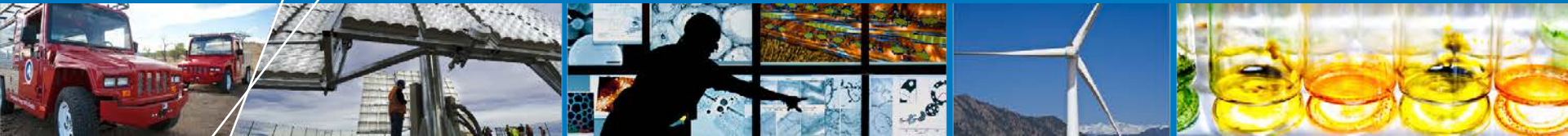


Fermentation and Electrohydrogenic Approaches to Hydrogen Production



2012 Annual Merit Review and Peer Evaluation Meeting

Pin-Ching Maness

National Renewable Energy Laboratory

May 16, 2012

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: 10/2012*
- Percent complete: N/A

Budget

- Total project funding: \$2,310K (includes \$332K subcontract)
- Funding received in FY11: \$400K
- Planned funding for FY12: \$350K

Barriers

Barriers addressed

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

Partners

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

*Project continuation and direction determined annually by DOE

Relevance

- **Objective:** Develop direct fermentation technologies to convert renewable lignocellulosic biomass resources to H₂.
 - Optimize sequencing fed-batch bioreactor (hydraulic and solid retention time) using cellulose (Task 1).
 - Develop genetic tools to improve H₂ molar yield (Task 2).
 - Integrate microbial electrolysis cell (MEC) reactor with fermentation to improve H₂ molar yield (Task 3).
- **Relevance:** Directly address the DOE barriers: feedstock cost (Task 1), H₂ molar yield (Tasks 1 & 3), and waste acid accumulation (Task 3) to improve techno-economic feasibility.

Characteristics	Units	2012 Status	2013 Target	2018 Target
Yield of H ₂ from glucose	Mol H ₂ /mol glucose	3.2	4	6
Feedstock cost*	Cents/lb glucose	12	10	8
Duration of production	Time	15 days	3 months	6 months

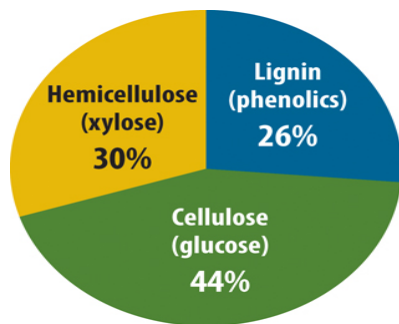
* DOE Office of Biomass Program status and target

Objectives/Approach/Milestone

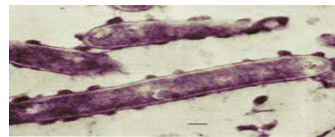
Task 1: Bioreactor Performance

- **Objective:** Address feedstock cost and improve the performance and durability of bioreactors for H₂ production via fermentation of lignocellulose.
- **Approach:** Optimize bioreactor in sequencing fed-batch mode by testing parameters such as cellulose loadings, hydraulic retention time, and acclimation of the cellulose-degrading bacterium *Clostridium thermocellum*.

