NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Fermentation and Electrohydrogenic Approaches to Hydrogen Production

2011 Annual Merit Review and Peer Evaluation Meeting

Pin-Ching Maness

May 11, 2011

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: 10/2011*
- Percent complete: N/A

Barriers

Production barriers addressed

- H_2 molar yield (AR)
- Waste acid accumulation (AS)
- Feedstock cost (AT)

Budget

- Total project funding: $1,910K (include $290K subcontract)
- FY10: $230K (include $60K subcontract)
- Funding allocated for FY11: $400K (include $60K subcontract)

Partners

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

*Project continuation and direction determined annually by DOE

Relevance

- **Objective:** Develop direct fermentation technologies to convert renewable lignocellulosic biomass resources to H_2.
  - Optimize fed-batch bioreactor (hydraulic and solid retention time) (Task 1).
  - Develop genetic tools to improve H_2 molar yield (Task 2).
  - Design and build a tubular type MEC and conduct performance evaluation (Task 3).
- **Relevance:** Address directly feedstock cost and H_2 molar yield barriers to improve technoeconomic feasibility.

Characteristics	Units	2013 Target	2011 Status
Yield of H_2 from glucose	Mole H_2/mol glucose	4	3.2
Feedstock cost*	Cents/lb glucose	10	12

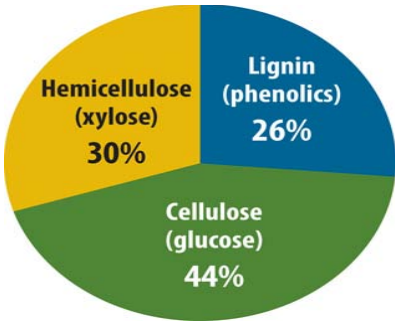
* DOE Office of Biomass Program status and target

Objectives/Approach/Milestone

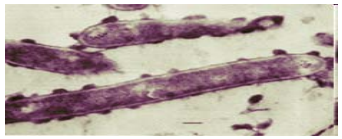
Task 1: Bioreactor Performance

- **Objective:** Address feedstock cost and improve the performance of bioreactors for H_2 via fermentation of lignocellulose.
- **Approach:** Optimize bioreactor in fed-batch mode by testing parameters such as amount and frequency of cellulose feedings and acclimation of the cellulose-degrading bacterium *Clostridium thermocellum*.

Lignocellulosic Biomass

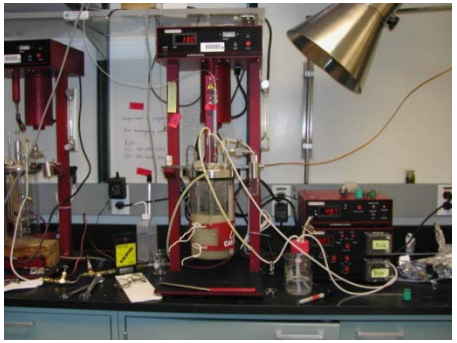


Fermentation



Clostridium thermocellum

Bioreactor Performance

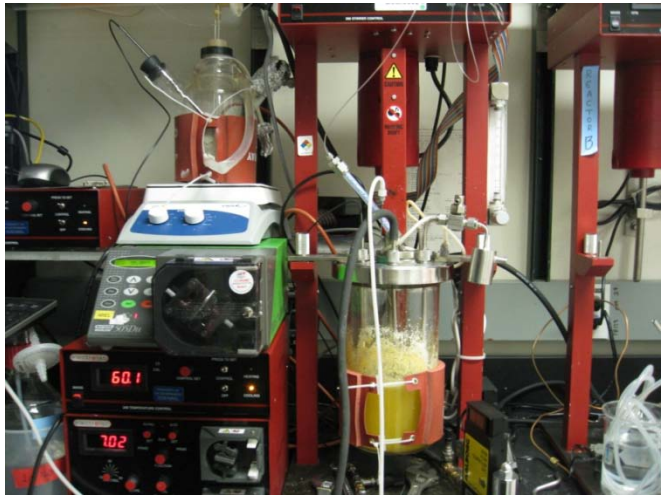


	Milestone	Completion Date	Status
3.2.1.2	Determine the solid and hydraulic retention time on rates and yield of H_2 in **fed-batch** reactor	4/11	On track

Task 1 – Technical Accomplishments

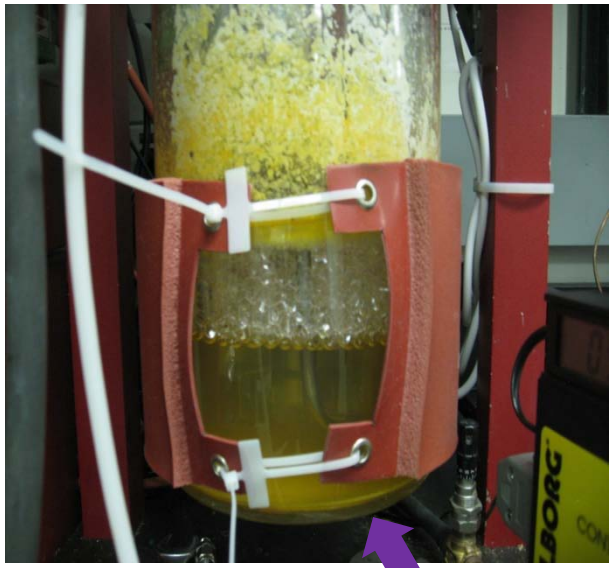
Fed-Batch Bioreactor Setup

- Work in FY10 determined the effect of substrate loadings on rates and yields of H_2, which guides the development of fed-batch fermentation.
- In a one-liter working volume bioreactor, we drained and replenished daily (hydraulic retention time = 24 h) with 500 mL fresh medium containing 2.5 g/L cellulose.



