National Renewable Energy Laboratory
Innovation for Our Energy Future

Fermentative and Electrohydrogenic Approaches to Hydrogen Production

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PD_18_Maness

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Overview

Timeline

Project start date: FY05

Project not funded in FY06

Project end date: 2013

Percent complete: N/A

Barriers

Production barriers addressed

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

Budget

Funding received in FY08: $680K

Funding allocated for FY09: $400K

Partners

Dr. Bruce Logan, Penn State University

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

Objectives/Relevance

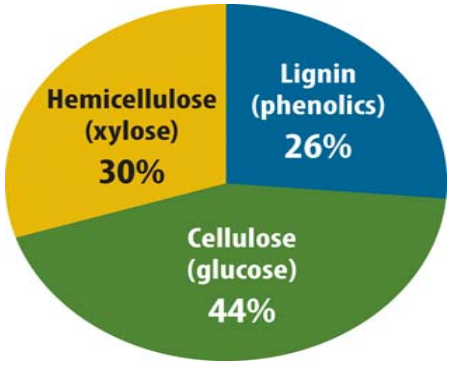
- **Objective:** Develop direct fermentation technologies to convert renewable, lignocellulosic biomass resources to H_2.
- **Relevance:** Address directly feedstock cost and H_2 molar yield to make the process cost competitive.
- Make positive impact on technical barriers and targets.

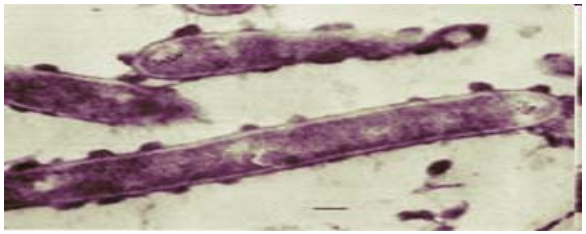
Characteristics	Units	2013 Target	2009 Status
Yield of H_2 from glucose	Mole H_2/mol glucose	4	8.52
Feedstock cost	Cents/lb glucose	10	12

Objectives/Approach/Milestone
Task 1: Bioreactor Performance

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

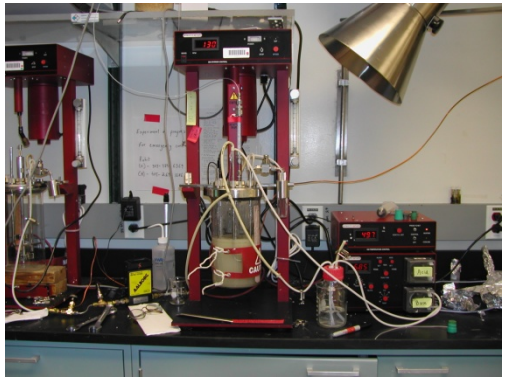
Lignocellulosic Biomass





Clostridium thermocellum

Bioreactor Performance



	Milestone	Completion Date	Status
3.2.3	Determine H_2 molar yield and mass balance using pretreated biomass	8/08	Completed

Task 1 – Technical Accomplishment

Investigated Fermentation of Various Substrates

- H_2 production rates and molar yields varied based on nature of the substrate and level of carbon loading.
  - Less recalcitrant substrates gives rise to faster rate
  - Higher carbon loading leads to higher rate of H_2 production
  - Lower carbon loading leads to higher H_2 molar yield.