Lignocellulosic Biomass

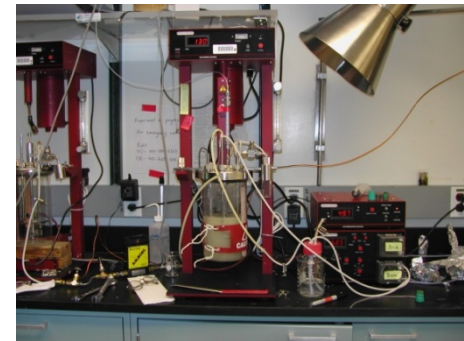


Fermentation



Clostridium thermocellum

Bioreactor Performance



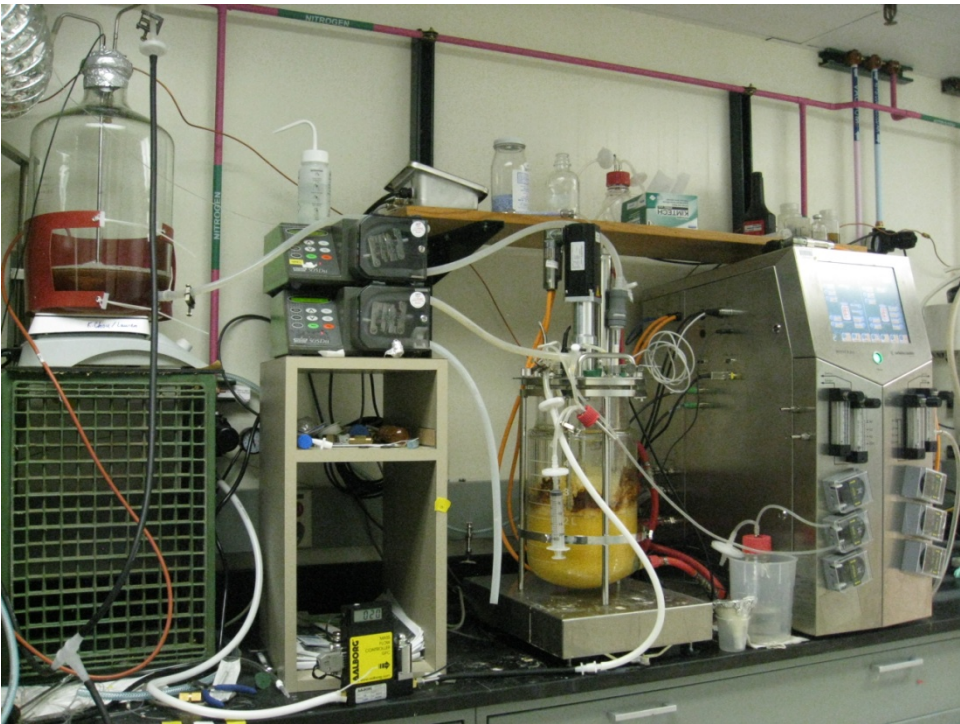
	Milestone	Completion Date	Status
3.2.1	Increase rate of H ₂ production by 20% and demonstrate scalability of sequencing batch reactor via optimizing hydraulic retention time, carbon loading, and volume of liquid replacement	5/12	Completed

Task 1 – Technical Accomplishments

Scale Up “Automated” Sequencing Fed-Batch Bioreactor

Automation features of the 5-L Sartorius bioreactor:

- Fermentation module executes the Fill, React, Settle, Decant, and Idle stages
- The Fill and Draw pumps are controlled by a scale positioned under the reactor



Settle, Drain, Feed



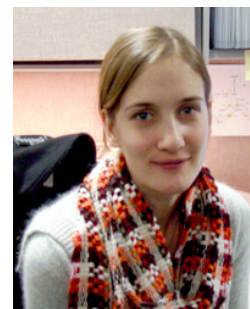
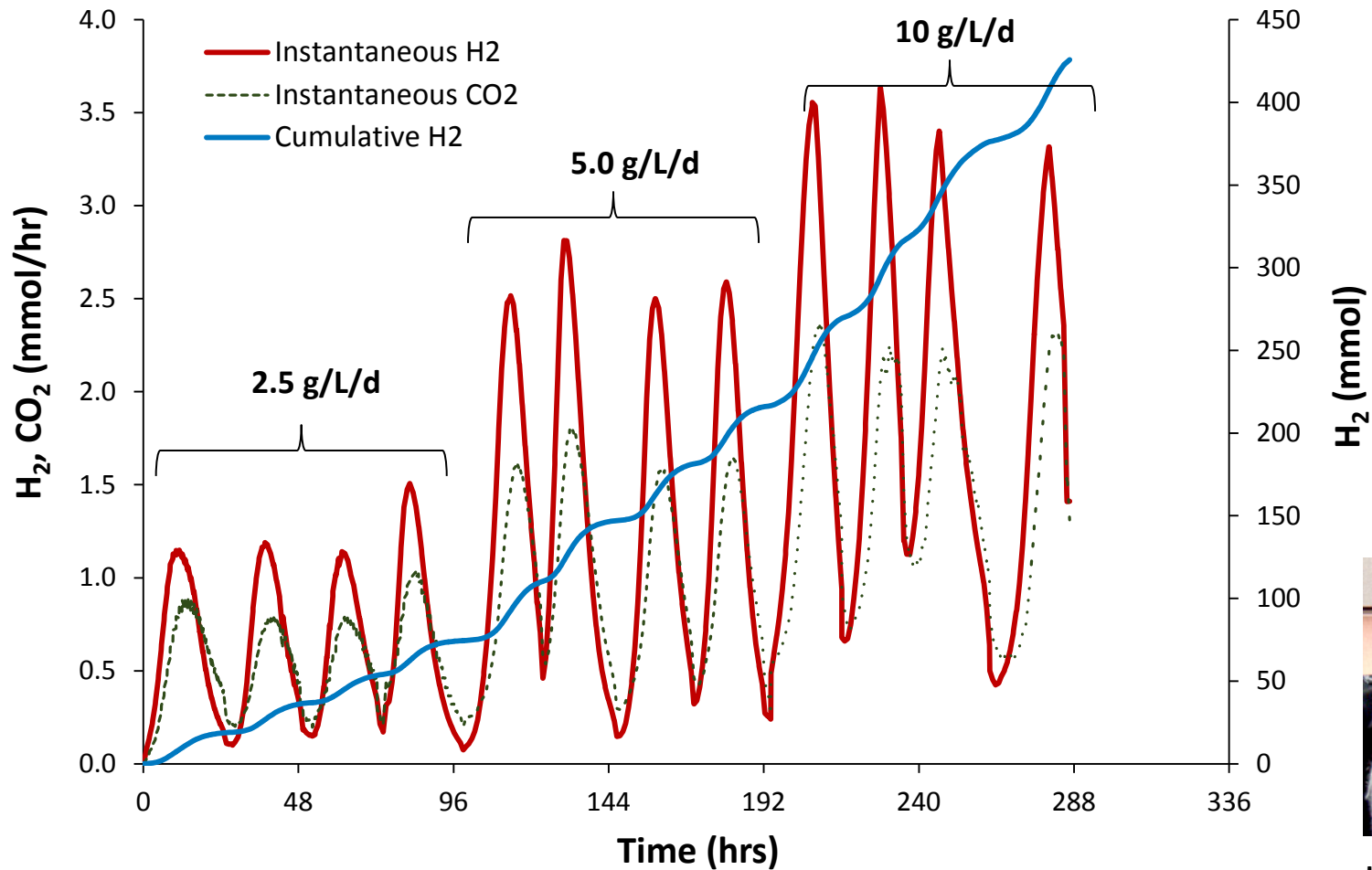
Cellulose settling retained acclimated microbes

