National Renewable Energy Laboratory
Innovation for Our Energy Future

Fermentation and Electrohydrogenic Approaches to Hydrogen Production

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Overview

Timeline

- Project start date: FY05
- Project not funded in FY06
- Project end date: 2012
- 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

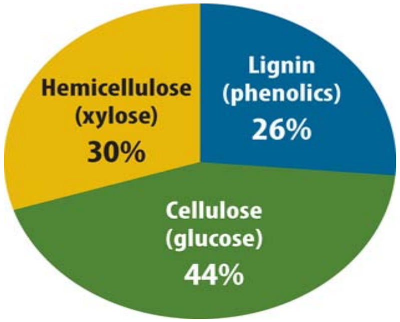
- **Objective:** Develop direct fermentation technologies to convert renewable, lignocellulosic biomass resources to H_2.
  - Determine effects of substrate loading on rates and yields (Task 1)
  - Develop genetic tools to improve H_2 molar yield (Task 2)
  - Develop continuous flow microbial electrolysis 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.

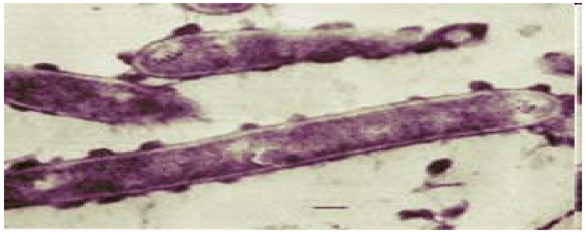
Characteristics	Units	2013 Target	2010 Status
Yield of H_2 from glucose	Mole H_2/mol glucose	4	1.6 - 2.0
Feedstock cost	Cents/lb glucose	10	12

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.

