



DOE Workshop on
**HYDROGEN PRODUCTION
VIA DIRECT
FERMENTATION**

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DOE—OFFICE OF HYDROGEN, FUEL CELLS AND
INFRASTRUCTURE TECHNOLOGIES

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Introduction

Direct fermentation of carbohydrate feedstocks by microorganisms is one of a number of potential technologies for producing renewable hydrogen. While hydrogen fermentations have been demonstrated in the laboratory, yields have been low and it is uncertain whether this technology can be developed to provide high yields of hydrogen and become economically competitive with gasoline or with alternative hydrogen production pathways. To explore the potential for this technology to meet cost targets for hydrogen production, DOE's Office of Hydrogen, Fuel Cells and Infrastructure Technologies (OHFCIT) sponsored a conceptual design and order-of-magnitude economic analysis for production of hydrogen by fermentation. The Neoterics/NREL study, "[Boundary Analysis for H2 Production by Fermentation](#)¹," suggests that a fermentation yield of 10 moles of hydrogen per mole of glucose and a glucose cost of 5 cents per dry pound will be required for this process to approach hydrogen costs that are competitive with traditional fuels.

The DOE Workshop on Hydrogen Production via Direct Fermentation was held to:

- 1) discuss the assumptions and findings of the Neoterics/NREL study and its implications for fermentation R&D targets;
- 2) identify technical barriers and challenges that must be overcome to achieve cost effective production of hydrogen via direct fermentation; and
- 3) explore ideas for breakthrough technologies and research and development (R&D) that could overcome these technical barriers and challenges.

Workshop Format

The one-day workshop brought together about 40 technical experts from across industry, universities, national laboratories, and other organizations (see List of Participants in Appendix A). As shown in the agenda (Appendix B), the workshop opened with two plenary presentations, "Feedstocks for Direct Fermentative Production of Hydrogen," and "Boundary Analysis for Hydrogen Production by Fermentation." A group discussion of the Boundary Analysis report was held, during which time participants offered comments on the report's assumptions and findings. Participants then divided into two pre-assigned breakout groups and parallel facilitated discussion sessions were held to address the topic "Challenges and Opportunities for Hydrogen Production via Direct Fermentation." The breakout group participants are shown in Appendix C.

¹ Eggeman, Tim (Neoterics International) and National Renewable Energy Laboratory. "Boundary Analysis for H2 Production by Fermentation." March 12, 2004.

Workshop Results

The comments and ideas of participants, as recorded during the workshop, are shown in Tables 1-9. Key common themes and findings include:

Boundary Analysis Report: Plenary Group Discussion (see Table 1)

- The participants generally supported the calculations and conclusions of the “Boundary Analysis for H₂ Production by Fermentation,” which concludes that fermentative hydrogen production from corn or other sources of glucose or readily fermentable carbohydrates has the potential to be commercially viable, assuming yields of 8-12 moles H₂/mole glucose and low-cost feedstocks (e.g., 5 cents per pound of glucose or less). The group recommended striking the sentence in the Conclusion section of the report that states “It is questionable whether deployment of resources needed to develop this method of hydrogen production is justified, given the fact that the long term goals of the Hydrogen Program require costs lower than those projected by in this analysis.” It is premature to come to such a conclusion, since there are many uncertainties surrounding how much the hydrogen yield can be improved through metabolic engineering and what cost targets will need to be met.
- Yields of more than 4 moles of hydrogen per mole of glucose have not been verified in a reproducible manner from any known organism.
- Corn-derived glucose was the only feedstock considered in the study. There are other feedstocks that may offer lower cost alternatives (e.g., sugar beets, waste products, glucose from hydrolysis of lignocellulosic biomass).
- The determination to limit the scope to a direct-fermentation-only system should be reconsidered to include multi-stage systems that might include direct fermentation as only one hydrogen- (and revenue-) producing step in the process.

Top-Rated Grand Challenges and Technical Barriers (see Tables 2 and 3)

- No known microorganism is capable of naturally producing more than 4 moles of hydrogen per mole of glucose at atmospheric pressures – the metabolic pathways have not been identified and the reaction is energetically unfavorable.
- Biomass feedstocks are too costly – need to develop low-cost methods for growing, harvesting, transporting, and pre-treating energy crops and/or biomass waste products.
- There is no clear contender for a robust, industrially capable organism that can be used as a platform for research to genetically alter its metabolism to produce more than 4 moles of hydrogen per mole of glucose.

