II.E.4 Fermentation Approaches to Hydrogen Production

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Objectives

- Perform H_2 fermentation using cellulolytic bacteria and corn-stover lignocellulose to lower feedstock cost.
- Perform pathway engineering to improve H_2 molar yield via fermentation.

Technical Barriers

This project addresses the following technical barriers from the Biological Hydrogen Production section (3.1.4.2.5) of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

(AI) H_2 Molar Yield
(AJ) Waste Acid Accumulation
(AK) Feedstock Cost

Technical Targets

Progress Toward Meeting DOE Technical Targets in Dark Fermentation

Characteristics	Units	2010 Target	2006 Status
Yield of H_2 from glucose	Mole H_2/mole glucose	4	2.4 (from cellulose)
Feedstock cost	Cents/lb glucose	10	13.5 (as of 2003)

- Yield of H_2 from glucose: DOE has a 2010 target of an H_2 molar yield of 4 using glucose as the feedstock. In fiscal year (FY) 2006 we achieved a molar yield of 2.3-2.4 using lignocellulose from corn stover as the feedstock, which is much more difficult to use yet more abundant and a realistic substrate.
- Feedstock cost: The DOE Biomass Program is conducting research to meet the 2010 target of 10 cents/lb biomass-derived glucose. NREL's approach is to use cellulolytic microbes to ferment cellulose and hemicellulose directly, which will result in lower feedstock costs.

Accomplishments

- Performed scale-up bioreactor experiments using *Clostridium thermocellum* and lignocellulose prepared from the steam explosion of corn stover under either neutral or acidic conditions. We measured H_2 molar yields of 2.3-2.4. This accomplishment provides a very good H_2 molar yield baseline from which to improve using a more difficult and yet less costly substrate.
- Demonstrated that biomass pretreatment conducted with and without acid supports fermentation equally well. Excluding the acid simplifies the biomass pretreatment process.

Introduction

Biomass-derived glucose feedstock is a major operating cost driver for economic H_2 production via fermentation. One of the cost contributors is the expense of the pure cellulase enzymes needed to hydrolyze cellulose to glucose. The DOE Hydrogen, Fuel Cells, and Infrastructure Technologies (HFCIT) Program will take advantage of the DOE Biomass Program's investment in developing inexpensive glucose from biomass to meet its cost target of 8 cents/lb by 2015. Meanwhile, one alternative and valid approach to addressing the glucose feedstock technical barrier AK is to use certain cellulolytic microbes that can ferment cellulose directly for H_2 production. Cellulose is the most abundant biopolymer on earth, and using it directly, in lieu of glucose, will address the glucose feedstock issue. In FY 2005, we screened and identified *C. thermocellum* strain 1.1.1, which grew the fastest and produced the most H_2 of strains kindly provided to us by Prof. Lee Lynd of Dartmouth College [1]. Our goal in FY 2006 is to demonstrate that strain 1.1.1 can ferment lignocellulose directly to H_2 to improve the economics of the process.

Another technical barrier to fermentation is the relatively low molar yield of H_2 from glucose (mol H_2/mol sugar; technical barrier AI), which results from the simultaneous production of waste organic acids and solvents. Biological pathways maximally yield 4 mole of H_2 per 1 mole of glucose (the biological maximum) [2]. Most laboratories have reported a molar yield of 2 or less [3, 4]. Another goal of this research is to perform molecular engineering of the model microbe to block competing pathways. This will redirect cellular metabolic energy toward maximal H_2 production while minimizing acid and solvent production. Blocking competing pathways via genetic engineering has been proven to improve H_2 molar yield in *Enterobacter aerogenes* [5]. Addressing technical barriers AI and AK will realize the potential of H_2 production via fermentation while overcoming technical barrier AJ (waste acid accumulation).