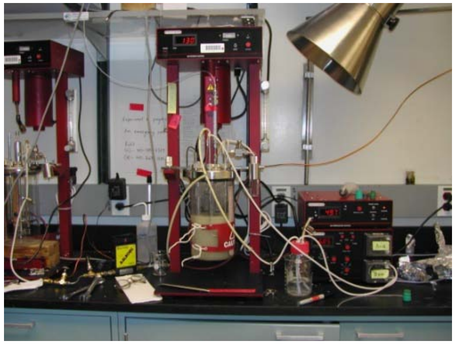
Lignocellulosic Biomass





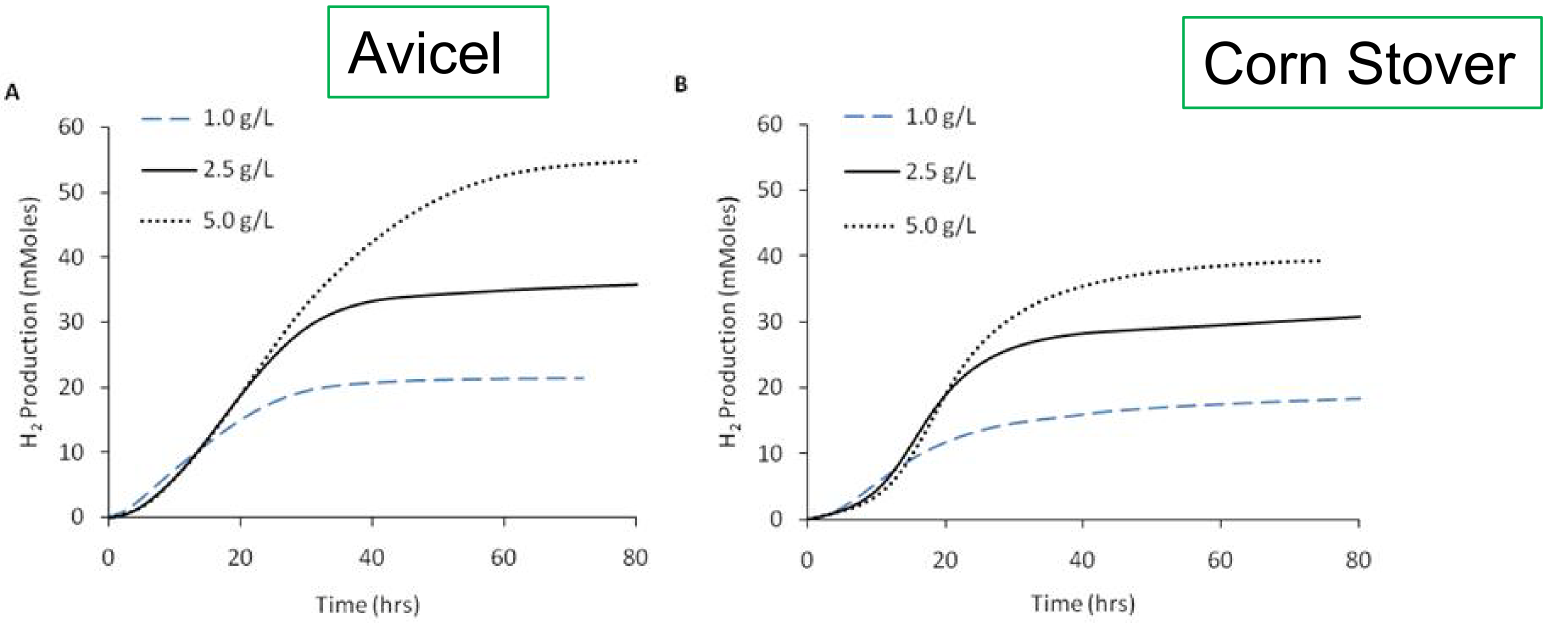
Clostridium thermocellum

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



- 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

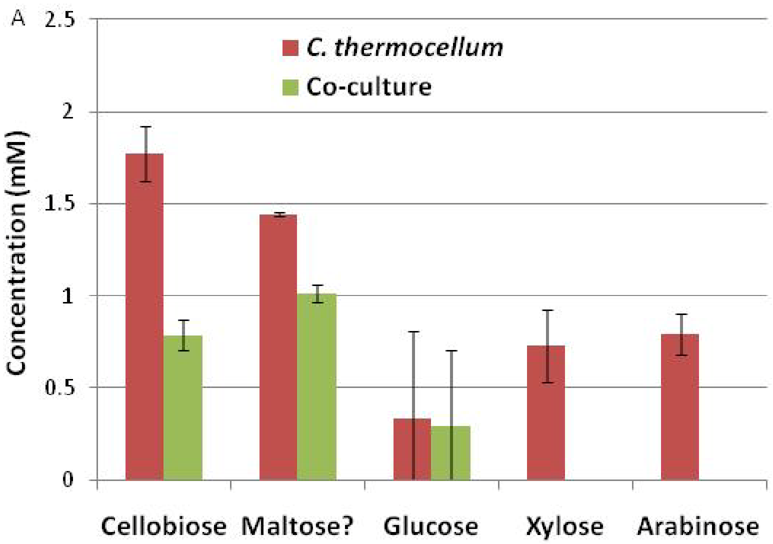
- H_2 production rates and molar yields varied with carbon loadings.
  - Higher carbon loading leads to faster rate of H_2 production
  - Lower carbon loading leads to higher H_2 molar yield.
  - The outcomes guide **fed-batch** bioreactor with daily feeding of **2.5 g/L.**

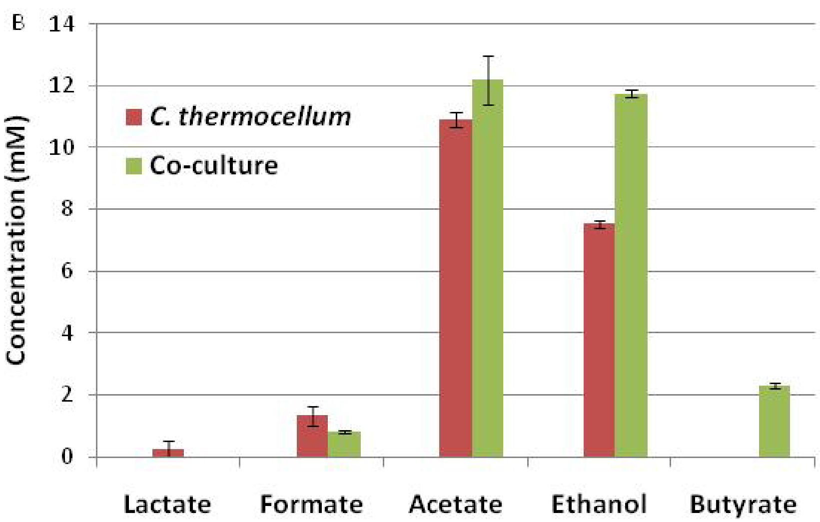
Substrate	G/L	Rate (mmol H_2/L/hr)	H_2 Molar Yield	Carbon Balance (%)
Avicel	1	0.58	3.2	74
Avicel	2.5	0.89	2.1	70
Avicel	5	0.98	1.6	70
Corn stover	1	0.51	2.8	70
Corn stover	2.5	1.06	2.0	94
Corn stover	5	1.21	1.2	51

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.