Top-Rated Scientific Advances and Technical Breakthroughs Needed (see Tables 4-7)

- **Metabolically Engineer an Organism that Can Produce High Yields of Hydrogen via Direct Fermentation**

Currently, there is no verified evidence that any naturally-occurring microbe can or will produce more than 4 moles of hydrogen per mole of glucose. Achieving higher yields is a critical make-or-break for direct hydrogen production via fermentation. Therefore, a crucial research objective is to create such an organism through metabolic engineering. The ideal outcome would be a microorganism that would be able to produce high hydrogen yields from an inexpensive feedstock. A number of high-priority technical breakthroughs and R&D activities are needed, including:

- Genetic tools to overcome the metabolic barrier by manipulating electron flux in hydrogen producing organisms (e.g., single host organism for transgenic expression of hydrogen pathways; genomic database; tailored genome shuffling/combinatorial tools; etc.)
- Coupling catabolism of glucose to reverse electron flow to hydrogenase (e.g., study genetics and regulation of electron flux pathway to hydrogenase; etc.)
- Eliminating unnecessary reactions that use hydrogen from glucose to reduce other fermentation products that compete with hydrogen production.
- Detailed study, modeling, and engineering of metabolic pathways used by hydrogen producing bacteria, including regulation of hydrogenases.
- Discovery of a method that would allow hydrogenase to function with a non-acetate associated terminal product
- Development of microbes that ferment multiple sugars and/or which can directly utilize cellulose/hemicellulose.

- **Explore Innovative Opportunities for Improving Fermentative Hydrogen Production System Economics**

It is possible that fermentative hydrogen production systems may be made more economical by combining them with other processes that create additional revenue streams. These systems could potentially use a diverse array of waste feedstocks that are less expensive than glucose. Examples might include a dark fermentation reactor followed by a photobiological reactor; a two- or three-stage system that utilizes all of the biomass feedstock (cellulose, hemicellulose and lignin); or a fermentation system that includes a second, “new microbe” stage for conversion of fermentation byproducts (e.g., organic acids) to hydrogen. These systems would need to be designed for optimal hydrogen production, since production of large quantities of hydrogen is the goal.

- **Genetic Engineering/Breeding of Energy Crops**

Biomass feedstock availability, geographic distribution, and cost are critical to the viability of any fermentative hydrogen production system. Genetic engineering and hybridization is needed to develop energy crops for higher productivity, lower input needs, more stress tolerance, and optimal composition for fermentative processing. Lower-cost techniques are also needed for harvesting (e.g., single-pass harvesting), pre-

processing (e.g., field processing for compacting), and pretreatment (e.g., acid hydrolysis) of biomass.

- **Develop Improved Bioreactor Design**

Current reactors do not perform optimally, especially under conditions required for industrial hydrogen production (i.e., robust, reliable performance and high sustained hydrogen yields). Research is needed to improve reactor designs and process parameters, including membrane technology to lower hydrogen concentrations within the reactor and improved techniques for mixing, pH and temperature control, and cell harvesting.

- **Fundamental Study of Complete Enzymatic Conversion to Simplify Process**

Although it produces high hydrogen yields, the current, laboratory-scale process for enzymatic conversion is too complex and too costly to scale up to a commercial process. In addition, enzymatic systems face similar thermodynamic hurdles for achieving high hydrogen yields as fermentation systems. Research that may be able to address some of the problems includes: combinatorial screening of enzymes, kinetic study of enzyme activity, and development of synthetic analogs of enzymes.