***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 Sequencing Fed-Batch Bioreactor

- Daily feedings of 2.5, 5.0, and 10 g/L/day of cellulose, four cycles each, with a hydraulic retention time of 48 h.



Lauren Magnusson

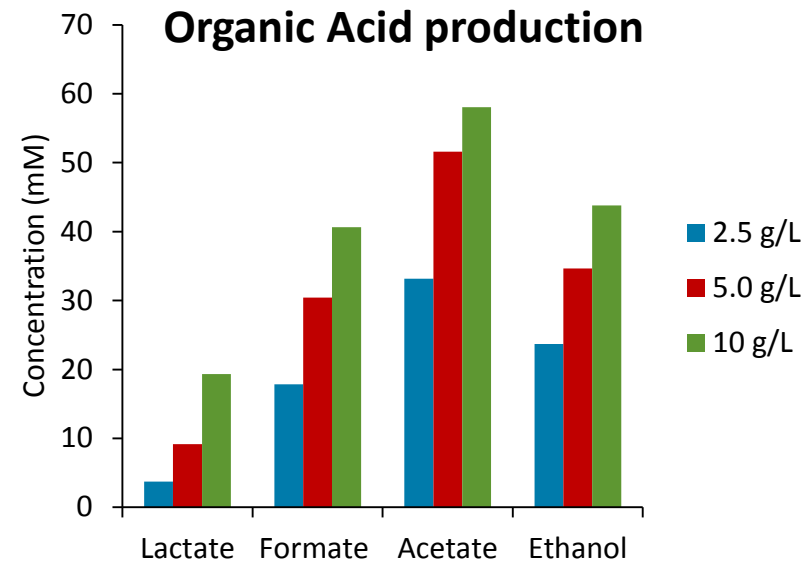
Task 1 – Technical Accomplishments

Hydrogen Production in Sequencing Fed-Batch Bioreactor

- Fed-batch mode adapted *C. thermocellum* to degrade cellulose faster
- The system is **scalable**, with both total H₂ output and volumetric rate of H₂ production proportional to amounts of cellulose feeding.

Cellulose Concentration	Amount of H ₂ produced	Max H ₂ production rate	H ₂ Yield*	H ₂ /CO ₂
(g/L/day)	(mmoles)	(mmol L ⁻¹ hr ⁻¹)	(mol H ₂ mol ⁻¹ hexose)	
2.5	18.5	1.2	1.2	1.2
5	35.3	2.6	1.1	1.3
10	51.9	3.5	0.8*	1.4

* Calculated based on substrate added, yield is higher if calculated based on substrate consumed.