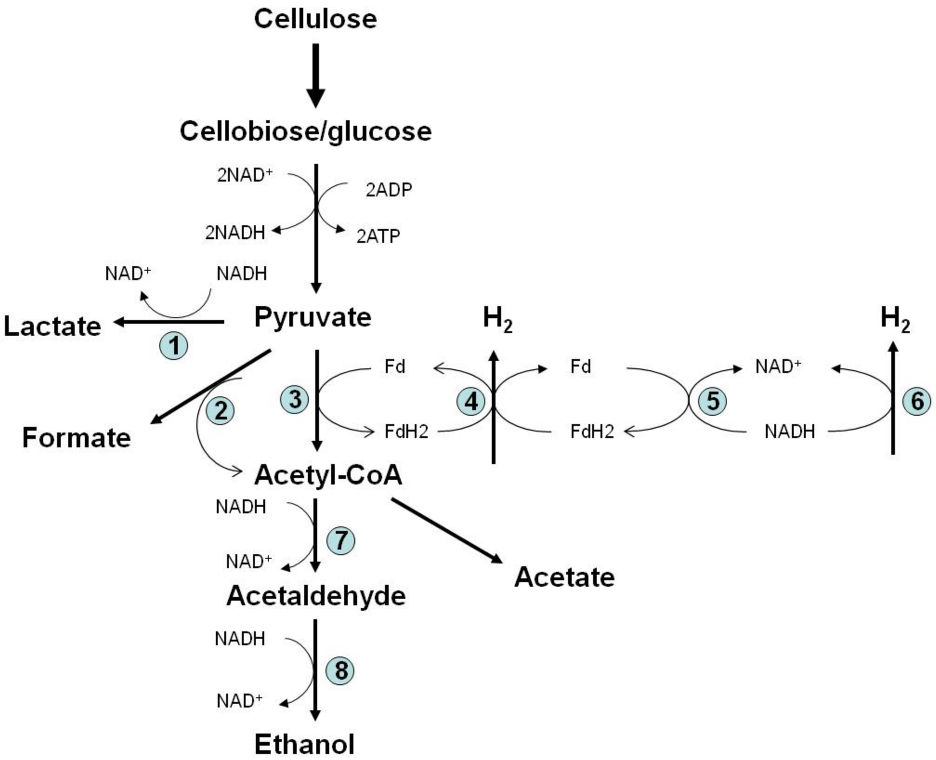
Culture	H_2 (mM)
C. thermocellum	10.53 +/- 6.19
Co-culture	13.23 +/- 4.70

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.

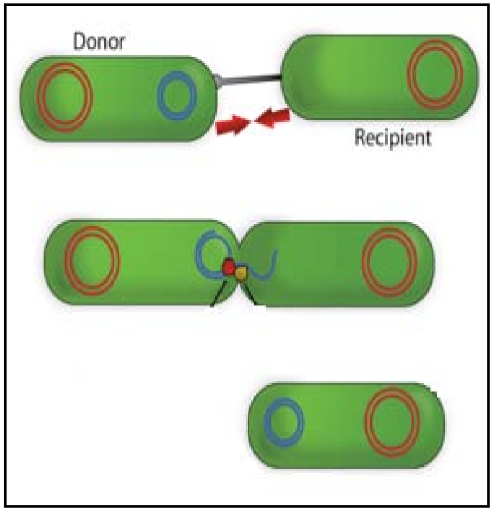


	Milestone	Completion Date	Status
3.2.2	Elucidate role of hydrogenase in *C. thermocellum*	6/10	In progress
3.2.5	Produce one genetic transformant in *C. thermocellum*	8/10	In progress