Acclimated microbes turned yellow

Settle, Drain, Feed



Settled microbes in yellow;
~80% recovery

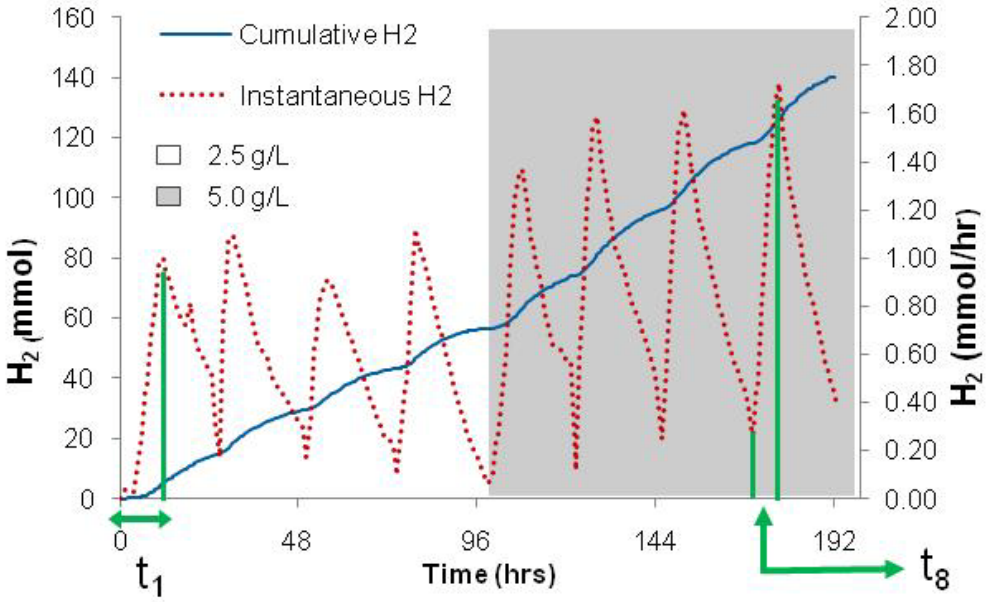
C. thermocellum **are immobilized on cellulose, allowing the bulk of the growth medium to be replaced without diluting the fully acclimated microbes.**

Task 1 – Technical Accomplishments

Hydrogen Production in Fed-Batch Bioreactor

Daily feedings of 2.5 (up to 96 h) and 5.0 g/L of cellulose conducted:

- Fed-batch mode adapted *C. thermocellum* to degrade cellulose, shown by a decrease in "time to peak H_2 production" (faster acclimation; t_1 to t_8).
- With faster rate of H_2 (> 53% increase), a smaller bioreactor can be built to reduce cost.
- Higher bacterial cell mass was observed; this will lead to higher H_2 output.



Batch	Cellulose Concentration	Time to Peak H_2 Production	Amount of H_2 Produced	Average H_2 Production Rate
	(g/L)	(t, h)	(mmoles)	(mmol L^{-1} h^{-1})
1	2.5	18:43	14.92	0.60
2-4		4:14	13.85	
5	5.0	8:09	17.57	0.92
6-8		5:27	22.11	

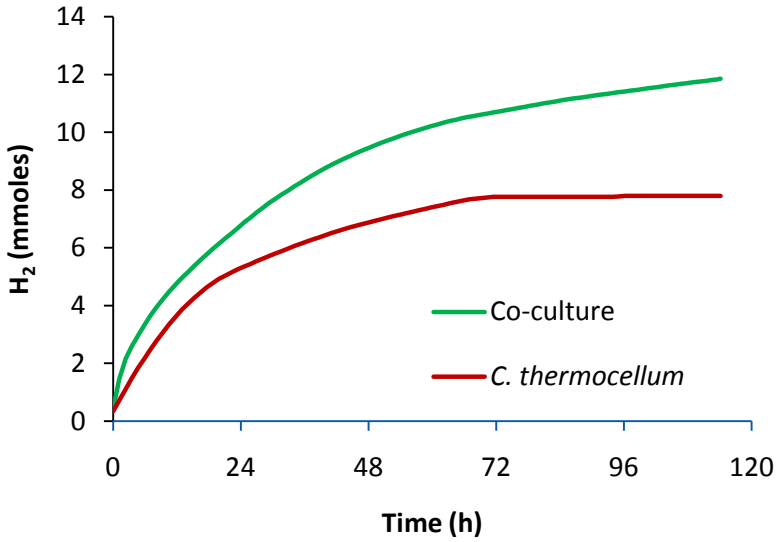
On track to complete Milestone "Determine the solid and hydraulic retention time on rates and yield of H_2 in fed-batch reactor (4/11).