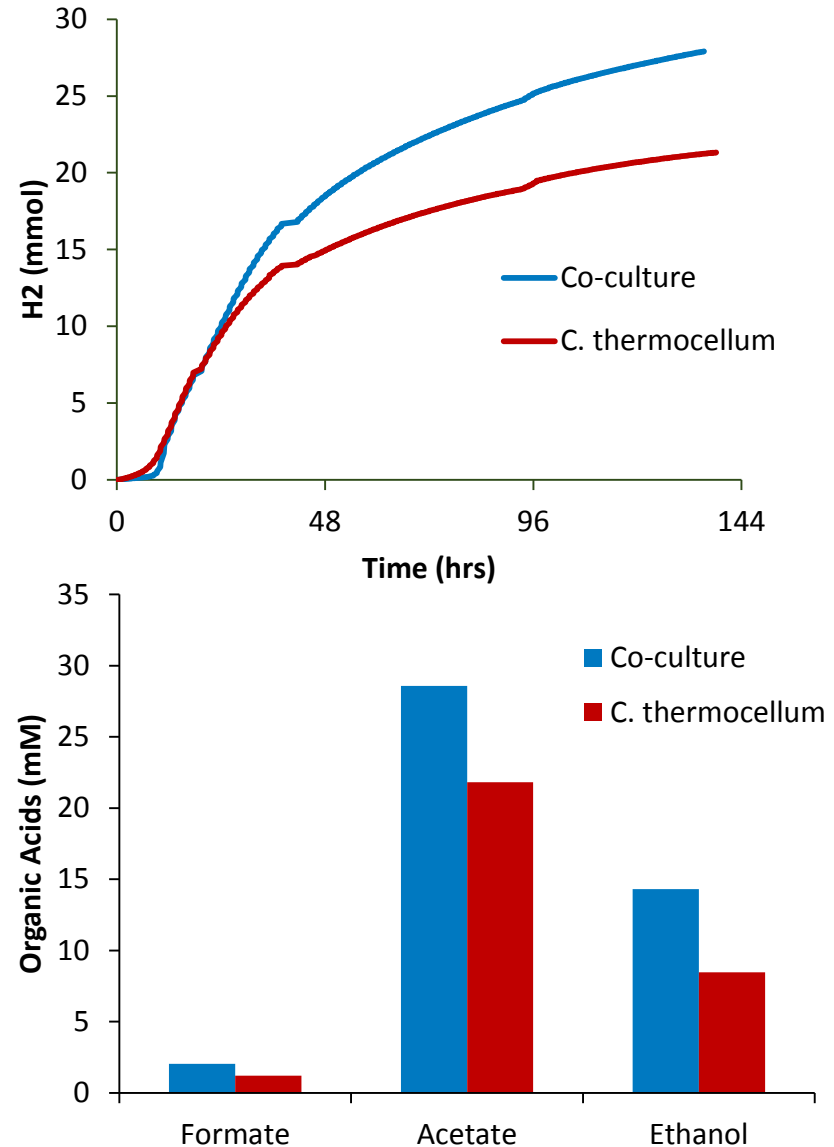
Completed milestone “Increase rate of H₂ by 20% and demonstrate scalability of sequencing batch reactor via optimizing hydraulic retention time, carbon loading, and volume of liquid replacement” (5/12).

Task 1 – Technical Accomplishments

H₂ From “Untreated” Corn Stover Using a Co-culture

- Biomass pretreatment is a cost driver for H₂.
- A co-culture containing *Clostridium thermocellum* (cellulose) and a *Clostridium* consortium (hemicellulose) enables:
 - Conversion of “raw” biomass to H₂
 - Improvement of biomass utilization.
- The co-culture produced **31%** more H₂ than *C. thermocellum* did alone.
- Consortium alone did not produce any H₂.
- H₂ output of co-culture on untreated corn stover is **74%** that of *C. thermocellum* alone on comparable amount of “pure cellulose”.

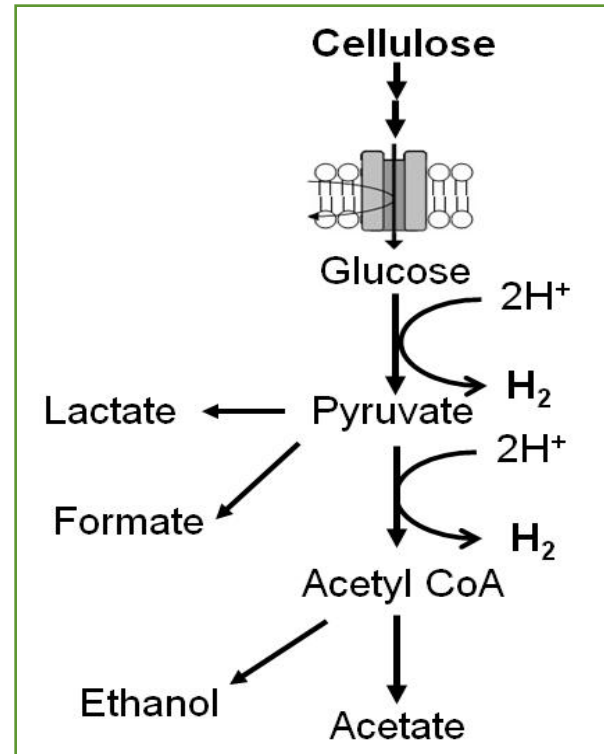
The utilization of untreated raw biomass decreases feedstock cost and hence lowers H₂ selling price.



Objectives/Approach/Milestone

Task 2 – Develop Genetic Methods for Metabolic Engineering

- **Objective:** Improve H₂ molar yield (mol H₂/mol hexose) via fermentation.
- **Approach:** Redirect metabolic pathways to improve H₂ molar yield via the development of genetic methods.
 - Optimize genetic protocols for “targeted” pathway mutagenesis.
 - Develop forward-evolution strategy for “global” pathway mutagenesis.