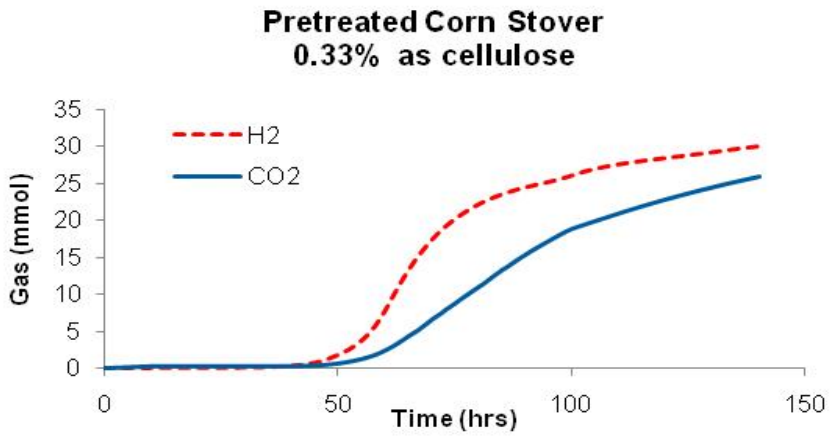
Substrate (%, w/v)	Hexose, mM	Temp (°C)	L H_2/L/Day	H_2 Molar Yield
Cellobiose (0.25%)	14.6 mM	55	2.94	1.1
Cellobiose (0.25%)	14.6 mM	50	1.65	1.64
Avicel (0.5%)	30.9 mM	50	1.44	1.51
Corn stover* (0.25%)	9.1 mM	50	0.25	1.67
Corn stover* (0.56%)	20.4 mM	55	0.55	1.33
Corn stover* (0.83%)	30.9 mM	55	1.21	Not determined

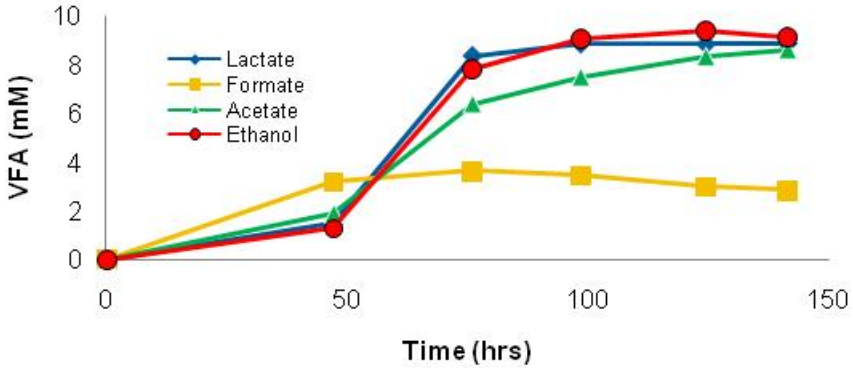
* Dilute acid (1.08% H_2SO_4) pretreated corn stover lignocellulose (59% cellulose; 25% lignin)

Task 1 – Technical Accomplishment

Optimized Lignocellulose Fermentation

- Lignocellulose (0.56%, 20.4 mM glucose) was added in bioreactor with controls in pH (7.0), temperature (55°C), and pressure.





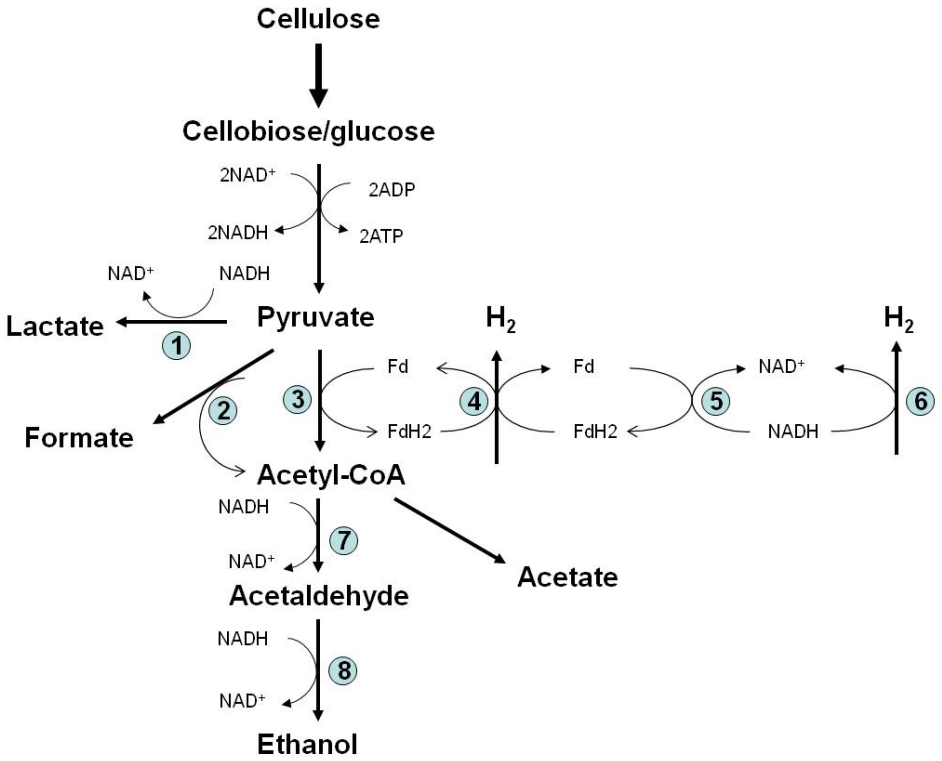
- H_2 molar yield: 1.33 mol H_2 mol^{-1} hexose
- Rate of H_2 production: 0.55 L H_2 L^{-1} D^{-1}
- Carbon mass balance 74.5%
  - CO_2: 23.62 mM
  - Succinic acid: 0.32 mM
  - Formic acid: 2.80 mM
  - Acetic acid: 7.13 mM
  - Lactic acid: 9.15 mM
  - Ethanol: 14.10 mM
- Carbon mass balance with cellobiose: 86%