TABLE 1. RESULTS OF GROUP DISCUSSION ON NEOTERIC/NREL BOUNDARY ANALYSIS REPORT

Cost Factors

- ◆ Accounting for variability in feedstock and associated downtime may increase costs.
- ◆ Currently, achieving 10 moles H₂/mole glucose is not technically feasible – achieving higher yields of hydrogen is a critical “make-or-break” research objective for fermentative hydrogen production.
- ◆ Enzymatic process results of 12 mole H₂/mole glucose did not account for many factors (e.g., metabolism, pressure of H₂).
- ◆ If we can change the 10 moles target to greater than 4 moles, we can drive research to break the glass ceiling in fermentation – metabolic engineering or bioprospecting may achieve targets.
- ◆ Definition of “successful” fermentation can be technical or economic. Technical = 10 moles H₂/mole glucose. Economic (could possibly) = integrate fermentation with other processes to make cost effective hydrogen, at 4 moles H₂/mole glucose or 8 moles H₂/mole glucose or approaching 10 moles H₂/mole glucose.
- ◆ Back out microorganism productivity to do a reality check and compare to ethanol process.
- ◆ Check utilization factor: used 80%, but could increase this value to improve economics.
- ◆ May not need as much steam to achieve sterile/sanitary conditions if organisms are robust.
- ◆ Instead of glucose, maybe use animal waste, MSW to reduce feedstock cost? (MSW is a difficult feedstock)
- ◆ Instead of using pure glucose, can you use an earlier product (e.g., starch, lignocellulose) to reduce cost?

ENGINEERING ISSUES	FERMENTATION		ALTERNATIVE PROCESSES	MODIFIED ANAEROBIC DIGESTION	DIGESTION/ PHOTO FERMENTATION COMBINATION	COMPLETE ENZYMATICAL CONVERSION	FEEDSTOCK BARRIERS
	MODIFIED AND NEW ORGANISMS	HYDROGENASE ISSUES					
<ul style="list-style-type: none"> production volumes <input type="checkbox"/> Cost for wastewater treatment (pushed to host in analysis presented) <input type="checkbox"/> Preventing interspecies hydrogen transfer (H₂ losses) in non-sterile systems 	<ul style="list-style-type: none"> rates to H₂ are too slow ◆◆ <input type="checkbox"/> Need for sterilization avoidance and/or low cost sterilization ◆ <input type="checkbox"/> Finding the right microbes to utilize cellulose and hemicellulose efficiently for H₂ production ◆ <input type="checkbox"/> Solventogenesis and acidogenesis metabolic shift <input type="checkbox"/> Identification of new microbes to make hydrogen, i.e., discovery of new microbes <input type="checkbox"/> Need to identify X-philes that will reduce need for sterilization 						

Breakout Group 2

TABLE 3. TECHNICAL BARRIERS TO COST-EFFECTIVE FERMENTATIVE HYDROGEN

◆ = MOST CRITICAL BARRIERS

BASIC SCIENCE	OPERATING PARAMETERS/ SYSTEM DESIGN	FEEDSTOCK
<ul style="list-style-type: none"> <input type="checkbox"/> Bacteria don't produce more than 4 mole H₂/mole glucose naturally ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Microbial pathways are not inherently designed to shuttle so many electrons to H₂, which is shuttled outside the cell (i.e., it's not natural to achieve >4 mole H₂ per mole glucose) ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Don't understand relationship of H₂ production and energy coupling ◆◆◆◆ <input type="checkbox"/> Don't have a way to reduce H₂ inhibition of hydrogenase ◆◆◆◆ <ul style="list-style-type: none"> - Don't understand basic chemistry of hydrogenase <input type="checkbox"/> Have not identified/engineered bacteria that use biomass directly (already some that use starch and xylan very well – thermotoga, pyrococcus) ◆◆◆◆ <input type="checkbox"/> Lack of a biocatalyst for producing more than 3.0 mole H₂/mole glucose by dark fermentation <input type="checkbox"/> No proven technology exists at present to come even close to the goal of ~10 mole H₂/mole glucose <ul style="list-style-type: none"> - We don't really understand all the pathways <input type="checkbox"/> Knowledge is lacking whether metabolic (fermentation) pathway(s) can be manipulated to produce >4 mole H₂/mole glucose <input type="checkbox"/> Insufficient understanding of metabolism of H₂ producing bacteria, and H₂ concentration tolerance <input type="checkbox"/> Doesn't seem like basic science is in place yet <input type="checkbox"/> Don't understand how H₂ is pumped through membranes 	<ul style="list-style-type: none"> <input type="checkbox"/> Lack of proper (single) bacterial platform ◆◆◆◆◆◆◆◆◆◆ <ul style="list-style-type: none"> - Selection and optimization of microorganism <input type="checkbox"/> Don't understand how integration of H₂ production with other energy/fuel coproducts (e.g., ethanol + H₂ production) could improve economics ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> What do you do with dead bacteria (reactor loop)? ◆◆◆◆◆◆◆◆◆◆ <ul style="list-style-type: none"> - At large scale, waste products must be negligible <input type="checkbox"/> Lack of reliable lab- to production-volume estimation methodologies ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Lack of a business pathway vs. conventional technologies ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Difficult to ferment broad range of feedstocks ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Lack of appropriate reactor design <input type="checkbox"/> Don't yet know the most cost-effective operating parameters (e.g., sterilization regimes) <input type="checkbox"/> Minimize the biomass of the bacteria by simplifying the biomass quality and promoting slow growth 	<ul style="list-style-type: none"> <input type="checkbox"/> Lack of sufficiently cheap feedstocks and pretreatment methodologies ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Lack of feedstock characterization to efficiently utilize glucose and xylose in fermentation (lowest cost sugar) ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Lack of near-term, mid-term and long-term goals/conversion cost targets ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Lack of understanding of production/purification of consistent sugar streams from a variety of raw materials for fermentation ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Increased fertilizer costs resulting from harvesting biomass that was formerly land-disposed or left in place (e.g., corn silage) ◆◆◆◆◆◆◆◆◆◆ <input type="checkbox"/> Cut feedstock cost 30% - decrease costs to grow, harvest, and transport and produce better yield of sugar precursors <input type="checkbox"/> Competition from production of higher-value chemicals from biomass