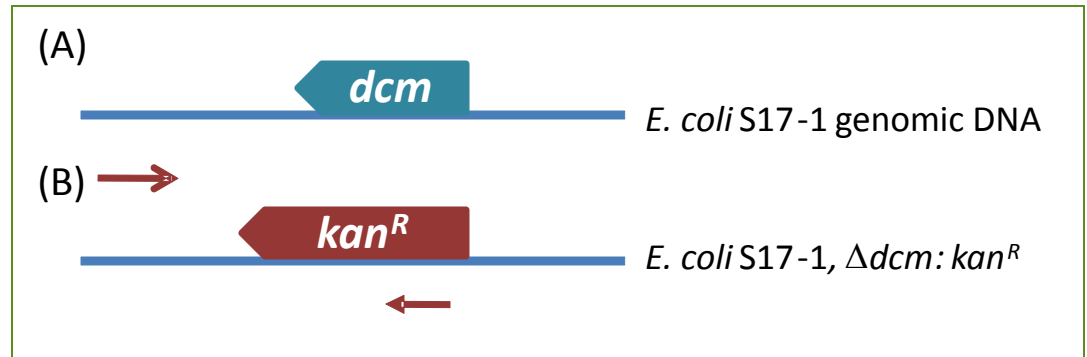
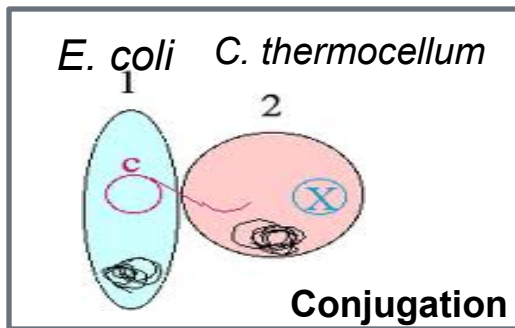


	Milestones	Completion Date	Status
3.2.2 (FY11)	Develop genetic protocols for the generation of the <i>pyrF</i> knockout mutant in <i>C. thermocellum</i> suitable for targeted mutagenesis to increase H ₂ molar yield	9/11	Completed
3.2.2 (FY12)	Produce one mutant in <i>C. thermocellum</i> defective in ALDH for pathway knockout construction (CPS Agreement Milestone)	9/12	On track

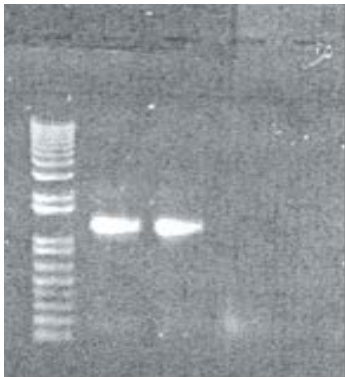
Task 2 – Technical Accomplishments

Develop Tools for Genetic Transformation

- **Objective:** Improve plasmid stability in *C. thermocellum*.
- NREL and Univ. of Manitoba developed proprietary plasmid and produced two mutants of *C. thermocellum*, yet the transformants were unstable.



colony
1 2 controls



- Generated **two** *E. coli* Δdcm mutants (1 and 2), confirmed via PCR
- *E. coli* and *C. thermocellum* have compatible DNA methylation system, both are *dam*⁺, *dcm*⁻.

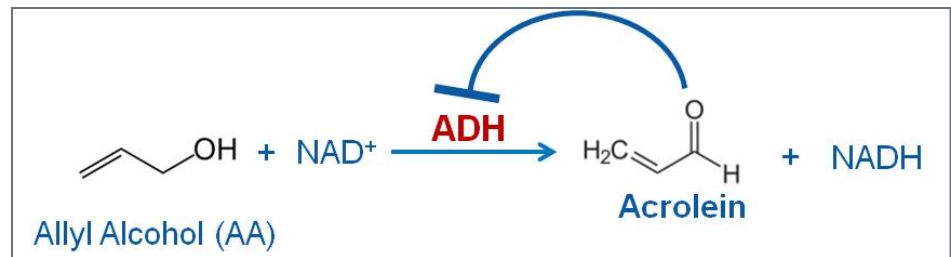
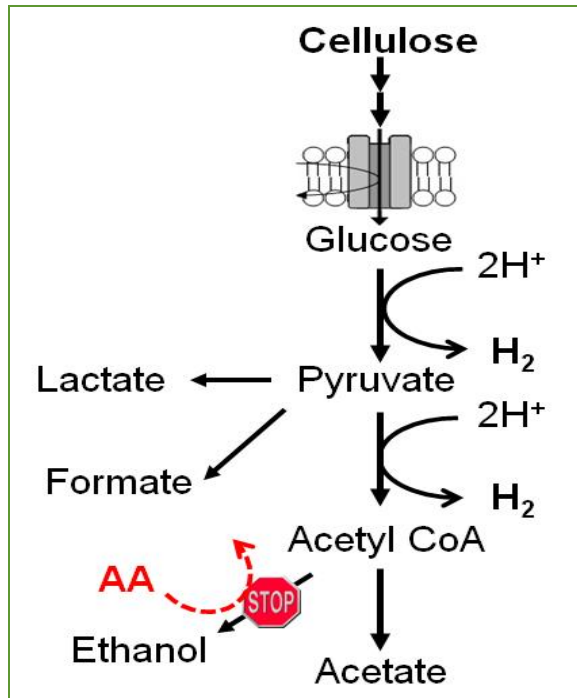