Approach

In FY 2006, our main goal has been to address technical barrier AK by fermenting lignocellulose directly using *C. thermocellum* 1.1.1 (identified in FY 2005). Most microbes that degrade cellulose also excrete a suite of hemicellulase enzymes to hydrolyze hemicellulose [6]. Together, cellulose and hemicellulose constitute up to 90% of the biomass and contain nearly 100% of the carbohydrate fractions [7]. Our strategy will simplify biomass pretreatment processes and thus significantly lower feedstock cost. Moreover, it will result in nearly complete utilization of most of the carbohydrate fractions of biomass for H_2 production; instead of using only the glucose component of biomass (mostly from cellulose), which is only 40%-60% for most lignocellulosic biomass.

The pathway engineering effort will resume in FY 2007 when full funding is restored.

Results

Corn Stover Pretreated with Steam Explosion

Using corn stover as the model biomass, we chose steam explosion as the pretreatment technology to evaluate its suitability for H_2 fermentation. Steam explosion is conducted by subjecting steam-saturated corn stover (200 pounds per square inch [psi]), with or without acid (1.2% sulfuric acid [H_2SO_4]), to a sudden release of pressure while forcing it through a small orifice, during which the flash evaporation of water causes the biomass to rupture [8]. This thermo-mechanical force breaks open the ultrastructure of biomass (corn stover) into an aqueous hemicellulose (hydrolyzate) and a solid lignocellulose fraction, both of which are then tested for fermentation. In this work, we are focusing on the fermentation of the lignocellulose solid because H_2 fermentation of the hydrolyzate is much easier and was demonstrated earlier in our laboratory [9].

Lignocellulose Fermentation: H_2 Molar Yield and Carbon Mass Balance Determination

To determine H_2 molar yield and carbon mass balance more accurately, we performed the fermentation in scale-up bioreactors with automated temperature (50°C) and pH (6.8) controls. The bioreactor was bubbled with nitrogen (N_2) gas (10 cc/min) to allow real-time sampling of H_2 and carbon dioxide (CO_2) via an on-line gas chromatograph. *Clostridium thermocellum* 1.1.1 was inoculated into a 1.3-liter (working volume) bioreactor fed with 0.5% (w/v) lignocellulose from the **neutral** steam explosion (220°C, 3 min) of corn stover [9]. Figure 1 displays the kinetics of H_2 and CO_2 production during a period of 170 hours. Carbon analysis indicated that almost 98.6% of the cellulose is consumed while approximately 91% of the xylan is metabolized; the latter was a contamination from the hemicellulose fraction of corn stover during steam pretreatment. Acetic acid (26.2 mM) is the dominant organic acid byproduct along with minor amounts of lactic (1.04 mM) and formic acids (0.59 mM), measured by high pressure liquid chromatography (HPLC). Carbon mass balance approaches 82.2% without accounting for the portion of the carbon substrate assimilated into new cell mass materials. Molar yield of H_2 from lignocellulose is near 2.3, comparable to that obtained with either glucose or Avicel®.

Similar fermentation was carried out using lignocellulose derived from the **acidic** steam explosion (190°C, 1 min) of corn stover (9). Figure 2 displays the kinetics of H_2 and CO_2 production during a period of 200 hours. Both H_2 and CO_2 production reach a stationary phase around 150 hours due to the depletion of lignocellulosic substrate. Carbon analysis indicated that almost all of the cellulose was consumed while approximately 90% of the xylan is metabolized; the latter was a contamination from the corn stover hemicellulose during steam pretreatment. Acetic acid (25.5 mM)

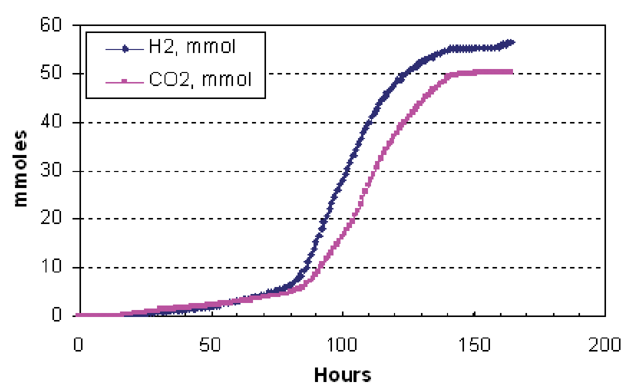

FIGURE 1. Kinetic H_2 and CO_2 Production from Lignocellulose (0.5%, w/v) Pretreated in *Neutral* Condition in a Bioreactor Inoculated with *Clostridium thermocellum* 1.1.1