Breakout Group 1
TABLE 4. SCIENTIFIC AND TECHNICAL BREAKTHROUGHS NEEDED TO OVERCOME BARRIERS TO HYDROGEN FERMENTATION

⊛ = TOP PRIORITY; ◆ = PRIORITY

COMPLETE ENZYMATIC CONVERSION	ALTERNATIVE PROCESSES	FERMENTATION			PROCESS COMBINATIONS	FEEDSTOCKS ADVANCES	ENGINEERING
		NEW/MODIFIED ORGANISMS	GENETIC TOOLS	METABOLISM			
<ul style="list-style-type: none"> □ Fundamental study of enzymatic conversion to simplify process ⊛⊛◆◆◆◆◆◆◆◆ 	<ul style="list-style-type: none"> □ Robust cell-free systems for metabolic control toward H₂ production ◆◆ □ A photo-synthetic green algal species that uses light and carbon streams in the biorefinery and produces H₂ to feed a fuel cell to power the biorefinery ◆ □ High-solid H₂ fermentation ◆ 	<ul style="list-style-type: none"> □ New microbes with cellulases engineered in microbes (vs. separate cellulases to make glucose) ◆◆◆◆ □ Organisms that ferment multiple sugars (fuel flexibility) ◆◆ □ Isolate more novel microbes and screen for H₂ production rates yields, and durability ◆ □ New microbe? Organism? discovery using combinational screening ◆ 	<ul style="list-style-type: none"> □ Genetic tools to manipulate electronflux in H₂ producing organisms ⊛◆◆◆◆◆◆◆◆ □ A single model organism (host) for transgenic expression of H₂ pathways ◆◆◆ □ Faster rDNA techniques for clostridial species ◆ □ Genetic tools for engineering thermophilic (and/or H₂ producing) microbes ◆ 	<ul style="list-style-type: none"> □ Through physiology, biochemistry, and genetics, couple catabolism of glucose to reverse electron flow to hydrogenase (6-8 moles H₂ by "fermentation" 9-10 moles H₂ from additional metabolic energy) ⊛⊛⊛◆◆◆◆◆◆◆◆ □ Discover a method to allow hydrogenase to function with a non-acetate-associated pathway terminal product ◆◆◆◆◆◆◆◆ □ Eliminate redundant genes to construct a robust micro-organism (a "bioengine") ⊛◆◆◆◆◆ □ Measure internal redox couples ◆◆◆◆◆ □ Develop full understanding of mechanisms ⊛◆ □ Construct hybrid microorganism by genome engineering and insert a good cassette for H₂ production into the bioengine ◆◆◆ □ Develop a complete understanding of H₂ transport out of the microbes ◆ □ Metabolic flux measurement ◆ 	<ul style="list-style-type: none"> □ Link fermentation to a second process that makes both economically possible ⊛⊛⊛◆◆◆◆◆◆◆◆ □ Microbial combinations, e.g., fermentation/ photo) ⊛◆◆◆◆◆◆◆◆ □ Optimize combined biological processes using different microbes ◆◆◆◆◆◆◆◆ □ Model any mixed cultures to determine behavior/stability ◆ □ Need to evaluate two stage process for improved economics (H₂ + CH₄ → reformation → H₂) ◆ 	<ul style="list-style-type: none"> □ Genetic engineering/ breeding of energy crops for <ul style="list-style-type: none"> - higher productivity - lower input needs - more stress tolerance - more favorable composition - more easily processed to sugars ⊛⊛◆◆◆◆◆◆◆◆ □ Pretreatments for converting polymers to monomers ◆ 	<ul style="list-style-type: none"> □ Basic studies on reactor designs, process parameters (HRT, SRT, mixing, pH, temperature, concentration), and microbial metabolic pathways ⊛◆◆◆◆◆◆◆◆ □ Engineering advances to reduce H₂ pressure or drive reaction ◆◆ □ New (or highly modified) bioreactors ◆ □ Evaluate and model the process kinetics to evaluate the practical feasibility using enriched culture ◆ □ Get value from bio-waste streams ◆

