Fermentative Approaches to Hydrogen Production

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DOE HFC&IT Program Review
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Project ID # PD18

Overview

Timeline

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

Budget

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

Barriers

- Production Barriers addressed
  - Barrier AI: H_2 Molar Yield
  - Barrier AK: Feedstock Cost

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 H_2

- The objectives in FY05 are to:
  - Screen and identify **cellulolytic microbes** which can produce H_2 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 H_2 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 H_2 production. NREL's Biomass Program is developing technologies to lower the cost of glucose from biomass to 8 cents per pound by 2015

Component	% Dry Weight
Cellulose	40-60%
Hemicellulose	20-40%
Lignin	10-25%

- ***Approach:*** Bio-prospect cellulolytic microbes that can convert cellulose and hemicellulose (xylose) directly, in lieu of glucose, to H_2 as an alternative and valid strategy to lower the feedstock cost barrier

Approach to Address H_2 Molar Yield (AI)

- ***Problem:*** Molar Yield of H_2 (mol H_2/mol sugar) is too low (2 to 2.5) due to the simultaneous production of other fermentation waste byproducts

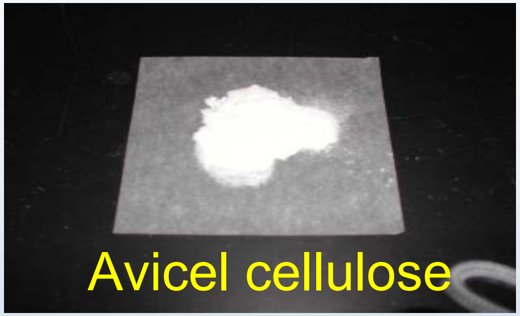
$$\text{Glucose} + 6H_2O \longrightarrow 6CO_2 + 12H_2$$

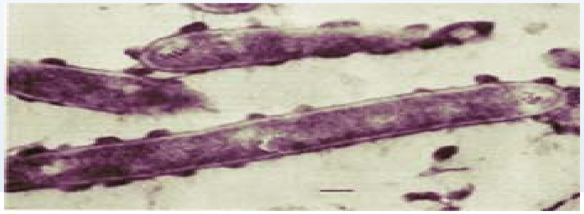
$$\text{Glucose} + 2H_2O \longrightarrow 2\text{Acetate} + 2CO_2 + 4H_2$$