Completed milestone "*Determining H_2 molar yield and carbon mass balance using pretreated biomass*" (8/08).

Objectives/Approach/Milestone

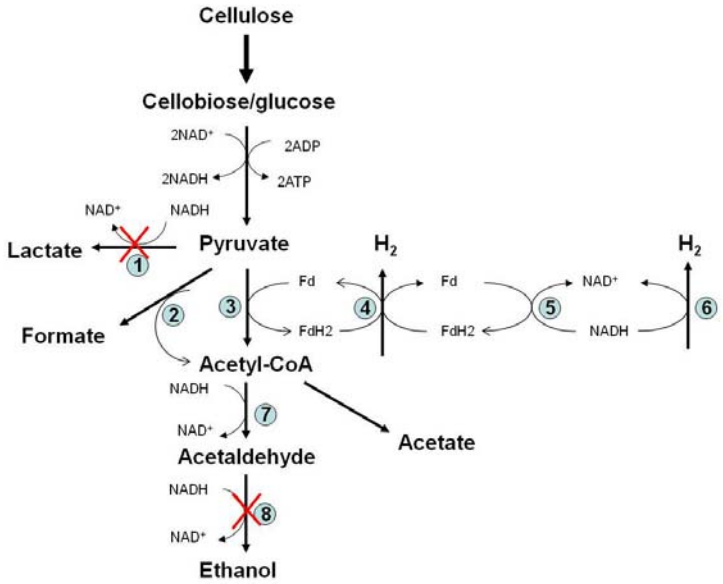
Task 2 – Develop Genetic Methods for Metabolic Engineering

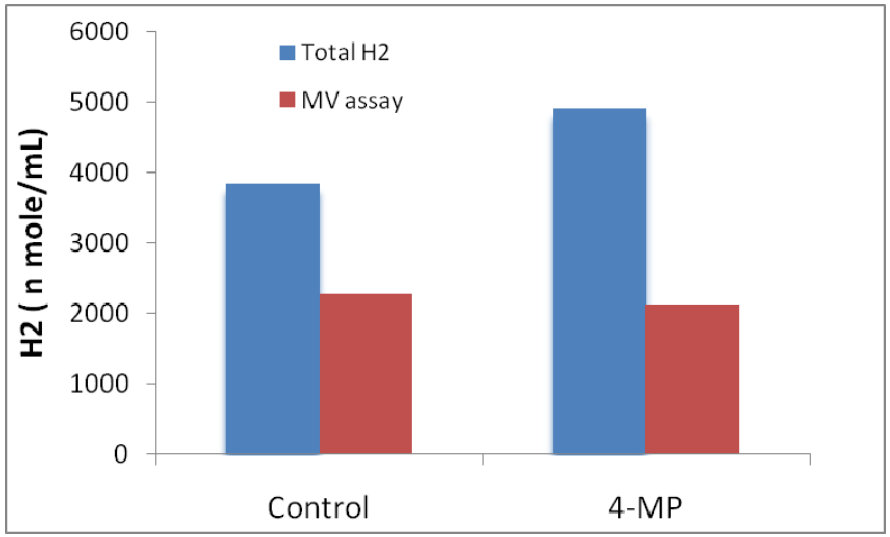
- **Objective:** Improve H_2 molar yield (mol H_2/mol hexose).
- **Approach:** Redirect metabolic pathways to maximize H_2 production via the development of genetic methods.