Breakout Group 2
TABLE 5. SCIENTIFIC AND TECHNICAL BREAKTHROUGHS NEEDED TO OVERCOME BARRIERS TO HYDROGEN FERMENTATION

BASIC SCIENCE	OPERATING PARAMETERS/SYSTEM DESIGN	FEEDSTOCK FOR H ₂ PRODUCTION
<ul style="list-style-type: none"> □ Model metabolic pathway of carbohydrate in “lab-rat” under O₂-limiting conditions to obtain the highest H₂ yield/mole glucose and analyze the consequences on other metabolic/regulation pathways → then create experimental designs ☼☼◆◆◆◆◆◆◆◆◆◆ - Conduct systems analysis of metabolism to predict and design experiments to analyze H₂ production vs. growth ◆ □ Overcome the thermodynamic barrier NAD(P)H → H₂ (+ 4.62 kJ/mole) ☼☼◆◆◆◆◆◆◆◆◆◆ - Reverse electron transport to drive H₂ production past barrier □ Increase knowledge by having DOE sequence genome of best H₂ producing bacterium ◆◆◆◆◆◆◆◆◆◆ - First need to identify the organism! ◆◆◆◆◆◆◆◆◆◆ □ Develop methods to minimize H₂ scavenging/inhibiting methanogens in fermentation ◆◆◆◆◆◆◆◆◆◆ □ Increase rate of H₂ production by deleting excess proteins so there are higher amounts of needed enzymes per cell mass ☼◆◆◆◆◆◆◆◆◆◆ □ Develop method for direct conversion of cellulose/hemicellulose to H₂ without going to depolymerization of feedstock ◆◆◆◆◆◆◆◆◆◆ □ Seek, identify and characterize alternative (extremophilic?) hydrogenases aimed at higher rates of H₂ production ◆ □ Increase H₂ yield by deleting all unnecessary reactions that use hydrogen from glucose ◆ - Acid production - Alcohols □ Make the bacteria edible by animals to dispose of it economically 	<ul style="list-style-type: none"> □ Develop methods for secondary conversion of byproducts to additional H₂ (e.g., organic acids to H₂) ☼☼◆◆◆◆◆◆◆◆◆◆ □ Build plants at 10x scale – including collection of biomass ◆ □ Process and economic model for converting sugars to ethanol and hydrogen – including tradeoffs and sensitivity analyses ◆ □ Development of a light delivery system for photofermentation ◆ □ System designed to harvest and utilize cell mass ◆ □ Is there a way of “tagging” and/or concentrating dead organisms for disposal? ◆ □ High cell density cultivation techniques that facilitate high volumetric throughput of feedstock per unit reactor area, without loss of reaction rate 	<ul style="list-style-type: none"> □ Optimized bioreactor design for high sustained H₂ production by immobilization and other methods ◆◆◆◆◆◆◆◆◆◆ - Optimal reactor design for H₂ removal (to keep H₂ concentration low) □ Manure breakdown: use products as a cheap pre-processing system ☼◆◆◆◆◆◆◆◆◆◆ □ Explore alternative methods for sterilizing or sanitizing reactor/reactants ◆◆◆◆◆◆◆◆◆◆ □ Develop better understanding of process kinetics, mass transfer, heat transfer, reactor design and scale-up issues ☼ □ 2-stage anaerobic fermentation (H₂ + CH₄ reactors) using any organic substrates (i.e., biomass, sludge, wastes) is by far the most economically and technically feasible technology on a full-scale demonstration. ☼ - Natural mixed culture system - No substrate pretreatment needed - CH₄ could be reformed to H₂ - Dead bugs from H₂ reactor digested by CH₄ reactor □ Minimize primary energy needed to support H₂ production process