$$\text{Glucose} \longrightarrow \text{Butyrate} + 2CO_2 + 2H_2$$

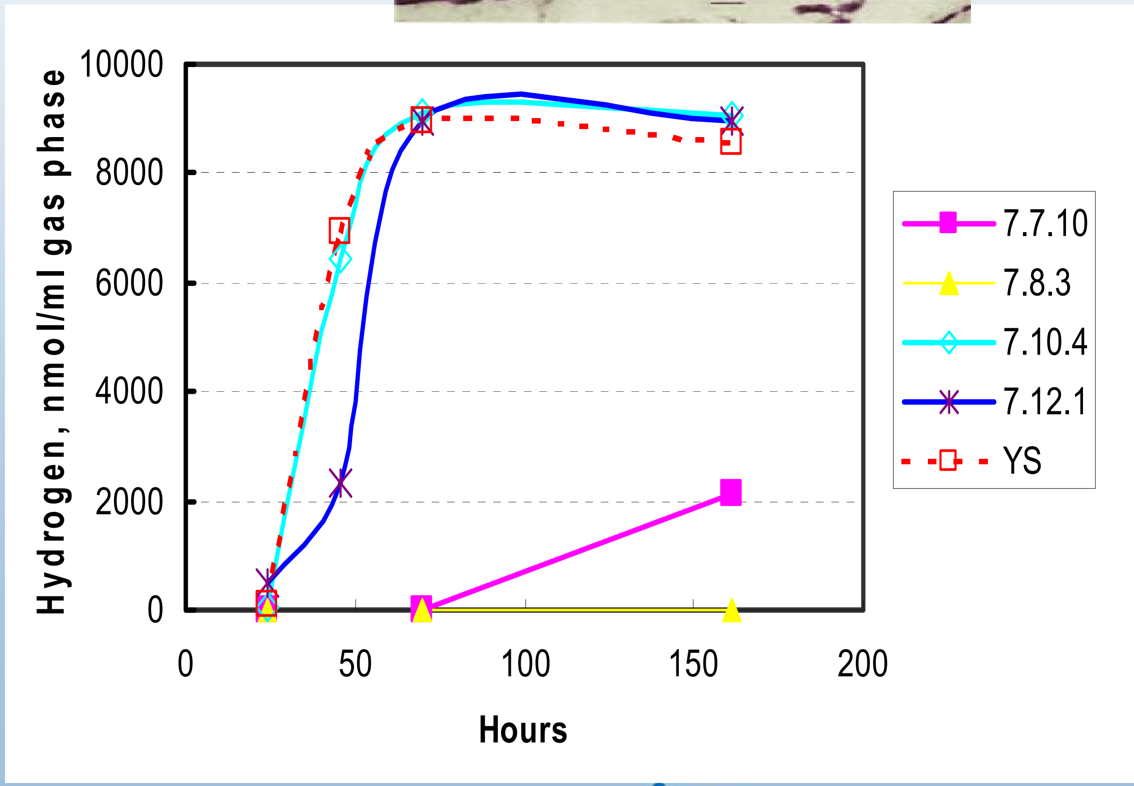
- ***Approach* (FY2006 and beyond):** Select a suitable cellulolytic microbe of known genome sequence for metabolic pathway engineering
  - Block competing pathways has been demonstrated in literature in improving H_2 molar yield

Technical Accomplishment/Progress – Screening 9 Strains of *Clostridium thermocellum*

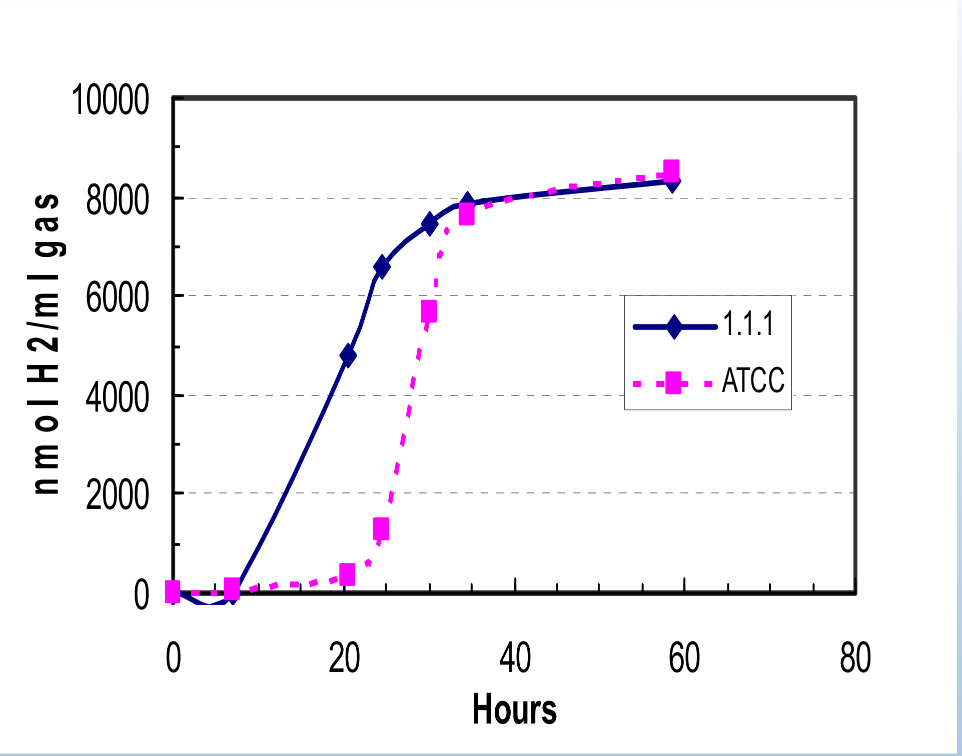




- Avicel® is the most recalcitrant cellulose
- Fermentation was carried out at 55 °C
- H_2 production resumes when the headspace H_2 was displaced with an inert gas