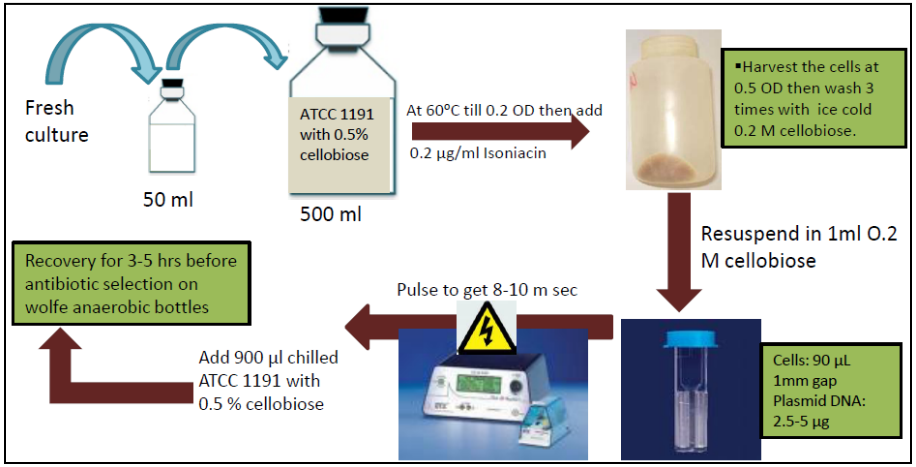
Task 2 – Technical Accomplishments

Developing Tools for Genetic Transformation

Conjugation



Electroporation

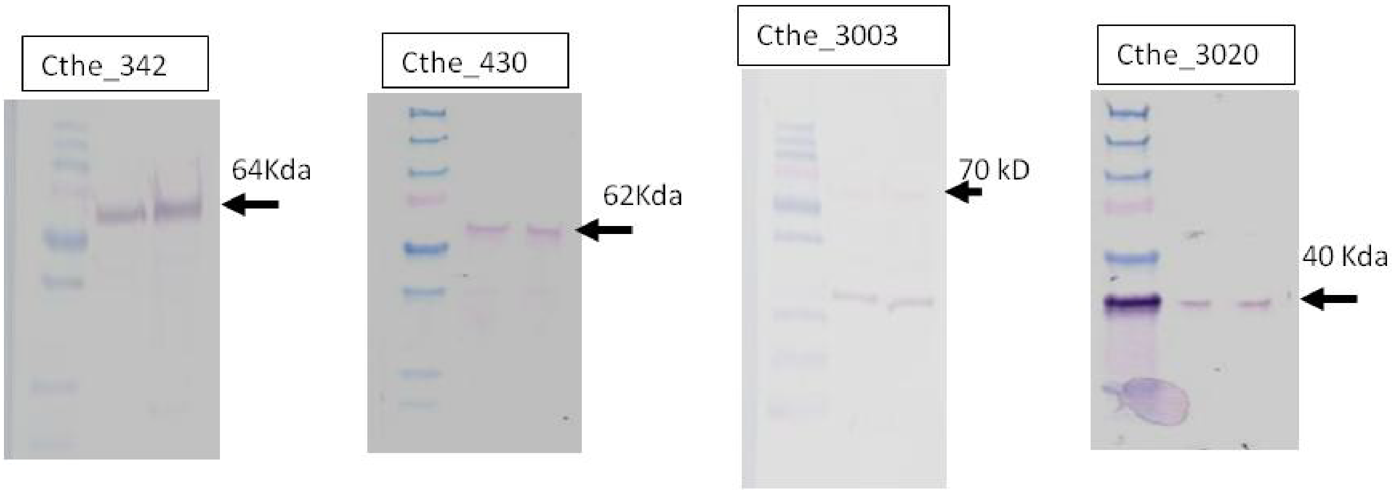


- We tested a proprietary protocol developed by the Oak Ridge National Lab using pIKM1 and pHV33 plasmids; the results were not successful.
- We conducted transformation and tested various parameters using a new electroporator that delivers high voltage to the cells.
- Work is under way to prepare protoplast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