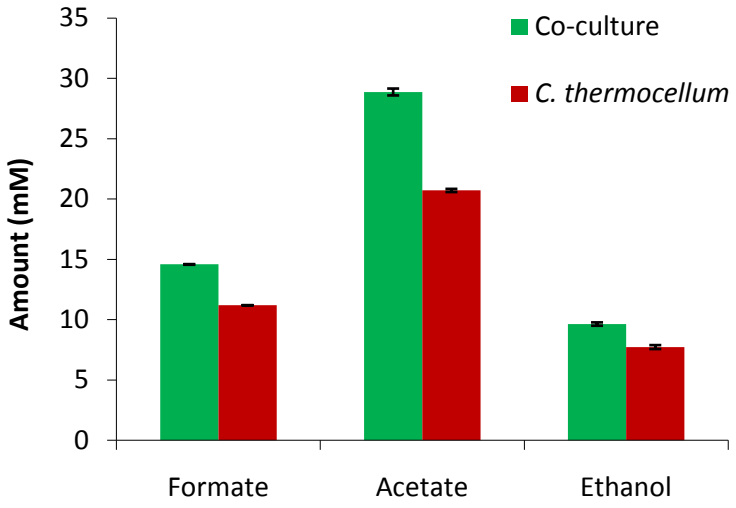
Task 1 – Technical Accomplishments

H_2 From "*Untreated*" Corn Stover Using a Co-Culture

- We scaled up fermentation of untreated corn stover using a co-culture of *Clostridium thermocellum* and a *Clostridium* consortium
- *C. thermocellum* hydrolyzed both cellulose and hemicellulose, with the latter utilized by the consortium.
- The co-culture produced **64%** more H_2 than *C. thermocellum* alone, suggesting better substrate utilization.
- The metabolite profiles also corroborate the synergy of the co-culture.

The utilization of untreated biomass lowers feedstock cost.

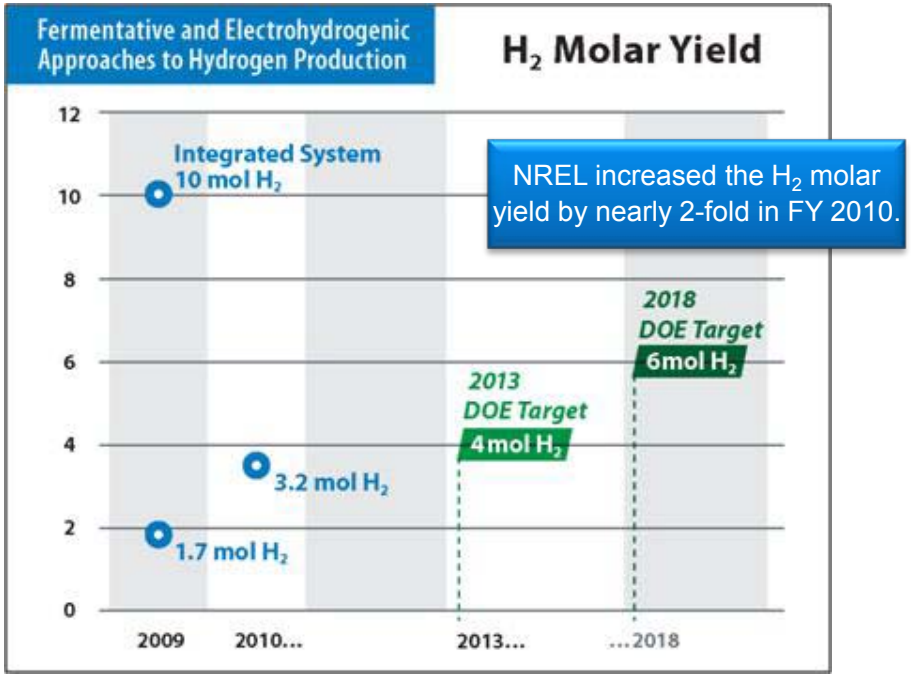




Objectives/Approach/Milestone

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.
  - Design plasmids and optimize transformation protocols.
  - Create mutant host suitable for targeted mutagenesis.



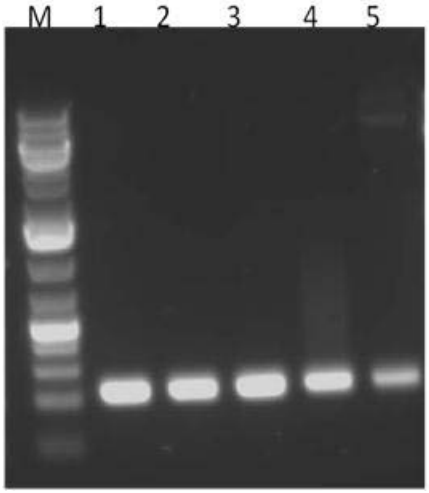
	Milestone	Completion Date	Status
3.2.2	Produce one genetic transformant in *C. thermocellum* (FY10)	9/10	Completed
3.2.2	Obtain one mutant of *C. thermocellum* lacking the *pyrF* gene as the platform host for targeted mutagenesis (FY11)	9/11	On track

Task 2 – Technical Accomplishments
Develop Tools for Genetic Transformation

- NREL co-developed genetic tools (with the University of Manitoba): proprietary plasmid and transformation protocols, and obtained two mutants of *C. thermocellum*.
- Transformation was verified by (1) growth in antibiotic (chloramphenicol,100 µg/mL); (2) PCR of the antibiotic gene; and (3) retransformation in *E. coli*.
- This will lead to blocking competing pathways to improve H_2 molar yield.