Technical Accomplishment/Progress – Screening 9 Strains of *C. thermocellum*



cellulose

- 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)

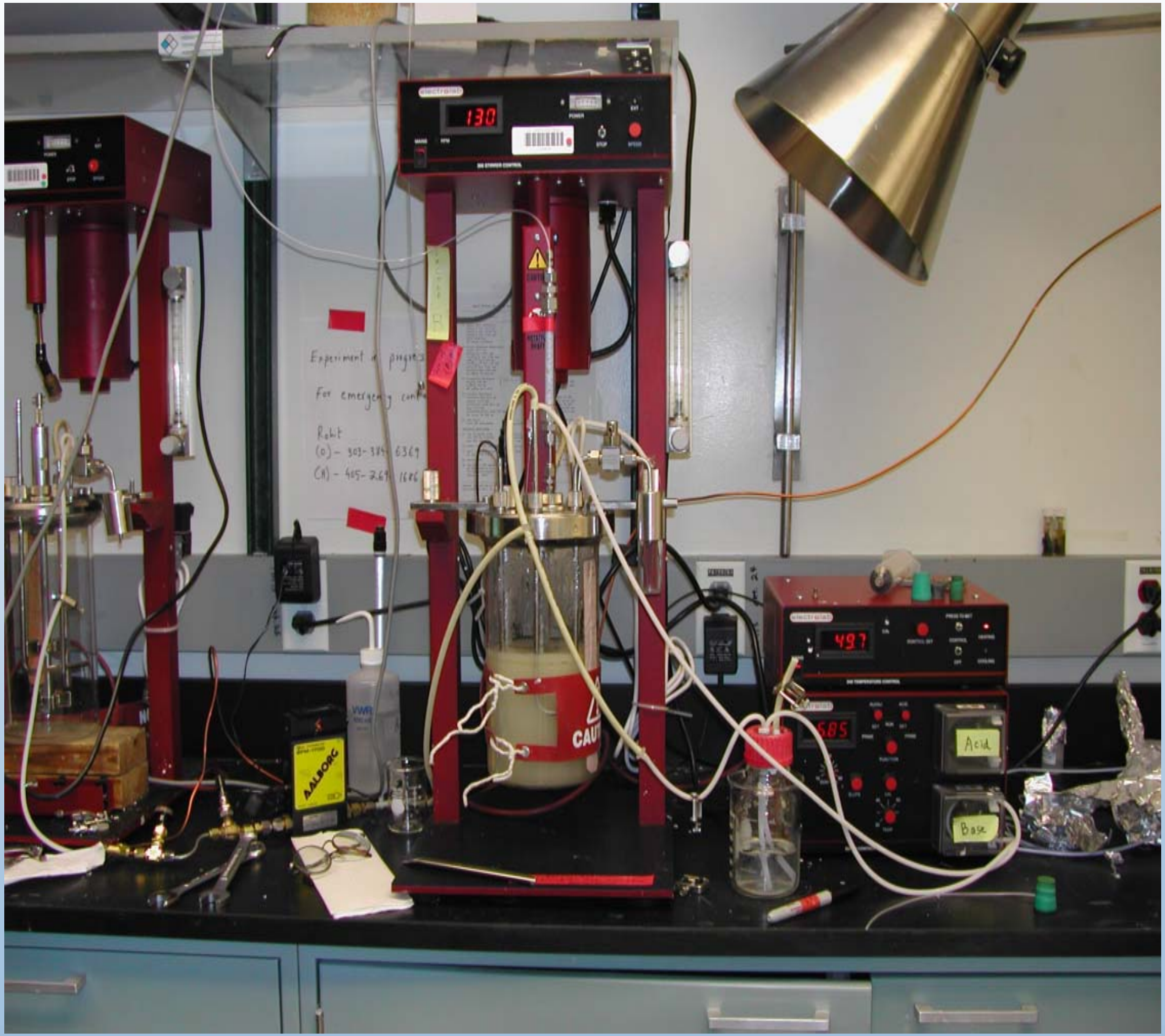
Technical Accomplishment/Progress – Identified the Suitable H_2 Producer

