Fermentative and Electrohydrogenic Approaches to Hydrogen Production

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National Renewable Energy Laboratory

Bruce Logan
Penn State University (Subcontract)

May 20, 2009

Project ID #:
PD_18_Maness
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 H₂.

- **Relevance:** Address directly feedstock cost and H₂ molar yield to make the process cost competitive.

- Make positive impact on technical barriers and targets.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Units</th>
<th>2013 Target</th>
<th>2009 Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of H₂ from glucose</td>
<td>Mole H₂/mol glucose</td>
<td>4</td>
<td>8.52</td>
</tr>
<tr>
<td>Feedstock cost</td>
<td>Cents/lb glucose</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>
Objectives/Approach/Milestone

Task 1: Bioreactor Performance

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

### Lignocellulosic Biomass

- **Hemicellulose (xylose)**: 30%
- **Lignin (phenolics)**: 26%
- **Cellulose (glucose)**: 44%

### Bioreactor Performance

- **Clostridium thermocellum**

### Milestone Completion

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Completion Date</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.3</td>
<td>8/08</td>
<td>Completed</td>
</tr>
</tbody>
</table>
Task 1 – Technical Accomplishment
Investigated Fermentation of Various Substrates

- H₂ 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 H₂ production
  - Lower carbon loading leads to higher H₂ molar yield.

<table>
<thead>
<tr>
<th>Substrate (%, w/v)</th>
<th>Hexose, mM</th>
<th>Temp (°C)</th>
<th>L H₂/L/Day</th>
<th>H₂ Molar Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellobose (0.25%)</td>
<td>14.6 mM</td>
<td>55</td>
<td>2.94</td>
<td>1.1</td>
</tr>
<tr>
<td>Cellobose (0.25%)</td>
<td>14.6 mM</td>
<td>50</td>
<td>1.65</td>
<td>1.64</td>
</tr>
<tr>
<td>Avicel (0.5%)</td>
<td>30.9 mM</td>
<td>50</td>
<td>1.44</td>
<td>1.51</td>
</tr>
<tr>
<td>Corn stover* (0.25%)</td>
<td>9.1 mM</td>
<td>50</td>
<td>0.25</td>
<td>1.67</td>
</tr>
<tr>
<td>Corn stover* (0.56%)</td>
<td>20.4 mM</td>
<td>55</td>
<td>0.55</td>
<td>1.33</td>
</tr>
<tr>
<td>Corn stover* (0.83%)</td>
<td>30.9 mM</td>
<td>55</td>
<td>1.21</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

* Dilute acid (1.08% H₂SO₄) 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.

- H₂ molar yield: 1.33 mol H₂ mol⁻¹ hexose
- Rate of H₂ production: 0.55 L H₂ L⁻¹ D⁻¹
- Carbon mass balance 74.5%
  - CO₂: 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 H₂ molar yield and carbon mass balance using pretreated biomass” (8/08).
Objectives/Approach/Milestone
Task 2 – Develop Genetic Methods for Metabolic Engineering

- **Objective**: Improve $\text{H}_2$ molar yield (mol $\text{H}_2$/mol hexose).

- **Approach**: Redirect metabolic pathways to maximize $\text{H}_2$ production via the development of genetic methods.

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Completion Date</th>
<th>Status</th>
</tr>
</thead>
<tbody>
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<td>3.2.2</td>
<td>6/08</td>
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</tr>
<tr>
<td>3.2.5</td>
<td>3/09</td>
<td>Completed</td>
</tr>
</tbody>
</table>

**Diagram Description**

- Cellulose $\rightarrow$ Celllobiose/glucose $ightarrow$ Pyruvate $ightarrow$ Acetyl-CoA
- Lactate $\rightarrow$ Formate $\rightarrow$ Ethanol
- Acetaldehyde $\rightarrow$ Acetate
- NAD$^+$, NADH, 2NAD$^+$, 2NADH, 2ADP, 2ATP
- $\text{H}_2$ production pathway diagram
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.

<table>
<thead>
<tr>
<th>Agar (%)</th>
<th>Number of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5%</td>
<td>3–8</td>
</tr>
<tr>
<td>1.2%</td>
<td>10–20</td>
</tr>
<tr>
<td>1%</td>
<td>25–40</td>
</tr>
<tr>
<td>0.8%</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>0.7%</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

> 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.

- 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).

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

Objectives/Relevance
Task 3 – Electrochemically Assisted Microbial Fermentation

Objective: Improve H₂ molar yield (mol H₂/mol hexose) by integrating dark fermentation with Microbial Electrolysis Cell (MEC) reactor to convert waste organic matter to additional H₂.

Biomass → Dark Fermentation → Acetic, formic, lactic, succinic acids and ethanol → MEC

- N₁ = 2 – 4 H₂
- N₂ = 5.8 - 7.6 H₂

N₁ + N₂ = 7.8 – 11.6 mol H₂ per mol sugar

One-stage process: slow
Two-stage process: fast
Approach/Milestone
Subtask 3: Electrochemically Assisted Microbial Fermentation

Milestone: Test H₂ production in MEC using waste effluent from the NREL lignocellulose fermentation system

Completion Date: 11/08
Status: Completed
Task 3 – Technical Accomplishments
Gas Production Using Real Fermentation Effluent

<table>
<thead>
<tr>
<th>Fermentation effluent</th>
<th>Total gas production (mL)</th>
<th>Gas composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H₂</td>
</tr>
<tr>
<td>Synthetic (Accl)</td>
<td>110 ± 10</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>Cellobose</td>
<td>105 ± 17</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>Corn stover 1</td>
<td>97 ± 16</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>2</td>
<td>90 ± 29</td>
<td>66 ± 8</td>
</tr>
</tbody>
</table>

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

predicted = 54% × acetate + 29% × ethanol + 12% × succinate + 4% × lactate + 1% × formate
Task 3 – Technical Accomplishments

Electrical Energy Efficiency

electrical energy efficiency = \frac{\text{energy in } \text{H}_2 \text{ produced}}{\text{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 H₂ Molar Yield

- **Fermentation:** 1.64 mol H₂/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 H₂ from fermentation effluent via MEC.
- **Combined Yield:** 9.95 mol H₂/mol hexose
  - The NREL-PSU integrated system exceeds DOE 2013 target of “4 mol H₂/mol hexose.”
  - Economic analysis in progress.

Completed milestone “Test H₂ 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 $\text{H}_2$ 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.