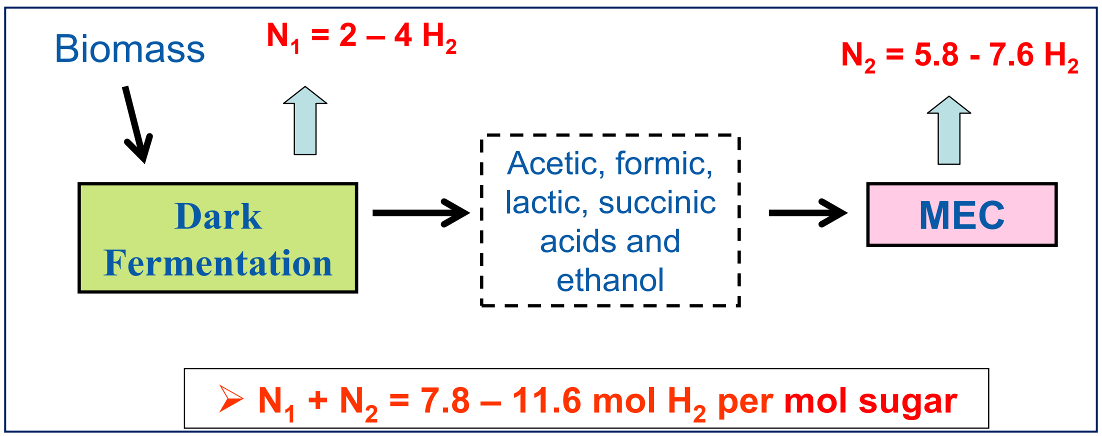
- Protein western blot revealed that HydA3 is not expressed amongst the four hydrogenases.
- Elucidating functions allows manipulations of growth conditions and/or hydrogenase genes to enhance H_2 production.

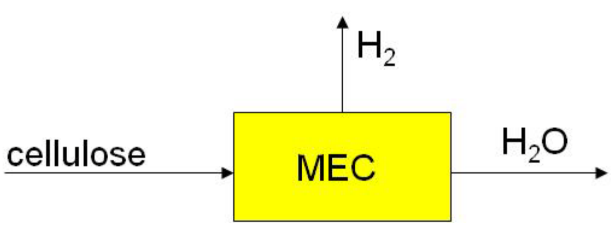
Meeting toward Milestone "*Elucidate role of hydrogenase in C. thermocellum*" (6/10).

Objectives/Relevance

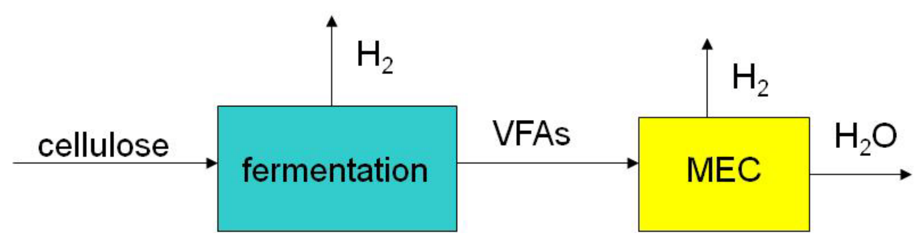
Task 3 – Electrochemically Assisted Microbial Fermentation