	Milestone	Completion Date	Status
3.2.2	Test effect of metabolic pathway inhibitors on H_2 production	6/08	Completed
3.2.5	Develop genetic tools and optimize screening methods for genetic engineering	3/09	Completed

Task 2 – Technical Accomplishment
Effects of Metabolic Pathway Inhibitor





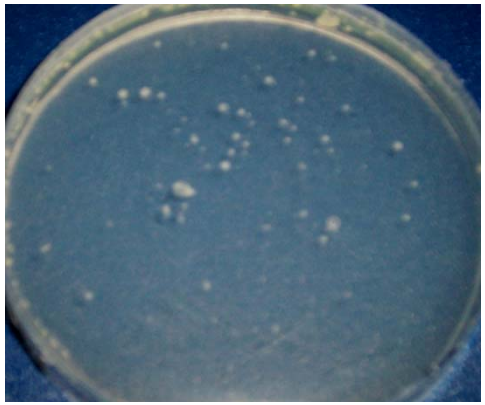
- Findings of the metabolic pathway inhibitors will guide development of the most effective genetic engineering strategies.
- Blocking the ethanol pathway and lactic acid pathway by 4-methyl pyrozole improved H_2 yield by **28%**.
- Blocking acetaldehyde (#7) or formate (#2) formation increased H_2 output by 81% and 58%, respectively (2008 AMR). In conclusion, blocking pathway #7 is the most effective strategy to improve H_2 production.

Completed milestone "*Test effect of pathway inhibitors on H_2 production*" (6/08).

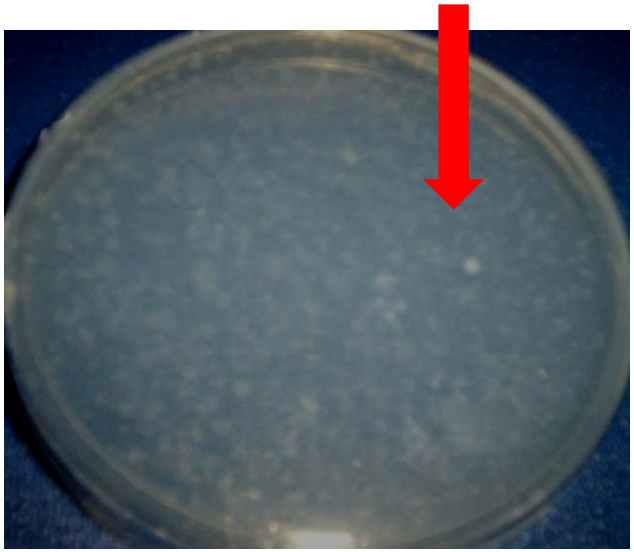
Task 2 – Technical Accomplishment
Develop Genetic Methods

- *Clostridium thermocellum* grew poorly on solid agar plate — a challenge for genetic engineering.
- We improved growth of *C. thermocellum* on solid agar plates by more than 100-fold to enable mutant selections.

