BioHydrogen (BioH2) Consortium to Advance Fermentative H_2 Production

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National Renewable Energy Laboratory

DOE Hydrogen and Fuel Cells Program
2020 Annual Merit Review and Peer Evaluation Meeting

Project ID: P179

Overview

Timeline and Budget

- Project start date: 10/1/2018
- FY19 DOE Funding: $1.2M

	FY19	FY20 to-date
NREL	$600K	$300K
LBNL	$200K	$100K
PNNL	$200K	$100K
ANL	$200K	$125K
Total	**$1.2M**	**$625K**

Barriers

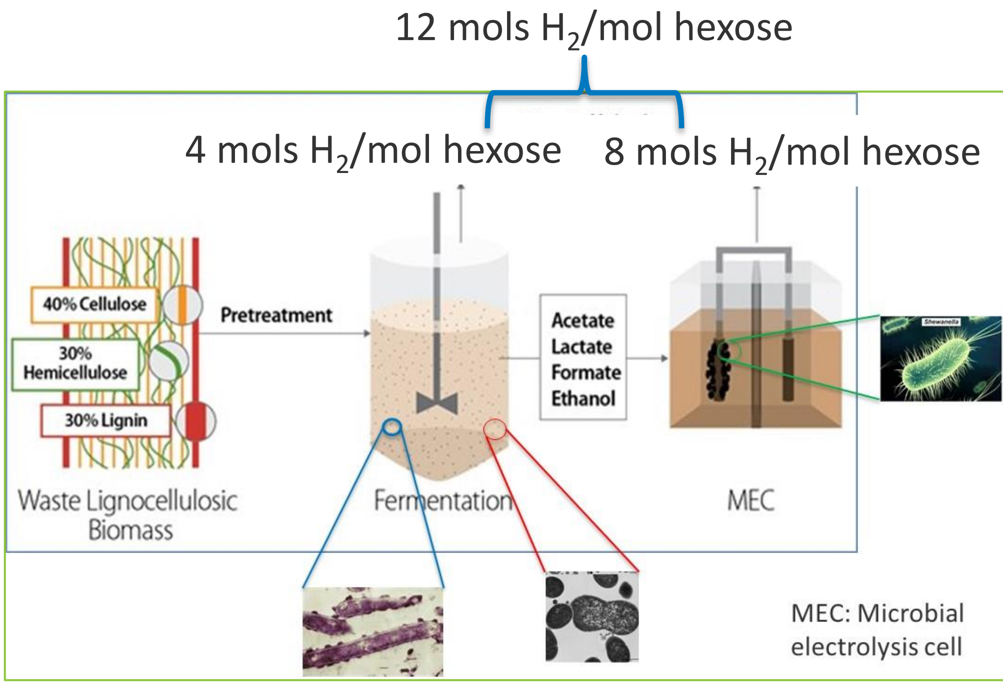
- H_2 molar yield (AX)
- Feedstock cost (AY)
- System engineering (AZ)

Consortium Partners

- Dr. Steven Singer, Lawrence Berkeley National Lab (LBNL)
- Dr. Alex Beliaev, Pacific Northwest National Lab (PNNL)
- Dr. Amgad Algowainy, Argonne National Lab (ANL)

Relevance

Overall Objective: Develop a high-solid loading microbial fermentation technology to convert renewable lignocellulosic biomass resources into H_2 and integrate microbial electrolysis cell (MEC) to meet DOE H_2 production cost goal.



