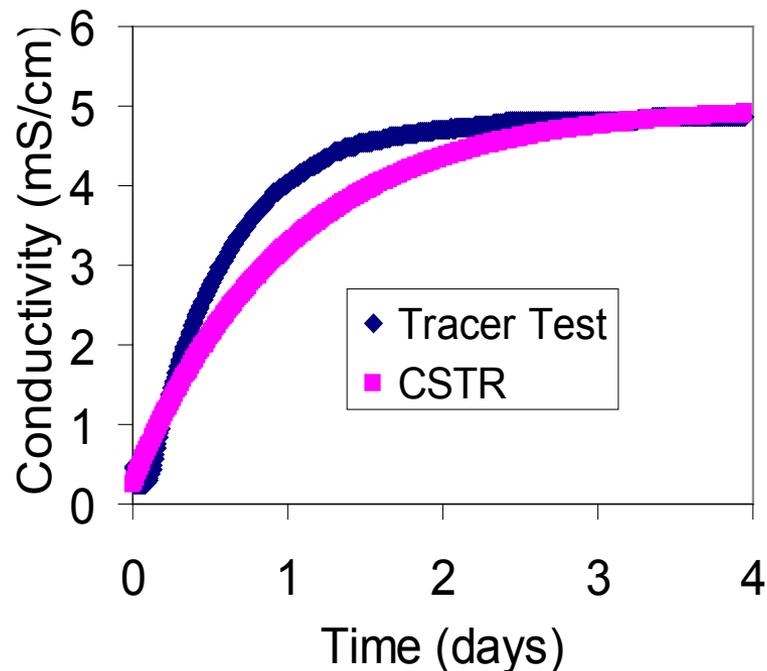
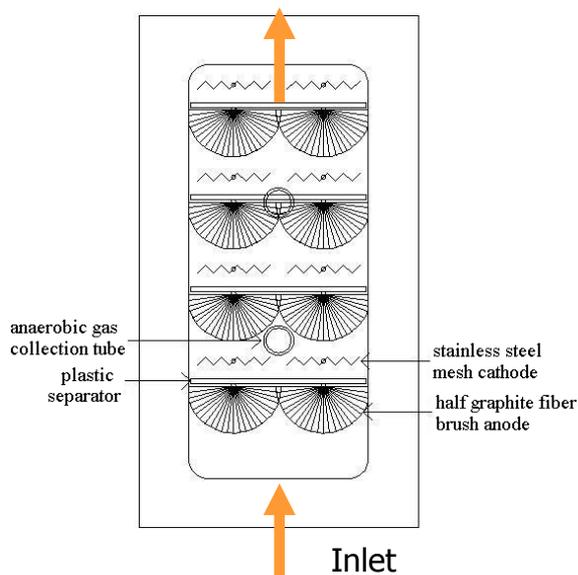
Plastic Separator



Stainless Steel Mesh Cathodes

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.

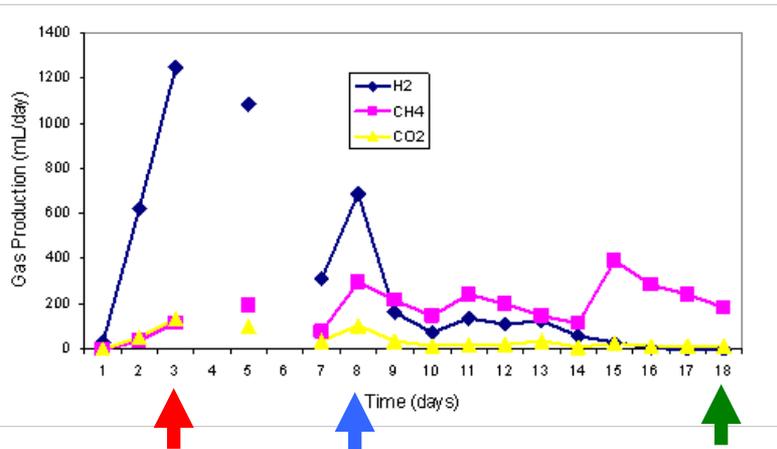
Completed Milestone “Perform hydraulic test of synthetic effluent” (4/10)