Agar (%)	Number of colonies
1.5%	3–8
1.2%	10–20
1%	25–40
0.8%	>1000
0.7%	>1000



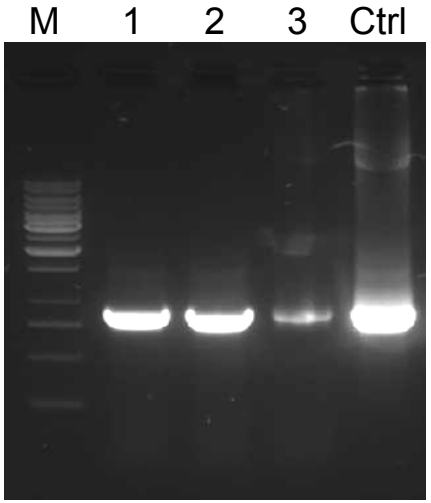
> 60 colonies

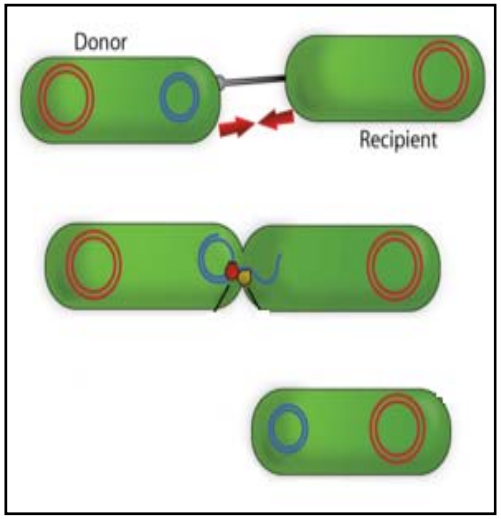


Task 2 – Technical Accomplishment

Developing Tools for Genetic Transformation

• Conjugation technique was successful in *Clostridium acetobutylicum* and *C. cellulolyticum,* thus improving chance of success.





M: Marker
1,2,3: Kan^r gene
Ctrl: Positive control

pIKM1 confirmation in *E. coli* S17-1

• Developed a colony formation protocol for *C. thermocellum* 27405 (July 2008).

• Obtained plasmid pIKM1 from Dr. Wiegel (University of Georgia) and helper plasmid RP4 from Dr. Wolk (Michigan State University) (Sept 2008).

• Transferred the pIMK1 plasmid into an *E. coli* host and confirmed by PCR and restriction digestion (Oct 2008).

• Gene transfer to *C. thermocellum* via conjugation was not successful (Dec 2008).

• Obtained *E. coli* strain S17-1 with a chromosomally integrated helper plasmid and confirmed plasmid pIKM1 in *E. coli* (Mar 2009).

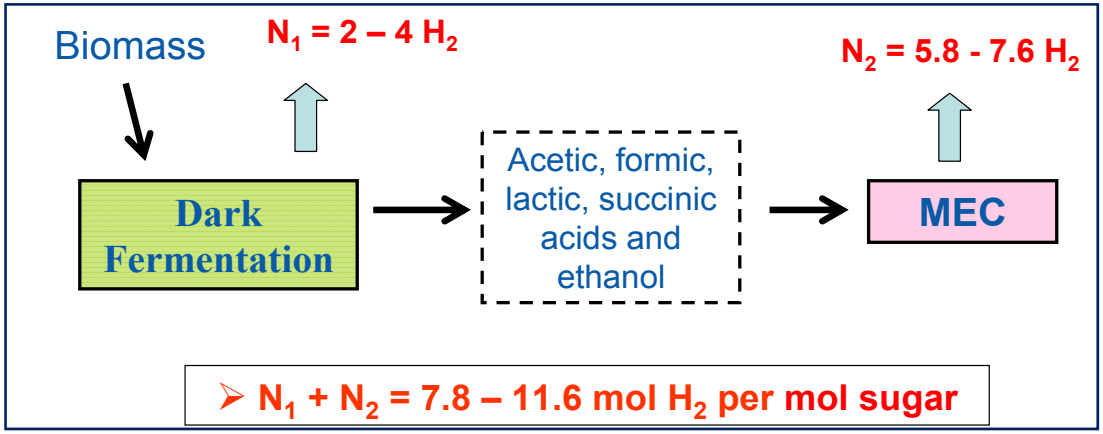
• Conjugation with *C. thermocellum* is under way (In progress).

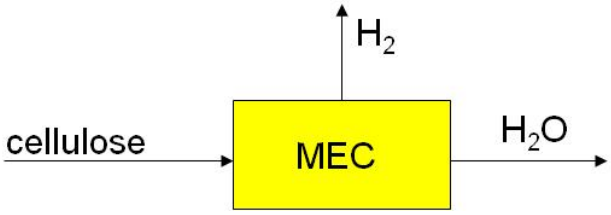
Completed milestone "*Develop genetic tools and optimize screening methods for genetic engineering*" (3/09).