Cell growth:

1. Transformant in antibiotic
2. Transformant without antibiotic
3. Control cells in antibiotic
4. Control cells without antibiotic.



PCR of the antibiotic gene:

- Lanes 1-2: transformant #1;
- Lanes 3-4: transformant #2;
- Lane 5: plasmid control.

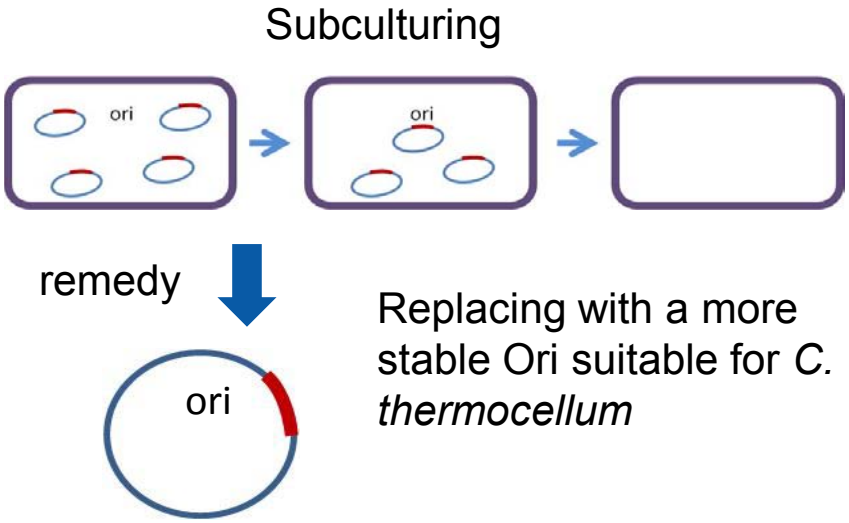
<u>Completed</u> Milestone "*Produce one genetic transformant in Clostridium thermocellum*" (8/10).

Task 2 – Technical Accomplishment

Develop Targeted Pathway Mutant

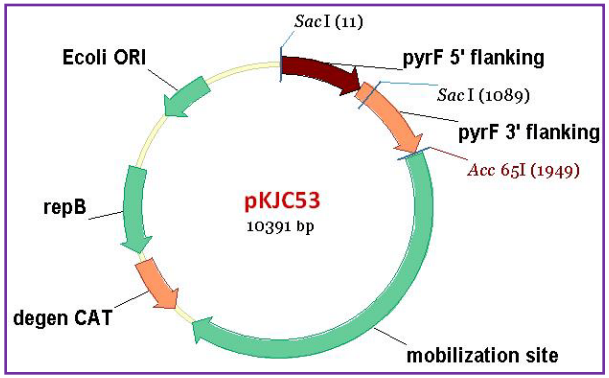
Improve plasmid stability

- We discovered that the proprietary plasmid is not stable in the transformant. Work is underway to replace its origin of replication (ori) to improve its long-term stability.



Block pyruvate-to-formate pathway

- Blocking the pyruvate-to-formate pathway (with hypophosphite) increased H_2 production by 81% (FY09) – proof of concept.
- Work is ongoing to create a Δ*pyrF* mutant host to generate the targeted pathway mutant via an effective suicide method.

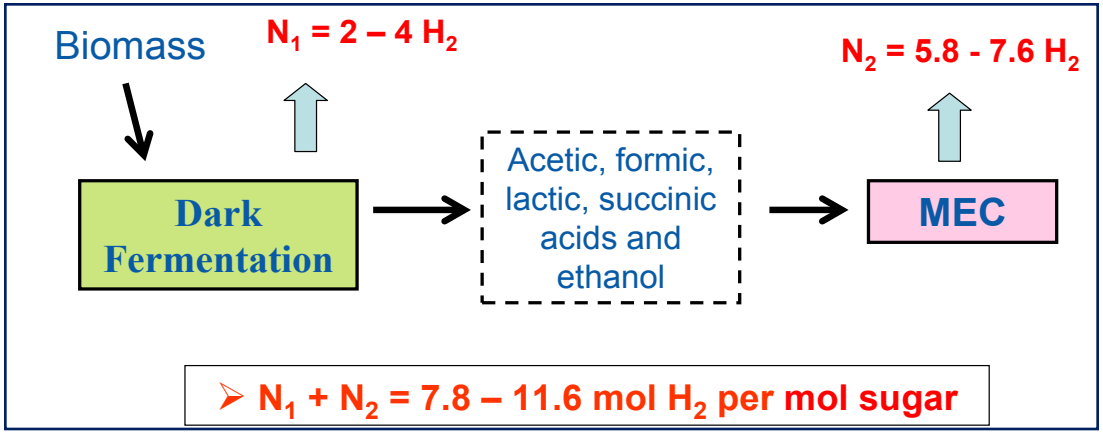


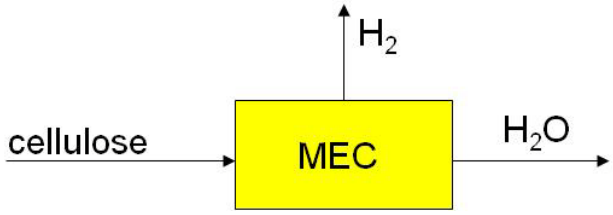
<u>On track</u> to complete Milestone "*Obtain one mutant of C. thermocellum lacking the pyrF gene as the platform host for targeted mutagenesis*" (9/11).