Task 3 – Technical Accomplishments

MEC Performance



Overall Performance:



Energy Recovery Considering Only H₂:

Q (m ³ /m ³ /d)	Day	η_E (%)	η_s (%)	η_{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/m³

Energy Recovery Considering H₂ and CH₄:

Day	W _{H₂} (kJ)	W _{CH₄} (kJ)	W _{H₂+CH₄} (kJ)	η_E (%)	η_s (%)	η_{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 CH₄ (891 kJ/mol vs. 286 kJ/mol for H₂) allows for more energy recovery from a smaller volume

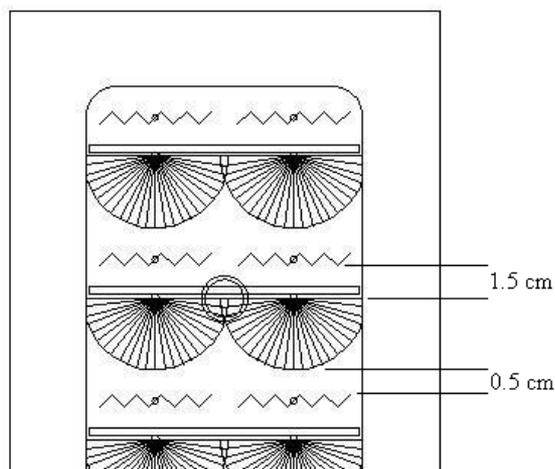


Task 3 - Technical Accomplishments

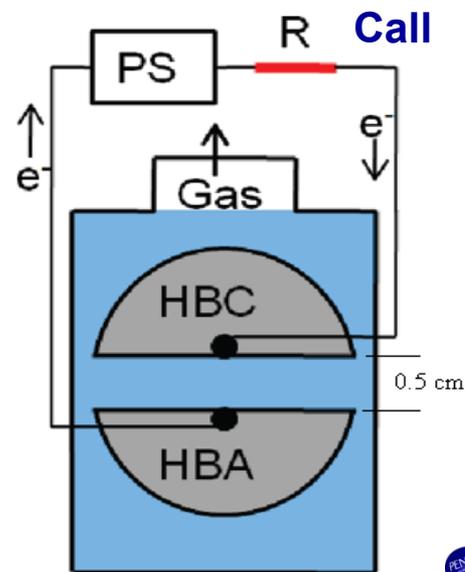
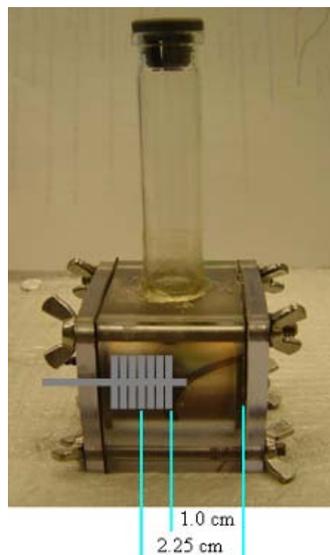
Scalability: Comparison Based on Cathode Current

	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 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 Shiyong 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 H₂ molar yield).

Proposed Future Work

Task 1:

- Repeat 1 and 5 g/L substrate experiments (both avicel and corn stover) for carbon consumption and H₂ 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 H₂ 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 H₂ production (FY10/11).

Summary

Task 1:

- Determined effects of substrate loading on H₂ 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 H₂ performance in the reactor using a continuous flow system.
- Achieved up to 0.53 m³/m³-d at a cathode surface area of 0.15 m²/m³.
- Current slightly lower than expected based on cathode surface area; this could be improved by reducing electrode spacing.