Objectives/Relevance

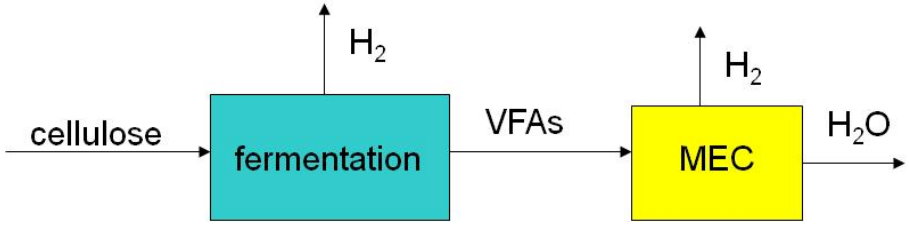
Task 3 – Electrochemically Assisted Microbial Fermentation

- **Objective:** Improve H_2 molar yield (mol H_2/mol hexose) by integrating dark fermentation with Microbial Electrolysis Cell (MEC) reactor to convert waste organic matter to additional H_2.





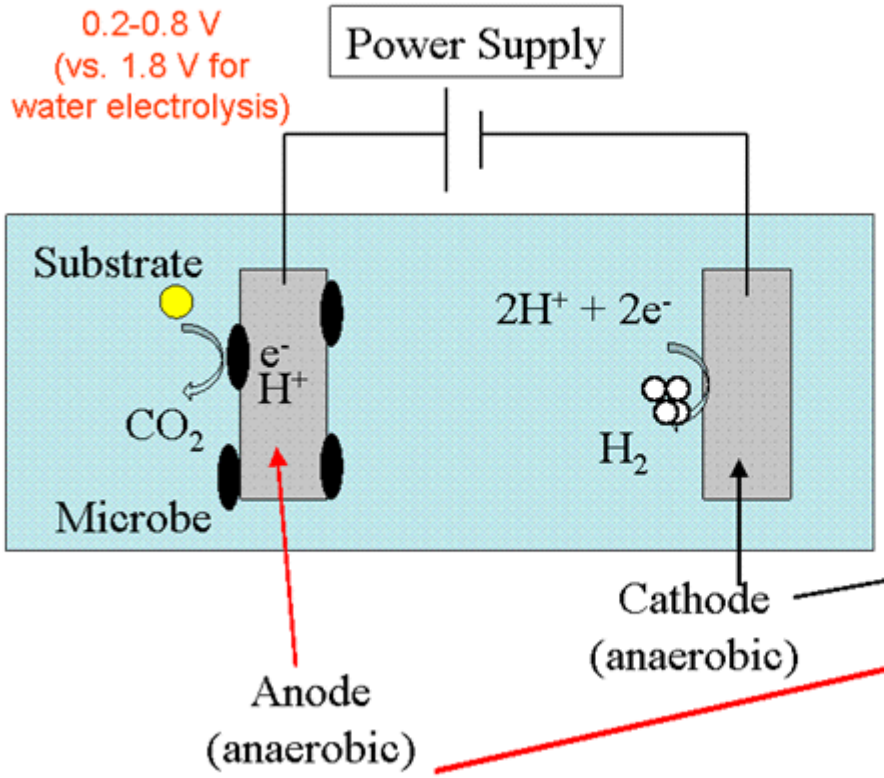
One-stage process: slow



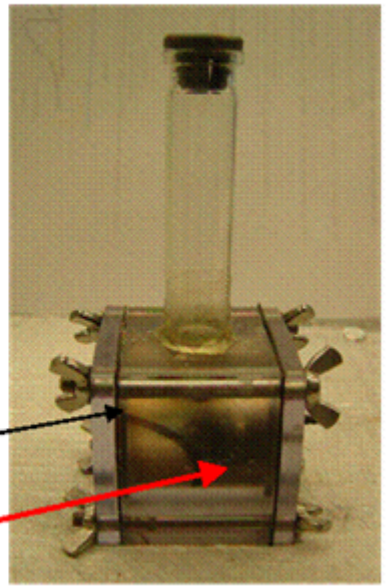
Two-stage process: fast

Approach/Milestone

Subtask 3: Electrochemically Assisted Microbial Fermentation



single-chamber MEC

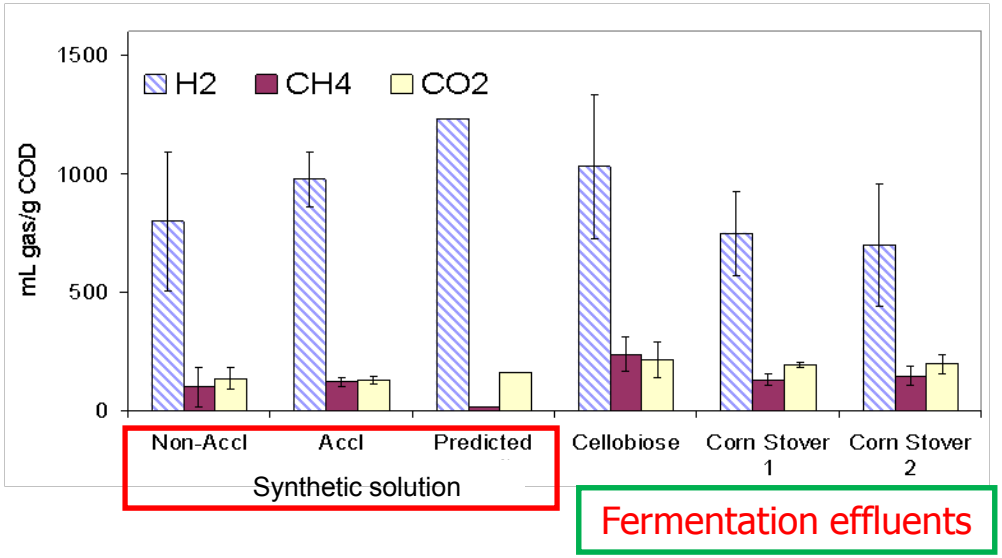


	Milestone	Completion Date	Status