- **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

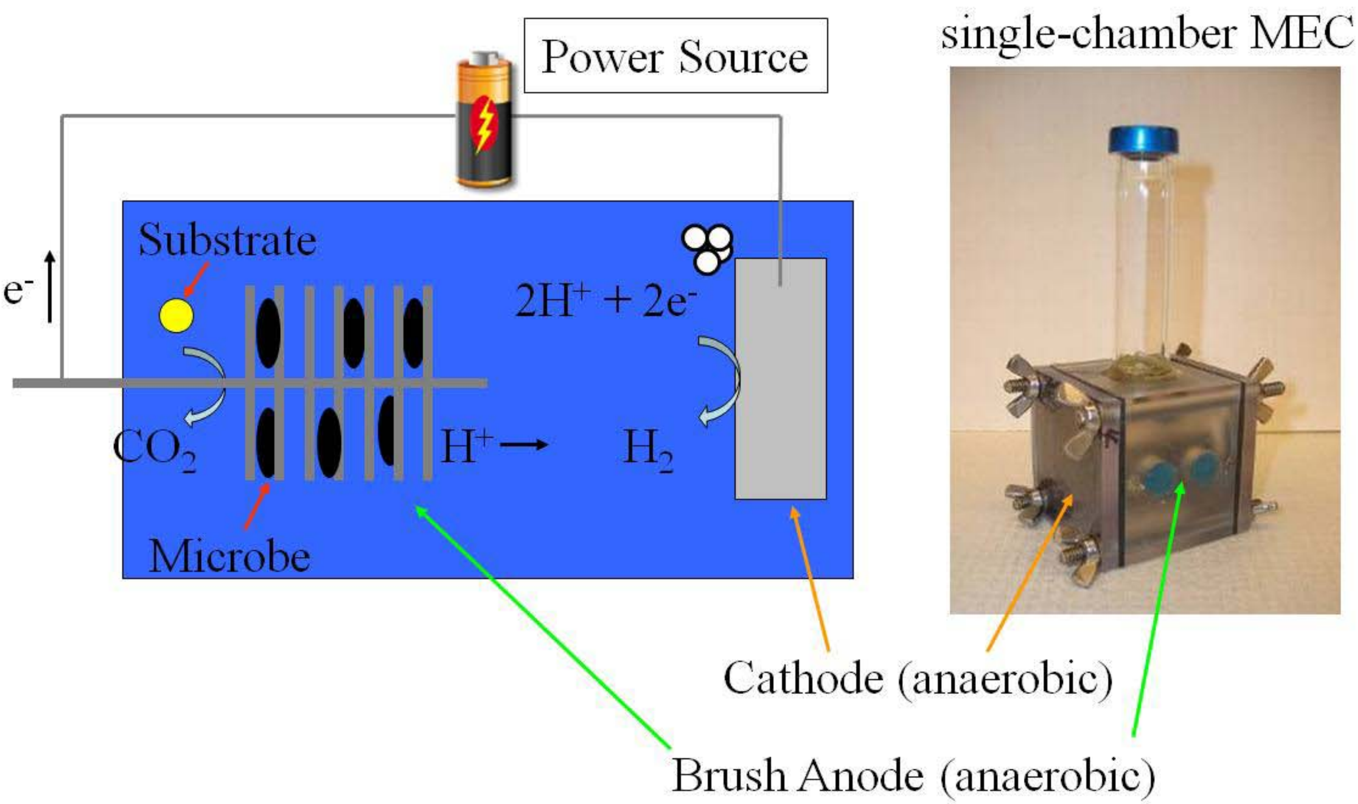


Two-stage process: fast

Approach/Milestone

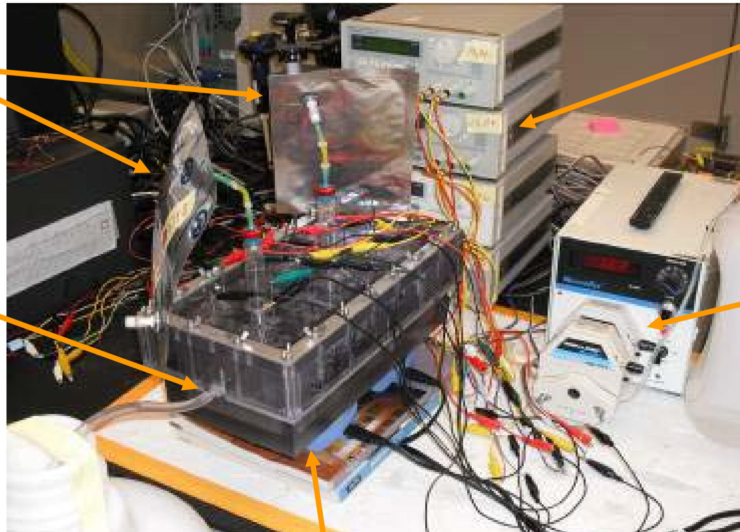
Subtask 3: Electrochemically Assisted Microbial Fermentation

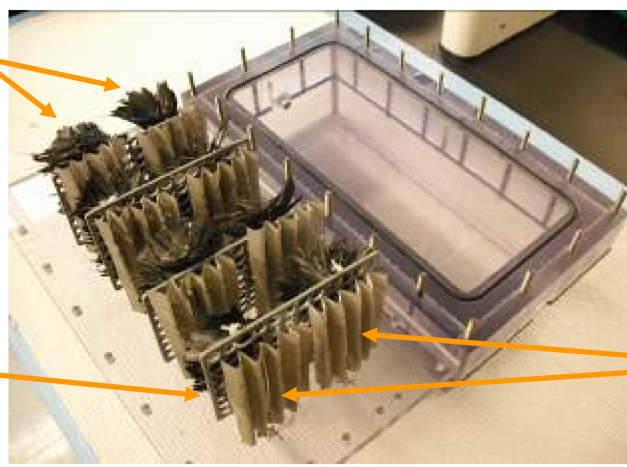
-Produce H_2 -Non-spontaneous (+ΔG) reaction