Objectives/Relevance

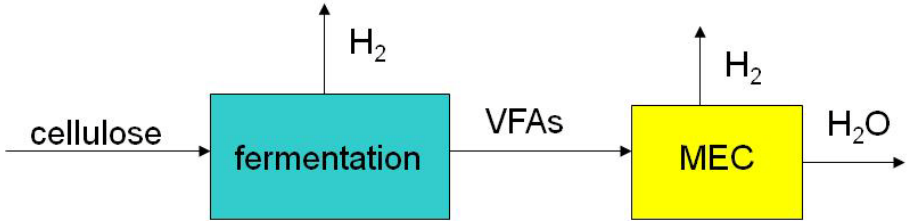
Task 3 – Electrochemically Assisted Microbial Fermentation

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

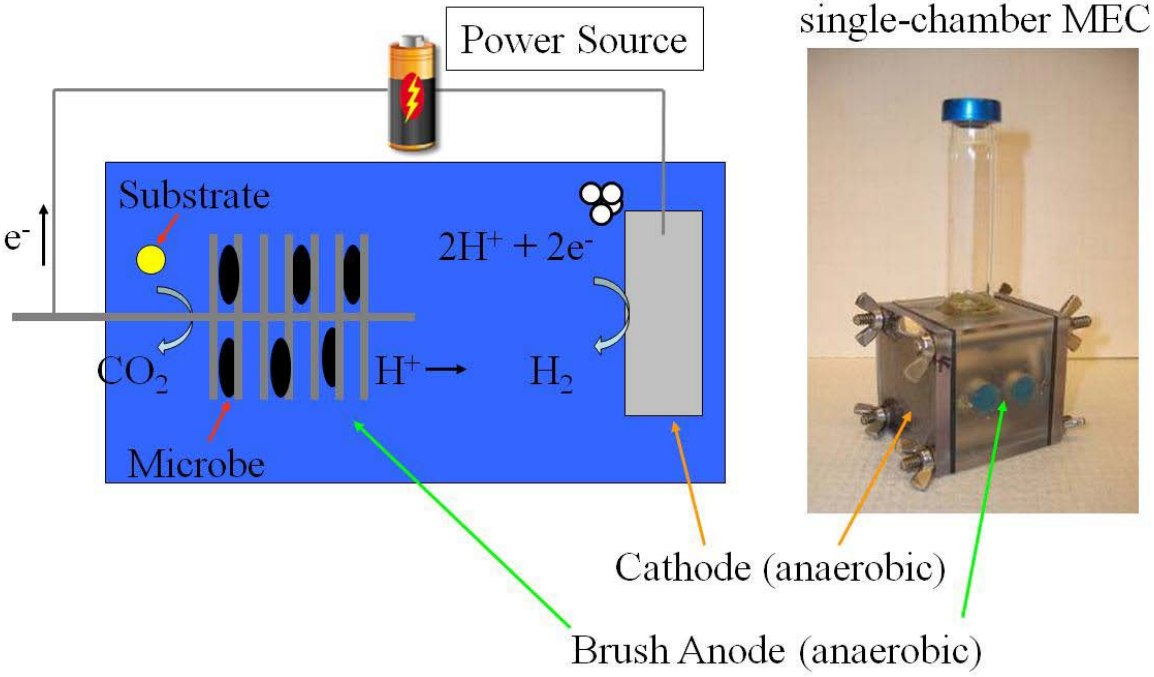


Two-stage process: fast

Approach/Milestone

Subtask 3: Electrochemically Assisted Microbial Fermentation

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

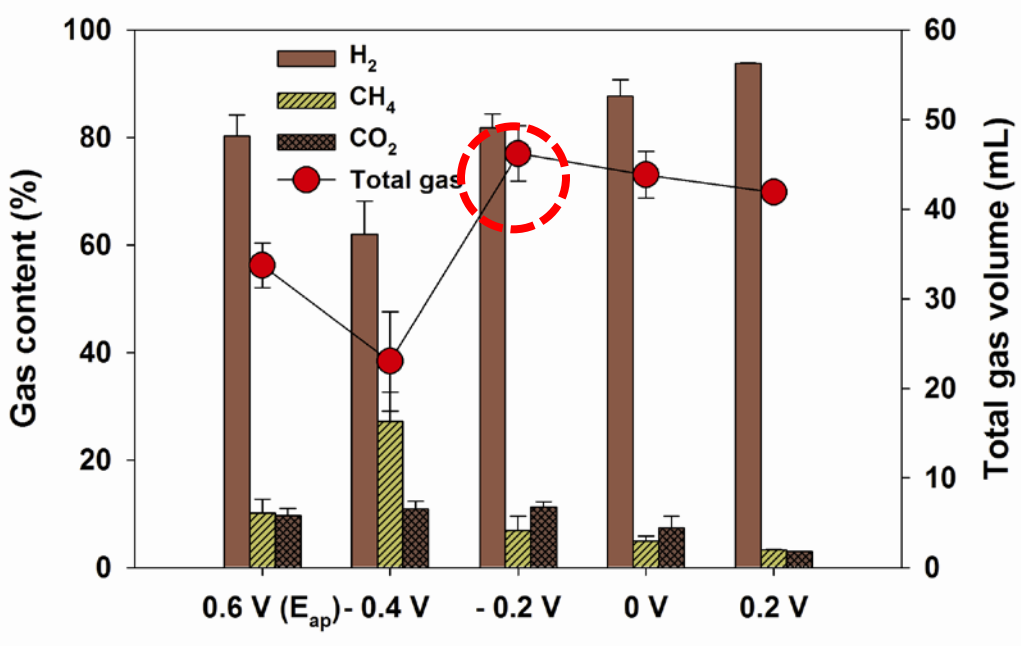


	Milestone	Completion Date	Status
3.2.3	Prototype reactor operational (FY10)	9/10	Completed
3.2.3	Correlate removal of the subcomponents of the NREL fermentation effluent with current density and H_2 production (FY11)	9/11	On track

Task 3 – Technical Accomplishments

Methane Reduction by Setting Anode Potential

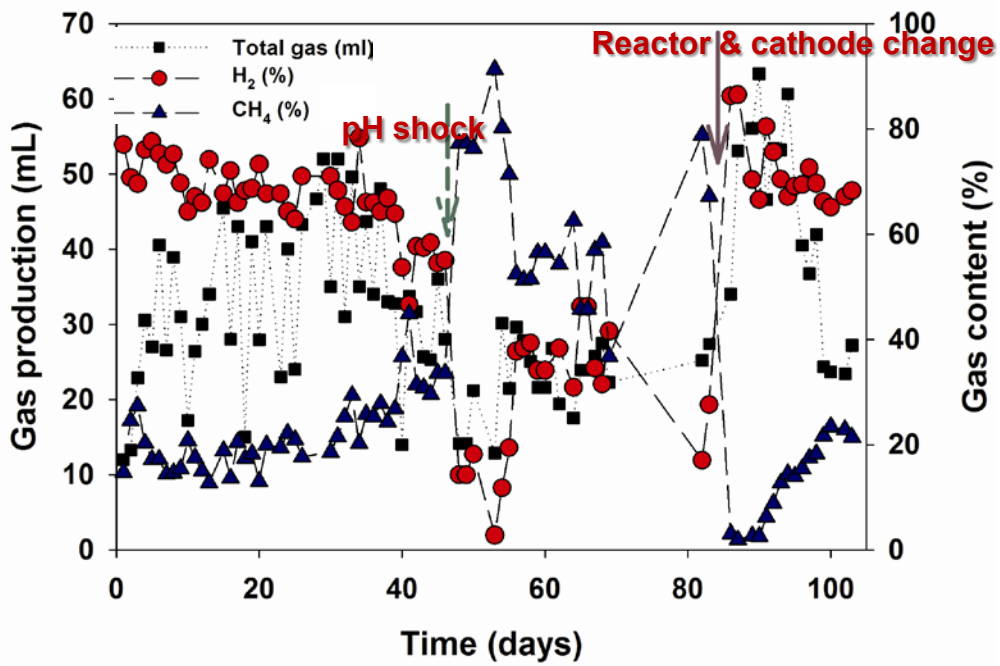
- Compare "boosting voltage (E_{ap})" with a power source (typically adding 0.6 V) with a set anode potential
- When adding 0.6 V, the anode is typically at -0.4 V, so we chose this and more positive anode potentials
- Highest gas production at **E_{an} = - 0.2 V**



V	Energy Input ($kWh/m^3_{reactor}$)	Energy Input (kWh/m^3_{H2})	η_{E+S} (%)	η_E (%)	η_S (%)	Cycle Time (h)
0.6 (E_{ap})	1.7	1.7	57	187	81	26
-0.4	1.2	2.9	27	114	34	40
-0.2	**3.0**	**2.3**	**59**	**143**	**97**	**16**
0	3.9	2.9	55	113	102	10
0.2	5.5	4.7	40	71	90	8

Task 3 – Technical Accomplishments

Methane Reduction by Setting Anode Potential



- Continuous operation at E_{An} = - 0.2 V

- Day 39, 68% H_2, 21% CH_4
- (average daily gas production 34 mL)
- → CH_4 increased up to 34% by Day 46.