Strains	Rate of H_2 Production*
ATCC	**1018**
1.1.1	**595**
YS	477
7.10.4	447
7.12.1	407
7.7.10	35
7.8.3	Traces
6.3.2	Traces
7.9.4	Traces

- Screened **9** strains of cellulolytic microbes
- ATCC strain has the highest rate. Work is underway to optimize its growth conditions to eliminate the 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**

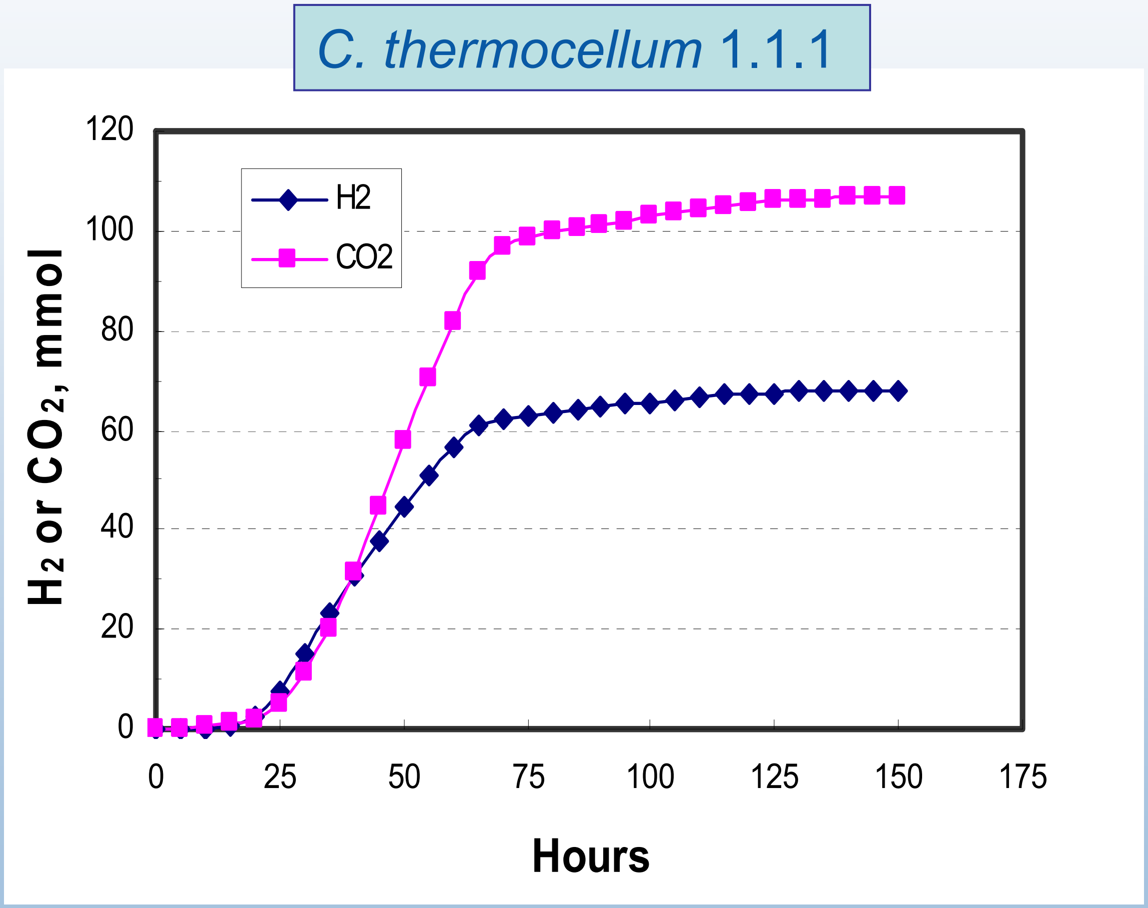
* nmol H_2/hr/ml culture gas phase

Bioreactor Configurations for Cellulose Fermentation



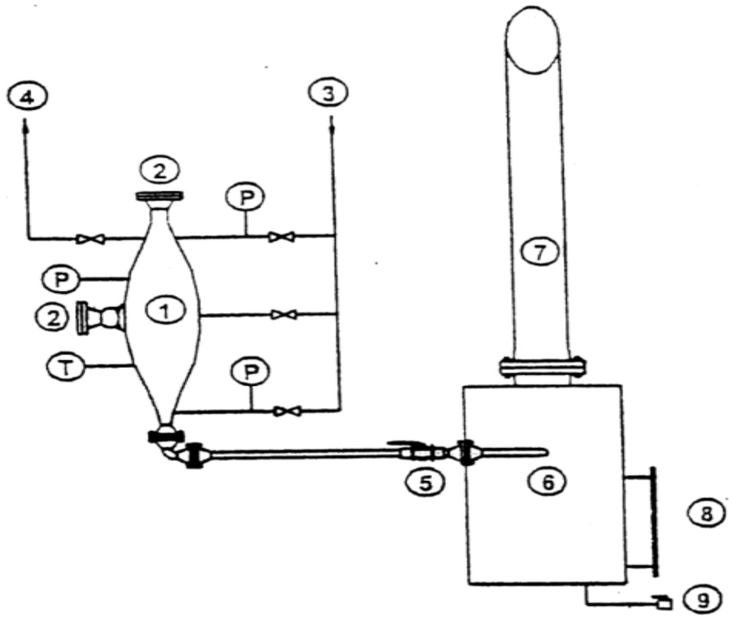
- pH and temperature controlled
- Operate two reactors simultaneously
- On-line continuous sampling of reactor gas phase via gas chromatography
- H_2/CO_2 is vented continuously,no pressure buildup

Technical Accomplishment/Progress: H_2 from Cellulose in Bioreactor



- **0.5% (w/v) Avicel was consumed completely**
- Fermentation waste byproducts are ethanol, and acetic acid with traces of lactic and formic acids
- Carbon mass balance approaching 90%
- **H_2 molar yield near 2**
- **First demonstration of H_2 molar yield data from cellulose**

Corn Stover Pretreatment: Steam Explosion



1 - Pressure Vessel; 2 - MSW feeding; 3 - Steam line; 4 - Safety vent;
5 - Release valve; 6 - Receiver; 7 - Waste steam exhaust pipe;
8 - Exploded material withdrawal door; 9 - Liquid collection line;
T - Temperature gauge; P - Pressure gauge

Liu et al. 2002. Biotech Bioengineering 77: 121-130.

Corn Stover

↓ Steam Explosion (acid or neutral)