Breakout Group 1

TABLE 6. ANALYSIS OF TOP-RATED SCIENTIFIC ADVANCES/TECHNICAL BREAKTHROUGHS

R&D NEEDED TO ACHIEVE THE BREAKTHROUGHS AND REDUCE H ₂ PRODUCTION COST	IMPACT ON HYDROGEN COST LOW 1 2 3 4 5 HIGH	TECHNICAL RISK/ RISK OF SUCCESS LOW 1 2 3 4 5 HIGH
1. GENETIC TOOLS TO MANIPULATE ELECTRON FLUX IN HYDROGEN PRODUCING ORGANISMS		
<input type="checkbox"/> Identify a single model (host) organism for transgenic expression of H ₂ pathways	4	2
<input type="checkbox"/> Develop a database (genomic, etc.) for hydrogen producing organisms: to identify genes and/or develop genetic models or model organisms	4	3
<input type="checkbox"/> Develop genome shuffling/combinatorial tools for <u>these</u> type of organisms	3	5
<input type="checkbox"/> Develop a robust transformation system for anaerobic hydrogen producing bacteria	4	4
2. COUPLE CATABOLISM OF GLUCOSE TO REVERSE ELECTRON FLOW TO HYDROGENASE		
<input type="checkbox"/> Study genetics and regulation of hydrogenase expression		
<input type="checkbox"/> Study regulation of electron flux pathway leading to hydrogenase		
<input type="checkbox"/> Develop tools and study metabolic flux (electron flux) in rDNA organisms expressing hydrogenases		
3. LINK FERMENTATION TO A SECOND PROCESS TO MAKE OVERALL PROCESS ECONOMICALLY FEASIBLE		
<input type="checkbox"/> Optimize dark fermentation on diverse feedstocks (wastes; partially heated biomass that produces cellulose and hemicellulose)	5 <i>We only have considered com/glucose here today; other large biomass streams exist.</i>	3 <i>Not challenge; just needs to be done.</i>
<input type="checkbox"/> Call for new innovative processes to add onto dark fermentation that produce additional H ₂ or an energy-value byproduct (electricity, methane, biofuels)	5 <i>Acetate and butyrase are "dead-end" fermentation and no single microbe shown to yet do it all in an oxygen-free dark fermentation environment.</i>	5 <i>We have not identified an optimal second process.</i>
<input type="checkbox"/> Economic evaluation of linking H ₂ (dark fermentation) and methane (CH ₄ reformed to H ₂).	5 <i>Technologies within reach; just not funding to test!</i>	1 <i>Paper study.</i>
4. GENETIC ENGINEERING/BREEDING OF ENERGY CROPS		
<input type="checkbox"/> Complete genomics of switch grass, poplar, others.	5	1
<input type="checkbox"/> Geonomics, proteomics, metabolomics to modify appropriate pathways to improve, productivity, composition, and stress tolerance; decrease input needs; and more easily process sugars.	5	5
<input type="checkbox"/> Harvesting and pre-processing (cost reduction). Examples: single pass harvesting – crop and	5	3