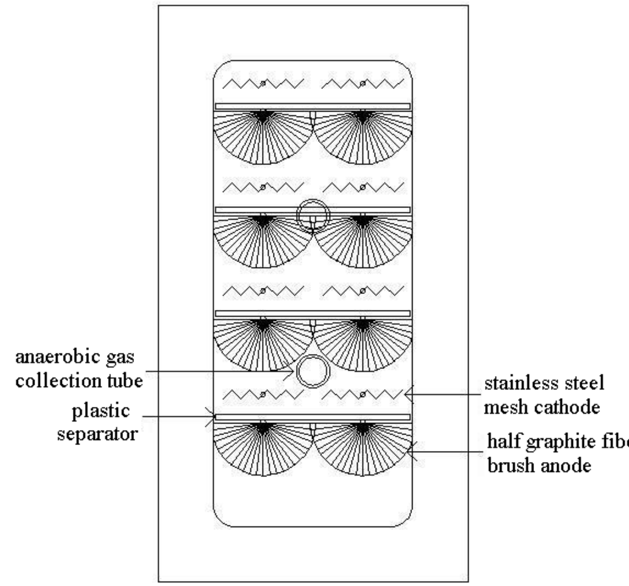
	Milestone	Completion Date	Status
3.2.3	Perform hydraulic test of synthetic effluent	4/10	Completed