Katherine Chou

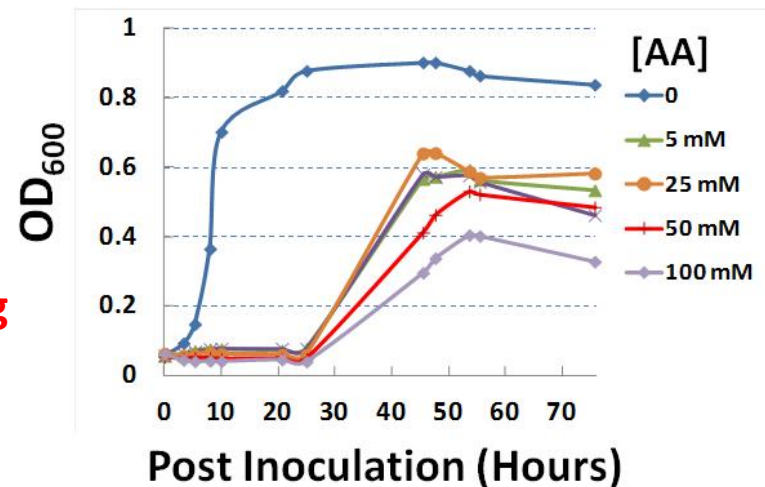
Task 2 – Technical Accomplishments

Forward-Evolution Strategy to Block Competing Pathway

- **Objective:** Develop an alternative forward-evolution strategy to block ethanol production – this approach had improved H₂ molar yield in the microbe *Enterobacter cloacae*.
- The native alcohol dehydrogenase (ALDH) converts allyl alcohol (AA) to the toxic product acrolein, which kills the cells. The survivors will have an inactive ALDH.



AA Killing Curves

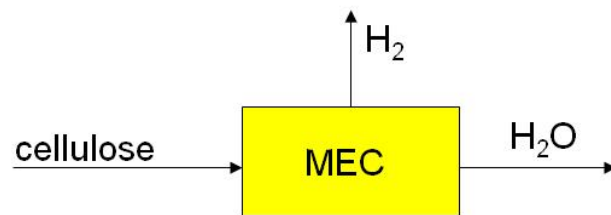
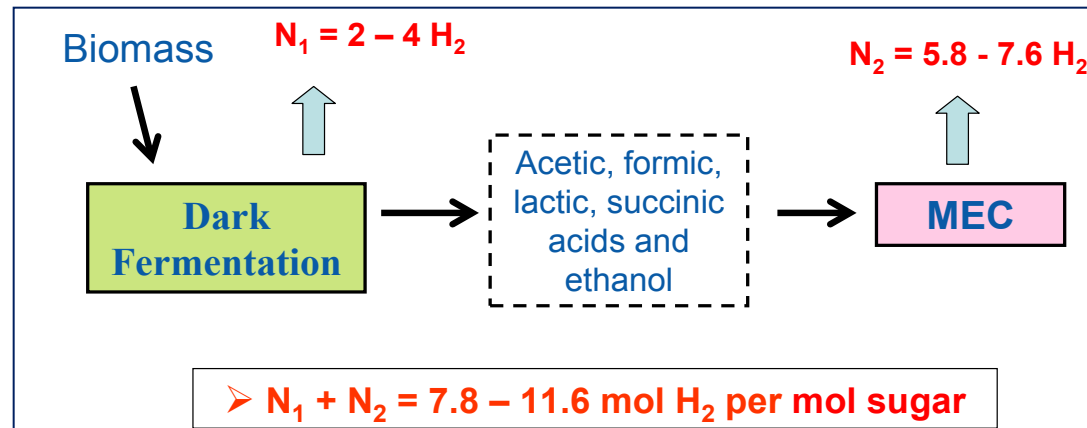


On track to complete milestone “Produce one mutant of *C. thermocellum* defective in ALDH for pathway knockout construction” (9/12).

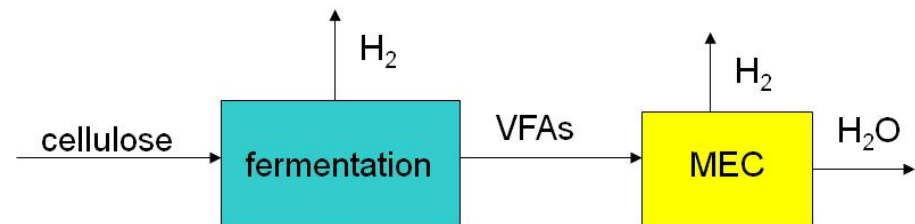
Objectives/Relevance

Task 3 – Electrochemically Assisted Microbial Fermentation

Objective: Improve H_2 molar yield (mol H_2 /mol hexose, N) by integrating dark fermentation with microbial electrolysis cell (MEC) reactor to convert waste biomass to additional H_2 (Bruce Logan, Penn State Univ.).



