Fermentative Approaches to Hydrogen Production

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DOE HFC&IT Program Review
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Project ID # PD18

This presentation does not contain any proprietary or confidential information
Overview

Timeline
• Project start date: FY 05
• Project end date: on going
• Percent complete: NA

Barriers
• Production Barriers addressed
  – Barrier AI: H₂ Molar Yield
  – Barrier AK: Feedstock Cost

Budget
• Total project funding
  – $200K (DOE share)
• Funding received in FY04: $0.00
• Funding for FY05: $200K

Partners
• Interactions/collaborations
  Dr. Bruce Logan, Dr. Jay Regan, Penn State University
  Dr. Lee Lynd, Dartmouth College
  Dr. David Levin, Univ. of Victoria (Canada)
Objectives

• The long-term goal is to assist DOE in developing direct fermentation technologies to convert renewable biomass resources to H₂

• The objectives in FY05 are to:
  – Screen and identify **cellulolytic microbes** which can produce H₂ directly from cellulose and hemicellulose, major constituents of biomass
  – Identify up to 3 suitable strains of fermentative microbes to select one from for pathway engineering to improve H₂ molar yield in FY06 and beyond (**FY05 Milestone**)
Approach to Address Feedstock Barrier (AK)

• **Problem:** Near 75 to 90% of lignocellulosic biomass is composed of sugars, ideal substrates for H₂ production. NREL’s Biomass Program is developing technologies to lower the cost of glucose from biomass to 8 cents per pound by 2015

<table>
<thead>
<tr>
<th>Component</th>
<th>% Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>40-60%</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>20-40%</td>
</tr>
<tr>
<td>Lignin</td>
<td>10-25%</td>
</tr>
</tbody>
</table>

• **Approach:** Bio-prospect cellulolytic microbes that can convert cellulose and hemicellulose (xylose) directly, in lieu of glucose, to H₂ as an alternative and valid strategy to lower the feedstock cost barrier
Approach to Address H₂ Molar Yield (AI)

- **Problem:** Molar Yield of H₂ (mol H₂/mol sugar) is too low (2 to 2.5) due to the simultaneous production of other fermentation waste byproducts

  \[
  \text{Glucose} + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 12\text{H}_2 \\
  \text{Glucose} + 2\text{H}_2\text{O} \rightarrow 2\text{Acetate} + 2\text{CO}_2 + 4\text{H}_2 \\
  \text{Glucose} \rightarrow \text{Butyrate} + 2\text{CO}_2 + 2\text{H}_2
  \]

- **Approach (FY2006 and beyond):** Select a suitable cellulolytic microbe of known genome sequence for metabolic pathway engineering
  - Block competing pathways has been demonstrated in literature in improving H₂ molar yield
Technical Accomplishment/Progress – Screening 9 Strains of *Clostridium thermocellum*

- Avicel® is the most recalcitrant cellulose
- Fermentation was carried out at 55 °C
- $\text{H}_2$ production resumes when the headspace $\text{H}_2$ was displaced with an inert gas
Technical Accomplishment/Progress – Screening 9 Strains of *C. thermocellum*

- Cellulose utilization is noted by a change in color

Strains were kindly provided to us by Profs. Lee Lynd (Dartmouth College) and Ed Bayer (Weizmann Institute of Science, Israel)
Technical Accomplishment/Progress – Identified the Suitable H₂ Producer

<table>
<thead>
<tr>
<th>Strains</th>
<th>Rate of H₂ Production*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>1018</td>
</tr>
<tr>
<td>1.1.1</td>
<td>595</td>
</tr>
<tr>
<td>YS</td>
<td>477</td>
</tr>
<tr>
<td>7.10.4</td>
<td>447</td>
</tr>
<tr>
<td>7.12.1</td>
<td>407</td>
</tr>
<tr>
<td>7.7.10</td>
<td>35</td>
</tr>
<tr>
<td>7.8.3</td>
<td>Traces</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Traces</td>
</tr>
<tr>
<td>7.9.4</td>
<td>Traces</td>
</tr>
</tbody>
</table>