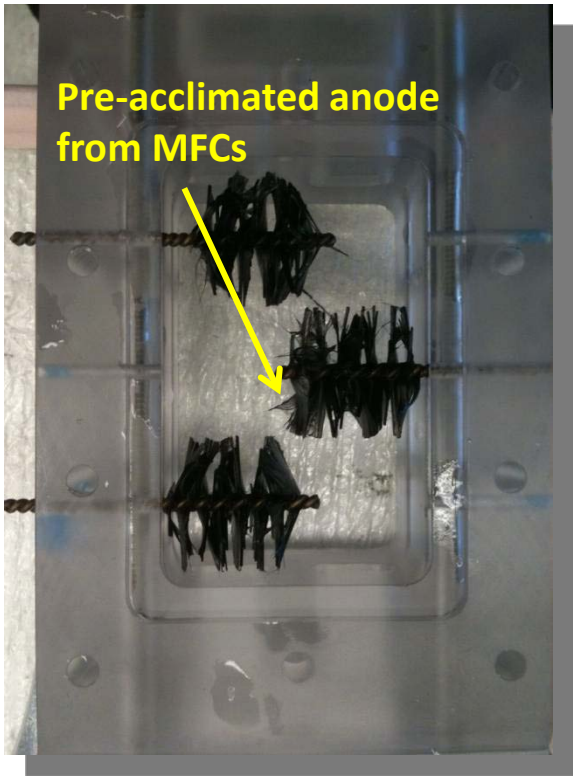
- pH shock tried… didn't work.

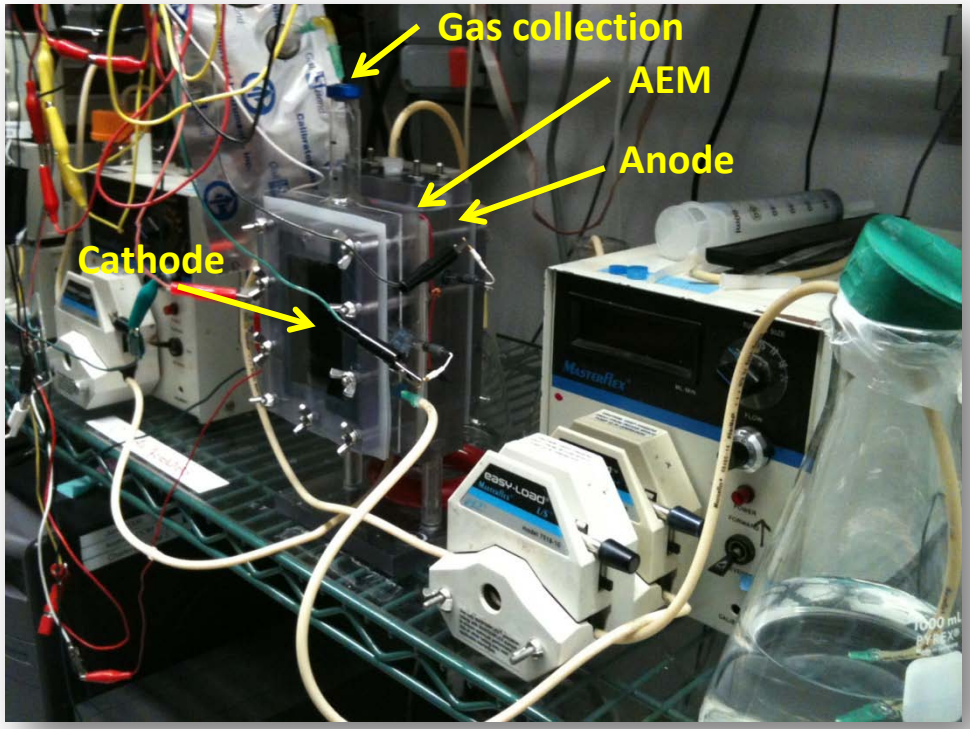
•What did work?
Placing the brush anode into new clean reactor (Day 85)→**86 % H_2, 3% CH_4**
→ Methanogens were not on anode, but in reactor assembly

Conclusion: **Setting anode potential can improve cell performance, but it was difficult to eliminate CH_4 completely in single-chamber MECs.**

Task 3 – Technical Accomplishments

Design New Tubular MECs



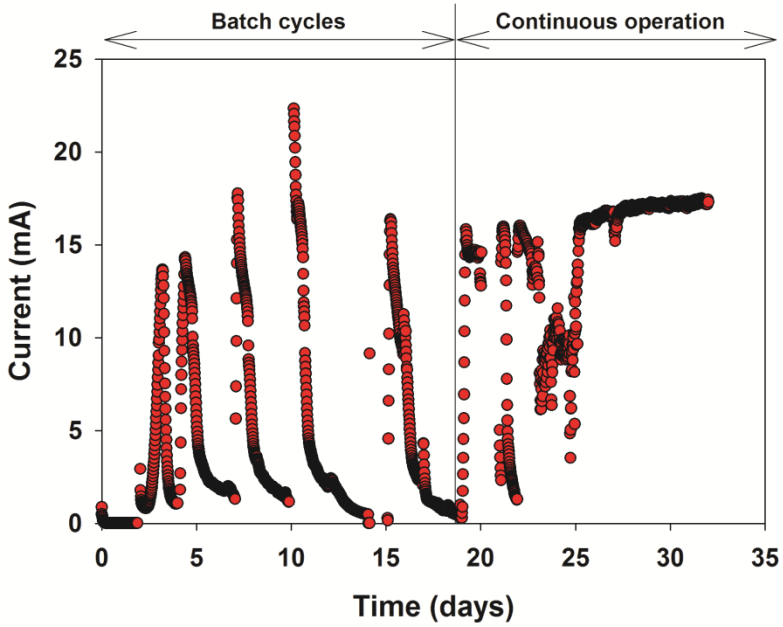


- A two chambered MEC with AEM , 0.9 V applied
- Anode chamber (135 mL) → 3 brush anodes, 1.5 g sodium acetate/L, HRT = 1 day
- Cathode chamber (147 mL) → Pt coated stainless steel, 50 mM PBS, HRT = 1 day