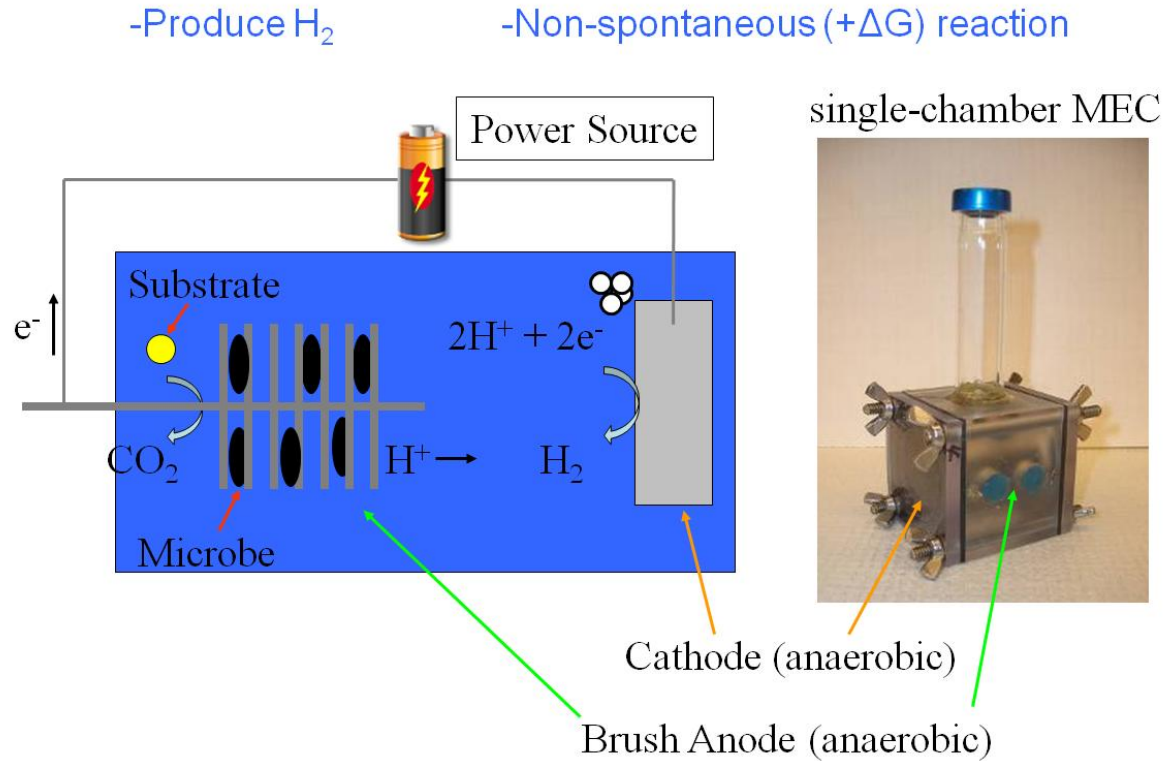
One-stage process: slow



Two-stage process: fast

Approach/Milestone

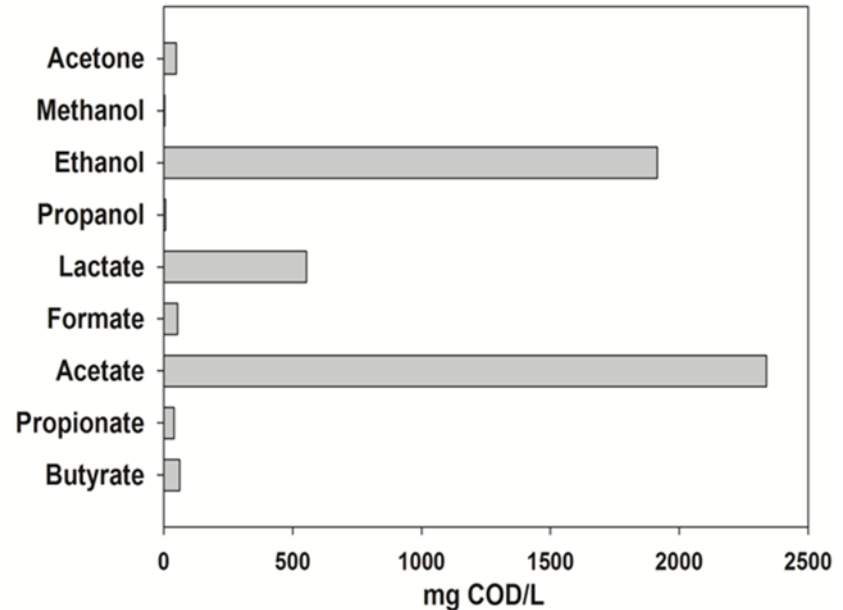
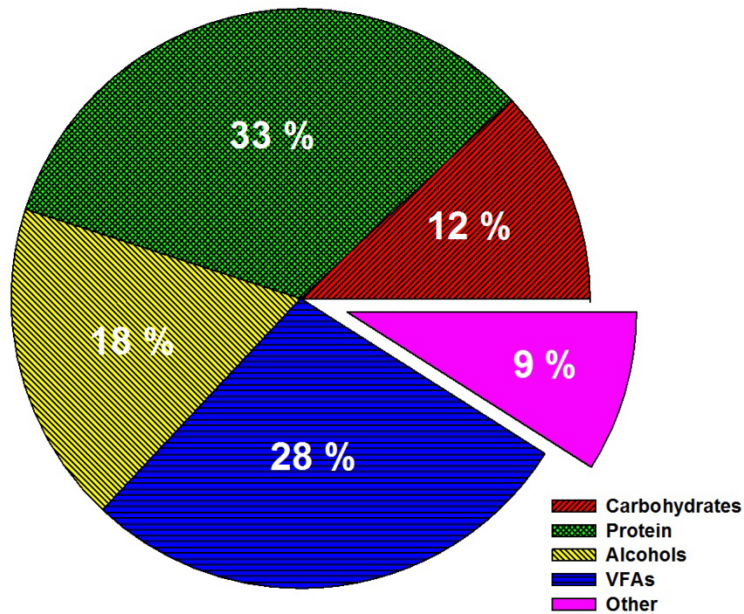
Task 3 – Electrochemically Assisted Microbial Fermentation