- Screened 9 strains of cellulolytic microbes
- ATCC strain has the highest rate. Work is ongoing to optimize its growth conditions to eliminate lag phase
- **Strain 1.1.1** was selected for scale-up experiment due to its fast growth rate in **cellulose**
- Screening effort is ongoing
- Using cellulose in lieu of glucose will meet the technical target of lowering the feedstock cost

* nmol H₂/hr/ml culture gas phase
Bioreactor Configurations for Cellulose Fermentation

- pH and temperature controlled
- Operate two reactors simultaneously
- On-line continuous sampling of reactor gas phase via gas chromatography
- H₂/CO₂ is vented continuously, no pressure buildup
Technical Accomplishment/Progress: H₂ from Cellulose in Bioreactor

- 0.5% (w/v) Avicel was consumed completely
- Fermentation waste byproducts are ethanol, acetic, and butyric with traces of lactic and formic acids
- Carbon mass balance approaching 95%
- H₂ molar yield near 2
- First demonstration of H₂ molar yield data from cellulose
Corn Stover Pretreatment: Steam Explosion

Corn Stover

Steam Explosion (acid or neutral)

Aqueous Hemicellulose
(5- and 6-carbon sugar oligomers)

Solid Lignocellulose
(cellulose & lignin)

Technical Accomplishment/Progress: \( \text{H}_2 \) from Corn Stover Lignocellulose Solids

- **C. thermocellum 1.1.1**

- Near 98% and 90% of cellulose and hemicellulose were consumed, respectively.

![Graph showing the production of \( \text{H}_2 \) over time with different conditions (Neutral and Acid).](image)

- **Neutral:** 220 °C, 3 min
- **Acid:** 190 °C, 1 min

- Solids before and after fermentation
Responses to Previous Year Reviewers’ Comments

- This new project started Oct 1, 2004 and has not been reviewed previously.
Future Work

**Remainder of FY2005:**
- Screen additional cellulolytic microbes such as *Clostridium cellulovorans*, *C. cellulolyticum*, etc.
- Further optimize fermentation parameters in scale-up bioreactor
- Determine carbon balance and H$_2$ molar yield
- Identify the best microbe of known genome sequence for metabolic engineering in FY2006 *(FY2005 Milestone)*

**FY2006:**
- With the selected model microbe, conduct metabolic profiling to determine the most effective strategy to re-direct biochemical pathways *(FY2006 Milestone)*
- Begin genetic engineering to block competing pathways to improve molar yield of H$_2$
Publications and Presentations

• Publications

• Presentations
  – The 10th Annual Meeting of Institute of Biological Engineering, Athens, GA. March 2005
  – Graduate Student Seminar Series, Dept. of Civil & Environ. Engineering, Penn State University, PA. April 2005
Hydrogen Safety

• The most significant hydrogen hazard associated with this project is the use of H$_2$-containing anaerobic glovebox for sample preparations under anaerobic environment

  – Anaerobic glovebox routinely contains 2-3% H$_2$ (in N$_2$), provided via a 10% H$_2$ gas cylinder (in N$_2$)
  – Inside glovebox are small electrical devices and power cords needed for sample preparations
Hydrogen Safety

- Our approaches to deal with this hazard are:
  - Install H₂/O₂ gas monitor inside the glovebox, with alarms set at 10% H₂ and 300ppm O₂ (Factory preset)
  - Maintain H₂ level inside the glovebox at 2-3% (in N₂)
  - Activate palladium catalyst frequently
  - The power cord is unplugged from the mains (outside) first prior to any (dis)connection inside the glovebox
  - Use a flammable gas detector to detect potential H₂ leaks out from the glovebox
  - NREL laboratory ventilation system provides 6 to 10 complete air exchanges per hour in the event of a catastrophic leak
  - The DOE Hydrogen Safety Review Team visited NREL in 2004 and we have incorporated their suggestions in our AOP.