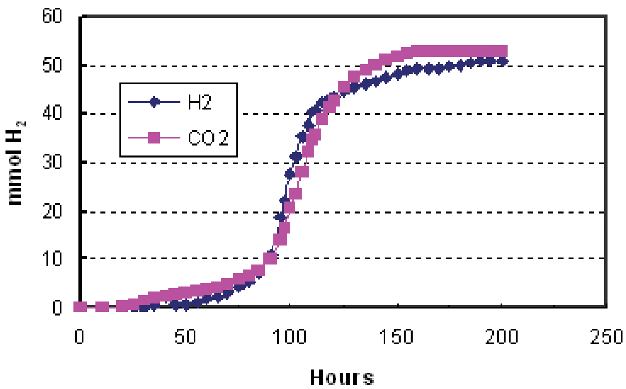

FIGURE 2. Kinetic H_2 and CO_2 Production from Lignocellulose (0.5%, w/v) Pretreated in Acidic Condition in a Bioreactor Inoculated with *Clostridium thermocellum* 1.1.1

was the dominant organic acid byproduct along with a minor amount of lactic acid (0.39 mM). Molar yield of H_2 from lignocellulose was near 2.4, which compared very favorably with results in literature using the more favorable glucose as the feedstock. Our findings also conclude that acid can be eliminated during steam explosion to simplify biomass pretreatment.

Metabolic Engineering

Due to a shortage in FY 2006 funding, our metabolic engineering effort was postponed until FY 2007.

Conclusions and Future Directions

- We demonstrated successfully that lignocellulose derived from steam explosion of corn stover under either neutral or acidic conditions worked equally well to support H_2 production.
- Neutral steam explosion is the treatment of choice due to its simplicity and less corrosivity.
- Using lignocellulose as the feedstock, we obtained H_2 molar yields near 2.3-2.4, which compared very favorably with those reported in literature using glucose as the substrate.
- Optimize bioreactor parameters in a scale-up fermentation process.
- Develop a genetic transformation system in *Clostridium thermocellum* for metabolic pathway engineering with the intent to improve H_2 molar yield.

FY 2006 Publications/Presentations

1. Zuo, Y., Maness, P. C., and Logan, B. E. 2006. Electricity Production from Steam-Exploded Corn Stover Biomass. Accepted for publication in *Energy and Fuels*.

2. Datar, R., Huang, J., Maness, P. C., Mohagheghi, A., Czernik, S., and Chornet, E. 2006. Hydrogen Production from the Fermentation of Corn Stover Pretreated with a Steam Explosion Process. Accepted for publication in *Intl. J. Hydrogen Energy*.

3. Maness presented US biological H_2 research activities during the International Partnership of H_2 Economy Workshop in Seville, Spain (Oct. 24-26, 2005).

4. Maness presented fermentation research to Xcel Tech Group (November 1, 2005).

5. Maness presented fermentation research at University of Minnesota (November 2-3, 2005).

6. Maness gave an invited presentation on fermentation and served as a Session Chair at the Materials Research Society Fall Meeting in Boston (December 2, 2005).

7. Maness presented fermentation research to Praxair, Inc. (December 12, 2005).

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9. Datar, R., Huang, J., Maness, P. C., Mohagheghi, A., Czernik, S., and Chornet, E. Hydrogen Production from the Fermentation of Corn Stover Pretreated with a Steam Explosion Process. Accepted for publication in *Intl. J. Hydrogen Energy.*