	Milestones	Completion Date	Status
3.2.3 (FY11)	Analyze subcomponents in the NREL fermentation effluent for H ₂ production using MEC	6/11	Completed
3.2.3 (FY12)	Correlate removal of the subcomponents of the NREL fermentation effluent with current density and H ₂ production	9/12	On track

Task 3 – Technical Accomplishments

Compositional Analysis of the NREL Fermentation Effluent



- 98% soluble chemical oxygen demand (sCOD), with only 2% particulate COD (225 mg/L)
- Alcohols and VFAs were 46% of the sCOD
- Alcohols mostly ethanol (1.9 g/L of 2.0 g/L), VFAs mostly acetate (2.3 g/L of 3.0 g/L)
- Unexpected observation: Proteins (3.6 g/L) > Carbohydrates (1.25 g/L) [sCOD basis]
- <9% of the sCOD was not identified by this analysis (could be less due to measurement errors)
- **The finding that most of the material is soluble means that the material will be more easily converted into current and hydrogen gas in the MEC.**



Jooyoun Nam

Task 3 – Technical Accomplishments

Delay of Penn State Subcontract Award

- Due to a budget rescission in FY 2011, the Penn State subcontract was cancelled in June 2011 (only 3 months of planned 12 months).
- Once resolved, the award of a new subcontract was further delayed due to legal issues.
- A new subcontract was finally awarded March 2012 and work has since resumed.
- DOE approved new due date of 9/2012 for Milestone 3.23 (originally due 9/2011).



Collaborations

- **Task 1 (Bioreactor):**

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

- **Task 2 (Genetic Methods):**

Drs. David Levin and Richard Sparling at the University of Manitoba, Canada. 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 Work



Task 1:

- Repeat sequencing fed-batch experiments (2.5, 5, 10, 15 g/L cellulose) and test hydraulic retention time (12 h, 24 h) for cellulose consumption, carbon balance, rates, and yield of H₂ (FY12).
- Optimize co-culture fermentation of untreated biomass, using the cellulosomes (containing cellulases and hemicellulases) secreted by *C. thermocellum* in a pretreatment step (FY12, FY13).
- Conduct sequencing fed-batch experiments using corn stover lignocellulose (FY13).

Task 2:

- Perform counter-selection to screen for *pyrF* knockout in *C. thermocellum*, suitable for deleting its pyruvate-to-formate pathway (FY12).
- Test the above mutant for H₂ rates, yield, and carbon balance (FY12, FY13).
- Select survivors of allyl alcohol killing for ethanol-knockout mutants (FY12).
- Target additional competing pathways to improve H₂ molar yield (FY13).

Task 3 (Penn State):

- Test performance of new MEC using fermentation effluent with boosted voltages (FY12)
- Describe performance of the bench scale MEC in terms of H₂ yields, H₂ production rates, and gas composition for the fermentation effluent (9/12 target), and enrich for H₂ production from protein (end FY12).
- Examine use of thermolytic solutions in scaled-up systems for enhanced H₂ production rates (FY13).

Summary

Task 1:

- Successfully scaled up and operated, in automation mode, the sequencing fed-batch reactor with cellulose feedings of 2.5, 5, and 10 g/L and hydraulic retention time of 48 h.
- The system is scalable, with total H₂ output and volumetric production rate proportional to cellulose loadings.
- Demonstrated that a co-culture could ferment untreated raw biomass and produced H₂ at 74% that of pure cellulose, hence could lower H₂ selling price.

Task 2:

- Improved genetic compatibility between *E. coli* and *C. thermocellum*, which significantly increased plasmid stability in *C. thermocellum*.
- Obtained *C. thermocellum* transformant suitable for targeted pathway mutations to improve H₂ molar yield. Mutant generation is underway.
- Devised a forward-evolution strategy to block the ethanol-production pathway aimed at improving H₂ molar yield.

Task 3:

- Analyzed components of the fermentation effluent; determined that about half of the organic matter is proteinaceous and that most is soluble organic matter. This should make it easier to treat in an MEC than particulate matter would be.
- Other tasks were not completed due to a lack of funding.