R&D NEEDED TO ACHIEVE THE BREAKTHROUGHS AND REDUCE H ₂ PRODUCTION COST	IMPACT ON HYDROGEN COST LOW 1 2 3 4 5 HIGH	TECHNICAL RISK/ RISK OF SUCCESS LOW 1 2 3 4 5 HIGH
residue; field processing for compacting; wet/silage/field or farm processing for high density transport; on-farm enzymatic pretreatment.		
<input type="checkbox"/> Processing plant processing, e.g., steam explosion, acid hydrolysis, and enzymatic.	4	3
5. FUNDAMENTAL STUDY OF ENZYMATIC CONVERSION TO SIMPLIFY PROCESS		
<input type="checkbox"/> Combinatorial screenings of enzymes	5 <i>Target should be doubling efficiency at half the cost</i>	5
<input type="checkbox"/> Kinetic study of enzyme activity	3 <i>Increases understanding used to engineer systems</i>	1
<input type="checkbox"/> Develop synthetic analogs of enzymes	4 <i>Cuts cost of enzymes significantly</i>	4 <i>Past experiences have difficulty replicating properties</i>

Breakout Group 2

TABLE 7. ANALYSIS OF TOP-RATED SCIENTIFIC ADVANCES/TECHNICAL BREAKTHROUGHS

R&D NEEDED TO ACHIEVE THE BREAKTHROUGHS AND REDUCE H ₂ PRODUCTION COST	IMPACT ON HYDROGEN COST LOW 1 2 3 4 5 HIGH	TECHNICAL RISK/ RISK OF SUCCESS LOW 1 2 3 4 5 HIGH
1. MODEL METABOLIC PATHWAY OF CARBOHYDRATE		
<input type="checkbox"/> Use E. coli metabolic models as a starting point and identify genes unique to model H ₂ organism; try plugging in unique gene functions to E. coli model to predict H ₂ output, then test experimentally. Goal: Minimize growth, maximize H ₂ production	5 <i>If successful, this research will maximize H₂ production over growth or other alternative side reactions/pathways.</i>	2 <i>Approaches/models are available but not well-developed; having a model will help determine whether goal can be realized.</i>
<input type="checkbox"/> Identify cellular processes that are required for H ₂ production under non-growing conditions	4 <i>Could identify genes/ processes that will be required to maximize H₂ production over growth.</i>	2 <i>Feasible to identify/quantify "senescence" genes (methods available) and identify their functions.</i>
2. SECONDARY CONVERSION OF BYPRODUCTS TO PRODUCE MORE HYDROGEN		
<input type="checkbox"/> Research on converting organic acids to additional hydrogen (e.g., photofermentation light delivery system; two-stage system integration; genetics)	5 <i>Secondary H₂ production is significant (~8-10 moles of H₂ can be produced).</i>	1 <i>Well demonstrated concept with only challenge of integration.</i>
<input type="checkbox"/> Biomass → Cellulose → Glucose → Fermentation H ₂ (Primary) <input type="checkbox"/> Hemicellulose → Xylose → Fermentation H ₂ (Secondary) <input type="checkbox"/> Lignin → Gasification → Syngas → Thermochemical H ₂ (Tertiary)	4 <i>Complete conversion of feedstock to H₂.</i>	3 <i>Xylose conversion to H₂ is moderately understood but lignin pathway will require significant R&D.</i>
<input type="checkbox"/> Organic Acids → H ₂ through direct biological conversion using acidophiles	3 <i>Additional yield of H₂.</i>	4 <i>Less understood pathway. Yield addition is unknown.</i>
3. OVERCOME THERMODYNAMIC BARRIER		
<input type="checkbox"/> Metabolic (pathway) engineering to reverse electron flow coupling additional energy to overcome thermodynamic barrier.	5 <i>Directly affects yields.</i>	3 <i>Pathways unknown.</i>
<input type="checkbox"/> Identify and transfer appropriate genes for improved hydrogen production - Clostridial hydrogenase, etc.	4 <i>Directly affects yields.</i>	3 <i>Heterologous expression.</i>
<input type="checkbox"/> Delete unnecessary genes and draining reactions.	4	3