3.2.2	Test H_2 production in MEC using waste effluent from the NREL lignocellulose fermentation system	11/08	Completed

Task 3 – Technical Accomplishments

Gas Production Using Real Fermentation Effluent

Fermentation effluent	Total gas production (mL)	Gas composition (%)		
		H_2	CH_4	CO_2
Synthetic (Accl)	110 ± 10	79 ± 3	10 ± 2	11 ± 1
Cellobiose	105 ± 17	69 ± 4	16 ± 4	14 ± 1
Corn stover 1	97 ± 16	69 ± 6	12 ± 3	19 ± 3
2	90 ± 29	66 ± 8	15 ± 5	19 ± 3



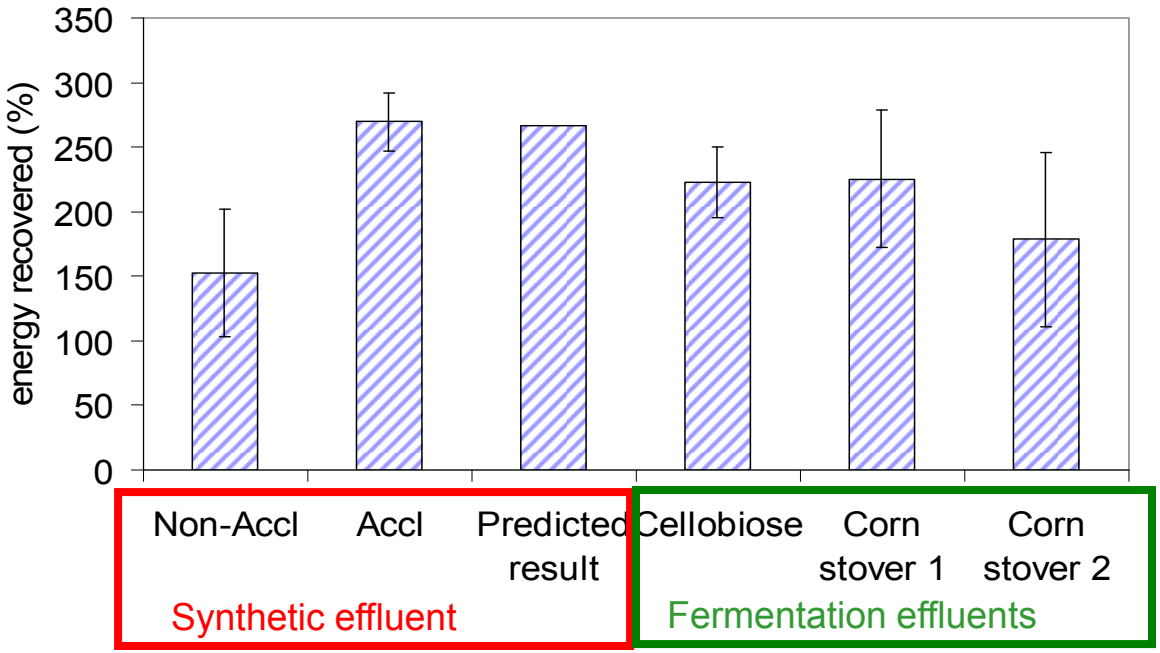
- Acclimated better than non-acclimated
- Acclimated less than predicted
- Cellobiose effluent performed better
- Some methane production in all tests.