Completed Milestone "*Design tubular MECs to reduce methane generation*" (9/10)

Task 3 – Technical Accomplishments

Tubular MEC Performance

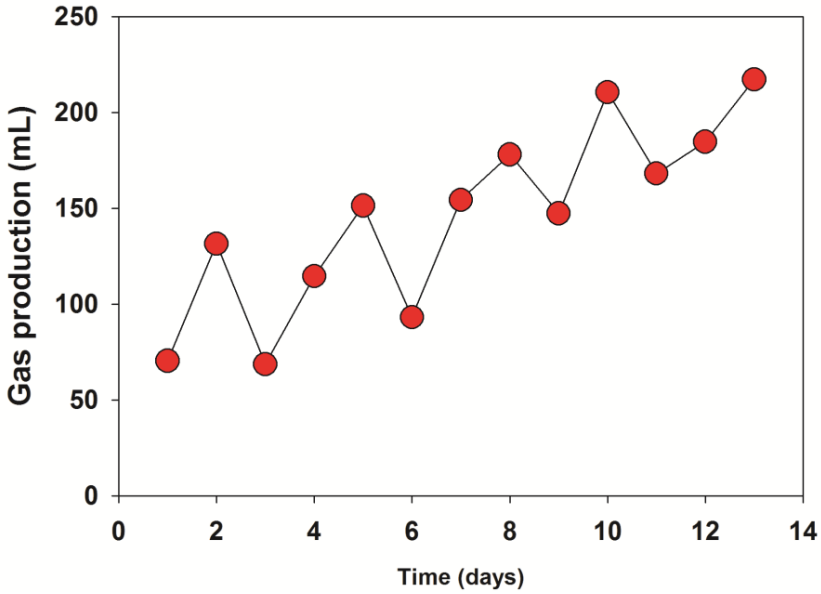


→ Almost pure H_2 (no CH_4, CO_2) with stable current

→CE 79-84%, r_{cat} = 88-107%, COD_{rem} > 90%
H_2 production rate 0.8 H_2 m^3/m^3-d (Day 13)

Stable current generation in a continuous mode → 17 mA, current density 60 A/m^3.

<Continuous gas generation>



Completed *"Test performance of the new system"* **(12/10).**
On track to complete Milestone *"Correlate removal of the subcomponents of the NREL fermentation effluent with current density and H_2 production"* **(8/11).**

Collaborations

- **Task 1 (Bioreactor):**

  Dr. Ali Mohagheghi, National Bioenergy Center at NREL (Biomass pretreatment and characterization)

- **Task 2 (Genetic Methods):**
  - Dr. Mike Himmel at NREL (funded by the DOE BER Program)
  - Drs. David Levin and Richard Sparling at the University of Manitoba, Canada (funded by the 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 Work

Task 1:

- Repeat fed-batch experiments (2.5, 5, and10 g/L cellulose) in one-liter bioreactor for cellulose consumption, carbon balance, rates and yield of H_2 (FY11).
- Scale up fed-batch experiment in 5-L bioreactor as above, testing hydraulic retention time and feeding strategy (FY11/12).
- Collect, analyze, and send fermentation effluent to PSU to generate H_2 via MEC integration (FY11/12).
- Type microbial community of the consortium if supported by the Program (FY12).

Task 2:

- Modify the proprietary plasmid to improve its long-term stability (FY11).
- Construct a *C. thermocellum* mutant host (*pyrF* knockout) and start deleting pyruvate-to-formate pathway (FY11).
- Test the above mutant for H_2 rates, yield, and carbon balance (FY11/12).
- Target additional competing pathways to improve H_2 molar yield (FY12).

Task 3:

- Complete analysis of fermentation effluent (FY11).
- Test performance of the new system with fermentation effluent (FY11).
- Conduct tests on performance with respect to hydrogen yields, hydrogen production rates, and gas composition for the fermentation effluent (FY11/12).

Summary

Task 1:

- Operated fed-batch reactor with substrate concentrations of 2.5 and 5.0 g/L and hydraulic retention time of 24 hr, and observed shorter acclimation time, improved rates of H_2, and elevated total cell biomass concentrations.
- Demonstrated that a co-culture (*C. thermocellum* and a *Clostridium* consortium) can ferment untreated corn stover and yielded 64% more H_2 than *C. thermocellum* alone. The outcomes work toward reducing feedstock cost.

Task 2:

- Developed genetic tools via collaboration and generated *C. thermocellum* mutants harboring the proprietary plasmid.
- Continue to improve stability of the plasmid to allow plasmid-based expression.
- Work is ongoing to generate a *C. thermocellum* mutant host for deleting competing pathway.

Task 3:

- Setting anode potentials in single chamber MECs could improve reactor performance, but did not completely eliminate methane generation.
- Higher gas production and H_2 composition were obtained at E_{An} = - 0.2 V compared to adding voltage of E_{ap} = 0.6 V.
- By designing and operating a tubular MEC composed of two chambers, pure H_2 was obtained. Achieved up to 0.8 H_2 m^3/m^3-d.