R&D NEEDED TO ACHIEVE THE BREAKTHROUGHS AND REDUCE H ₂ PRODUCTION COST	IMPACT ON HYDROGEN COST LOW 1 2 3 4 5 HIGH	TECHNICAL RISK/ RISK OF SUCCESS LOW 1 2 3 4 5 HIGH
4. OPTIMIZED BIOREACTOR DESIGN		
<input type="checkbox"/> Develop technology combining dark fermentation plus photofermentation.	<p style="text-align: center;">4</p> <i>Combined method will have high H₂ yield.</i>	<p style="text-align: center;">4</p> <i>Synchronizing the two reactions; avoiding the need for artificial light; new design for photoreactor</i>
<input type="checkbox"/> Develop membrane technology to lower H ₂ concentration in the reactor.	<p style="text-align: center;">5</p> <i>Avoid high H₂ concentrations; solves separation issues (save money on PSA); improves kinetics; prevents H₂ from being contaminated</i>	<p style="text-align: center;">3</p> <i>Membrane compatibility with active, aqueous bio system; manufacturability and stability; cost</i>
<input type="checkbox"/> Develop reactor design design to facilitate cell harvest	<p style="text-align: center;">2</p> <i>All mass can be used as nutrient; additional revenue stream</i>	<p style="text-align: center;">2</p> <i>Compatibility with selected cells; maintaining live cells and removing dead cells</i>

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Appendix B

DOE Workshop on Hydrogen Production via Direct Fermentation

June 9, 2004 ■■■ AGENDA ■■■ Baltimore, MD

Time	Activity
7:30-8:00 am	Registration and Continental Breakfast
8:00 am	Welcome and Opening Remarks , <i>Roxanne Danz, U.S. DOE/OHFCIT</i>
8:15 am	Review Workshop Agenda and Participant Introductions , <i>Shawna McQueen, Energetics Inc.</i>
8:45 am	Overview of U.S. Biomass Feedstocks for Fermentative Hydrogen Production , <i>Kelly Ibsen, National Renewable Energy Laboratory</i>
9:15 am	Overview of Fermentation Technology , <i>Tim Eggeman, Neoterics International</i>
9:30 am	BREAK
9:45 am	Overview of Neoterics/NREL Report “Boundary Analysis for Hydrogen Production by Fermentation,” <i>Tim Eggeman, Neoterics International</i>
10:15 am	Group Discussion of Assumptions and Findings of Neoterics/NREL Report , <i>Shawna McQueen, Energetics Incorporated (Session Moderator)</i> Questions to be considered include: <ul style="list-style-type: none"> • Are there other compelling options for direct fermentation that the study did not consider? • Are these the key cost factors (fermentation yield, cost of glucose, fixed capital, glucose concentration in the fermentation, etc.)? • What are some ways that these cost factors could be addressed? • How difficult will it be to meet fermentation yields of 10 moles of hydrogen per mole of glucose with reasonable costs for capital and feedstock?
11:30 am	LUNCH
1:00 pm	Convene in Parallel Breakout Groups: “Challenges and Opportunities to Hydrogen Production via Direct Fermentation” <i>Shawna McQueen and Ross Brindle, Energetics Incorporated (Session Facilitators)</i> <i>Focus Question #1: What are the key technical barriers or grand challenges to cost-effective fermentative hydrogen production?</i> <ul style="list-style-type: none"> - Brainstorm, Analyze, and Prioritize
2:15 pm	BREAK
2:30 pm	<i>Focus Question #2: What technology advances or scientific breakthroughs are needed to overcome these barriers and achieve the goals for fermentation yield and hydrogen cost?</i> <ul style="list-style-type: none"> - Brainstorm by Fermentation Pathway, Analyze, and Prioritize
3:45 pm	<i>Focus Question #3: Top 5 Analysis (in caucus groups)</i> <ul style="list-style-type: none"> - Divide into 5 caucus groups and provide additional detail on the “Top 5,” including R&D needed to achieve the breakthroughs and impact on yield and cost goals.
4:15 pm	<i>Prepare for Summary Session:</i> breakout groups elect a speaker to present results at closing summary session and assist with preparing highlights for presentation.
4:30 pm	Plenary Summary Session: Reports from Breakout Groups 1 and 2
4:50 pm	Closing Comments and Next Steps , <i>Roxanne Danz, U.S. DOE, OHFCIT</i>
5:00 pm	ADJOURN

Appendix C Breakout Group Participants

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