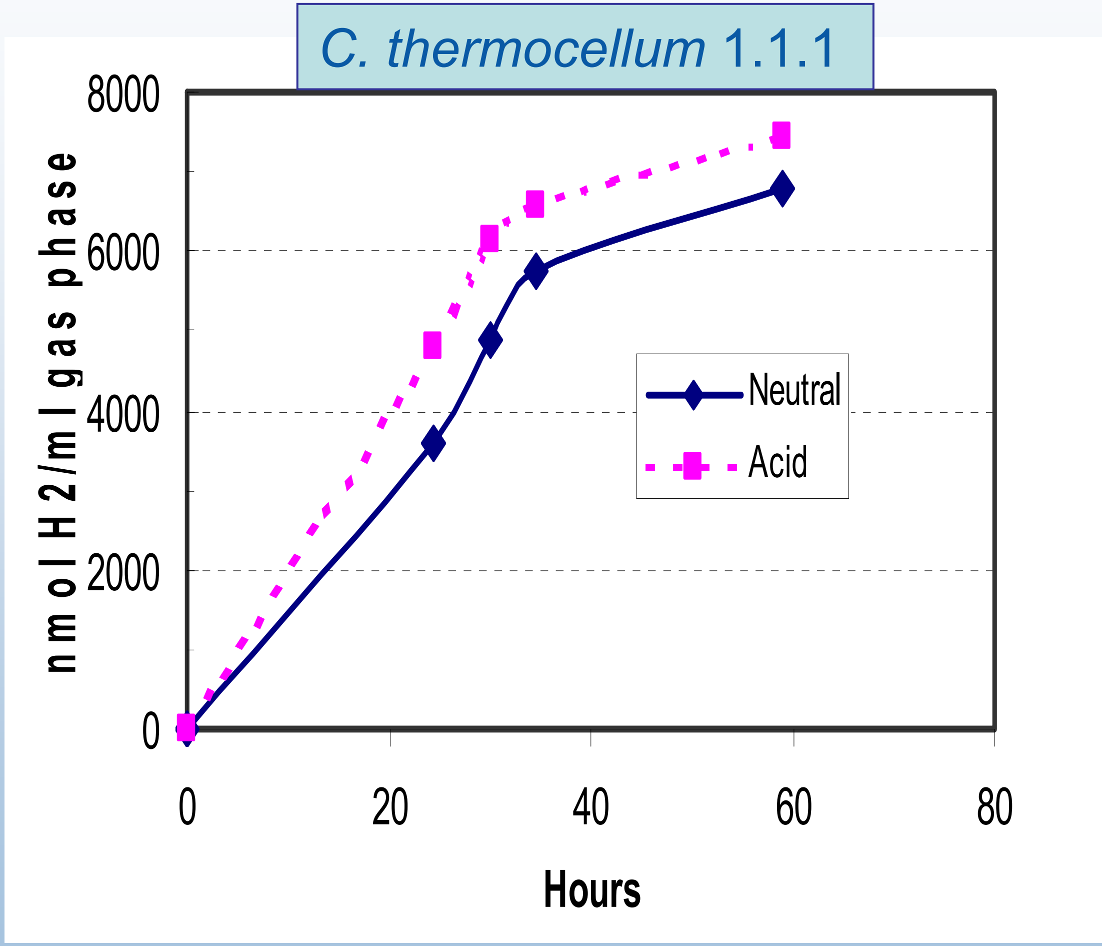
Aqueous Hemicellulose

(5- and 6-carbon sugar oligomers)

Solid Lignocellulose

(cellulose & lignin)

Technical Accomplishment/Progress: H_2 from Corn Stover Lignocellulose Solids



Solids before and after fermentation

- **Near 93% and 86% of cellulose and hemicellulose were consumed, respectively.**

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

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**
  - Datar, R., J. Huang, P. C. Maness, A. Mohagheghi, S. Czernik, and E. Chornet. Hydrogen production from the fermentation of corn stover biomass pretreated with a steam explosion process. Submitted to Environ. Sci. Technol.
  - Lee, J. Z., D. M. Klaus, P. C. Maness, and J. R. Spear. Characterization of the effect of butyrate on hydrogen production in photofermentation for use in Martian Resource Recovery. Submitted to Intl. J. Hydrogen Energy

- **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 H_2/O_2 gas monitor inside the glovebox, with alarms set at 10% H_2 and 300ppm O_2 (Factory preset)
  - Maintain H_2 level inside the glovebox at 2-3% (in N_2)
  - 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 H_2 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.