$$predicted = 54\% \times acetate + 29\% \times ethanol + 12\% \times succinate + 4\% \times lactate + 1\% \times formate$$

Task 3 – Technical Accomplishments

Electrical Energy Efficiency

$$electrical\ energy\ efficiency = \frac{energy\ in\ H_2\ produced}{energy\ input\ from\ power\ source}$$



- Acclimated 1.2 × greater than non-acclimated.
- Acclimated result achieved that predicted based on single substrates.
- Actual fermentation effluents ~200% efficiency.

Task 3 – Technical Accomplishments
Novel Integrated System Improved H_2 Molar Yield

- Fermentation: 1.64 mol H_2/mol hexose
  - Fermentation is fast and easily scalable, using recalcitrant cellulosic substrates.
- MEC: 6.88 mol/mol (based on actual cellobiose effluent)
  - First demonstration of H_2 from fermentation effluent via MEC.
- Combined Yield: 9.95 mol H_2/mol hexose
  - The NREL-PSU integrated system exceeds DOE 2013 target of "4 mol H_2/mol hexose."
  - Economic analysis in progress.

Completed milestone "*Test H_2 production in a MEC reactor using waste effluents from the NREL lignocellulose fermentation system*" (11/08).

Collaborations

- **Task 1:**

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

- **Task 2:**

  Drs. David Levin and Richard Sparling, University of Manitoba, Canada (Develop genetic tools for pathway engineering). Maness is an international collaborator in a recent grant award from the "*Genome Canada"* Program.

- **Task 3:**

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

Future Work

Task 1:

- Investigate effects of corn stover lignocellulose carbon substrate loading on rates and yield of H_2 .
- Optimize H_2 production in bioreactors using lignocellulose from pretreated switch grass.
- Conduct carbon mass balance and redox balance.

Task 2:

- Optimize conjugation protocols using a single *E. coli* strain containing both the helper plasmid along with the pIMK1 plasmid carrying the cargo genes.
- Develop electroporation protocols for *C. thermocellum*.

Task 3:

- Conduct continuous-flow MEC feeding NREL cellobiose fermentation effluent.
- Determine effects of temperature (lower temperatures to reduce methane production.
- Perform microbial community analysis.

Summary

Task 1:

- *Clostridium thermocellum* can produce H_2 from recalcitrant, abundant biomass sources such as pretreated corn stover and switch grass.
- Hydrogen molar yield and mass balance determined with various substrates.
- Raising temperature from 50 to 55°C improved H_2 production by 79%.

Task 2:

- Blocking waste-byproduct formation improved total H_2 output by 28%, which can guide the most effective pathway engineering effort.
- Obtained tools and developed protocols to initiate gene transformation.

Task 3:

- Developed an acclimated consortium tailored for mixed waste for H_2 production.
- Produced H_2 using both cellobiose and lignocellulose fermentation effluents, with a near 200% electrical energy efficiency.

H_2 molar yield of 9.95 achieved with the novel integrated system, exceeding 2013 DOE Technical Target.