Task 3 – Technical Accomplishments
2.5 L Continuous Flow MEC

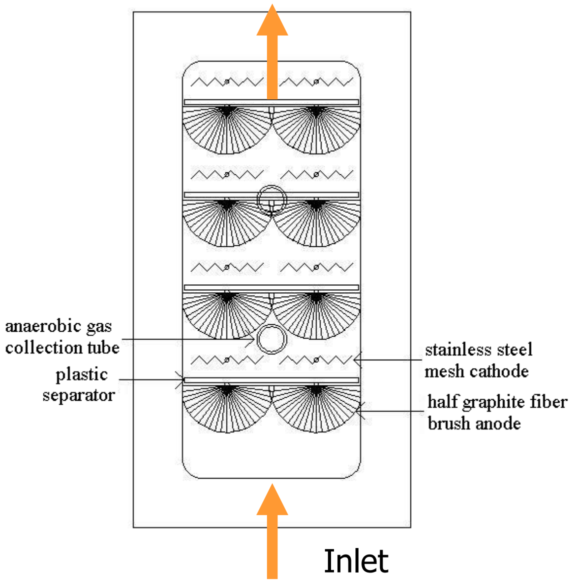


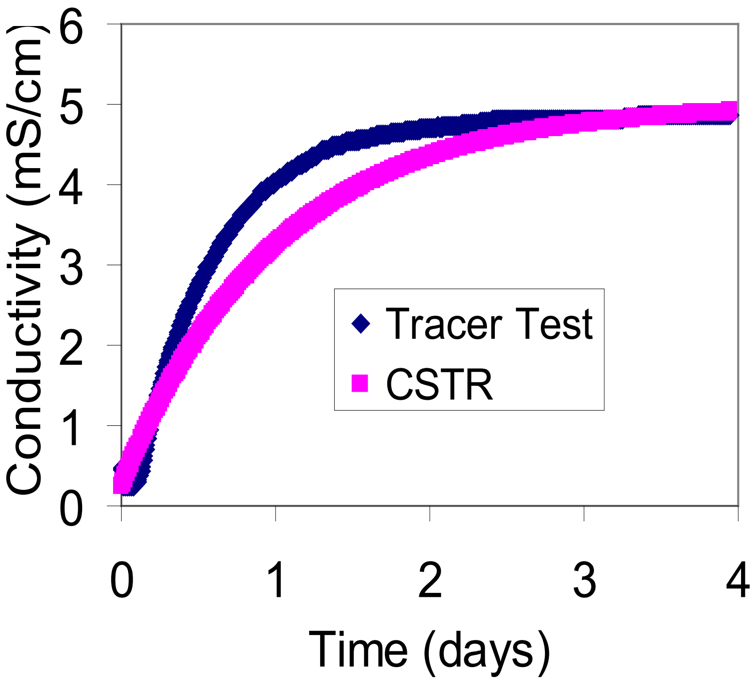


Schematic



Task 3 – Technical Accomplishments
Hydrodynamics of MECs



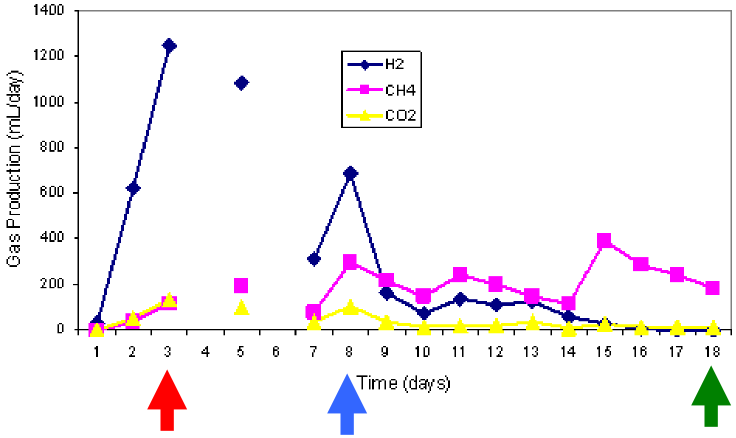


- Tracer conductivity increased more *quickly* than CSTR.
- Some short circuiting to outlet.
- May need to improve liquid flow using baffles.

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	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/m^3

Energy Recovery Considering H_2 and CH_4:

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

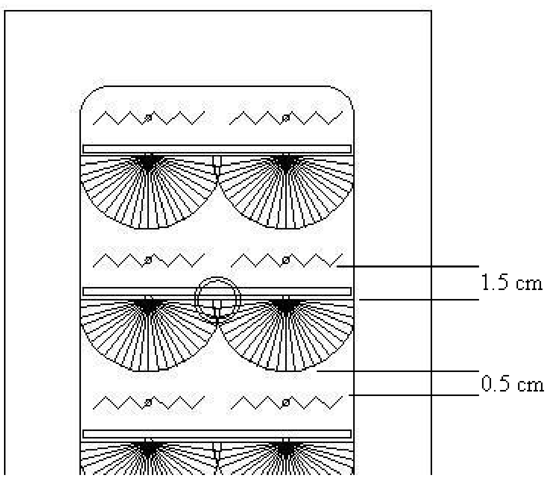
*Higher heat of combustion for CH_4 (891 kJ/mol vs. 286 kJ/mol for H_2) allows for more energy recovery from a smaller volume

Task 3 - Technical Accomplishments

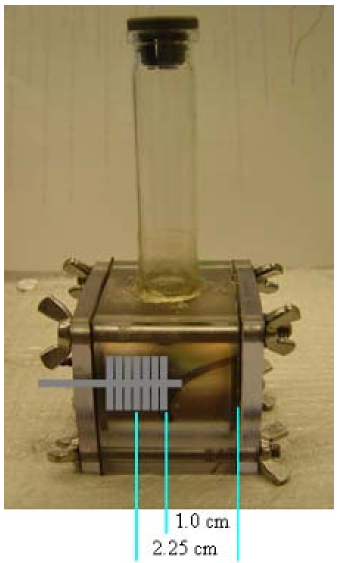
Scalability: Comparison Based on Cathode Current

	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



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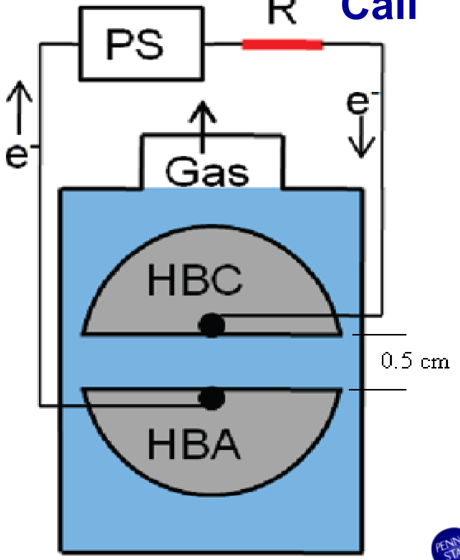

Call et al.

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.