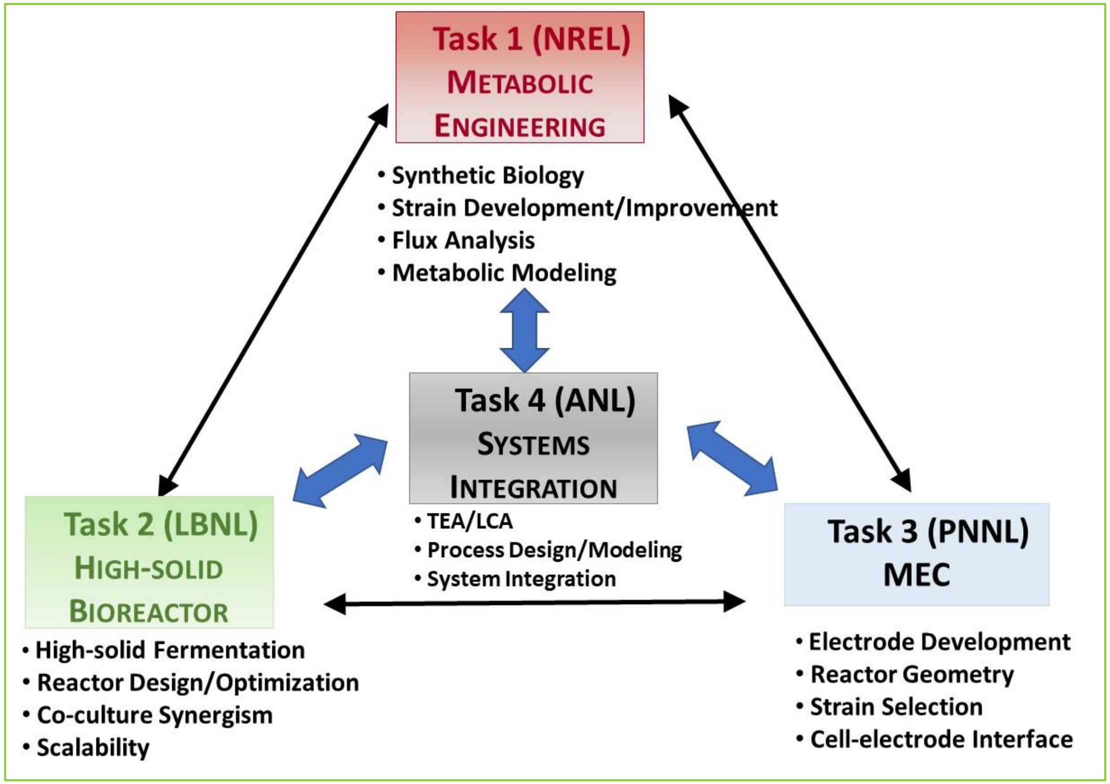
MEC: Microbial electrolysis cell

Current Project Year Objectives (October 2018 – March 2020)

- **Task 1. Strain Development and Improvement (NREL Lead)**
  - Lowering feedstock cost and improving hemicellulose (5-carbon sugar) to H_2 production via genetic engineering of *Clostridium thermocellum*. The strain can naturally break down and use cellulose.
- **Task 2. High-solids Bioreactor Development (LBNL Lead)**
  - Optimize bioreactor fermentations for *C. thermocellum* to improve mixing characteristics, substrate utilization, and H_2 production rates and yields under high-solids loading conditions.
- **Task 3. Microbial Electrolysis Cell (PNNL Lead)**
  - Engineer exoelectrogenic microbes (e.g., *Geobacter* spp., *Shewanella* spp.) that can efficiently couple oxidation of fermentation by-products for increased H_2 production and yield.
- **Task 4. System Integration, Techno-economic Analysis, and Life Cycle Analysis (ANL Lead)**
  - Design a conceptual, large-scale system to integrate the dark fermentation (DF) and MEC for bioH2 production. Model the overall system with Aspen Plus.

Relevance – BioH2 Consortium Synergy

Rationale: We assembled a highly productive and collaborative team of scientists from **four** National Labs whose research accomplishments and expertise lay down a strong foundation in addressing knowledge gaps and technical barriers for long-term success toward meeting the FCTO H_2 production cost goal.



Task 1 (NREL) METABOLIC ENGINEERING

- Synthetic Biology
- Strain Development/Improvement
- Flux Analysis
- Metabolic Modeling

Task 4 (ANL) SYSTEMS INTEGRATION

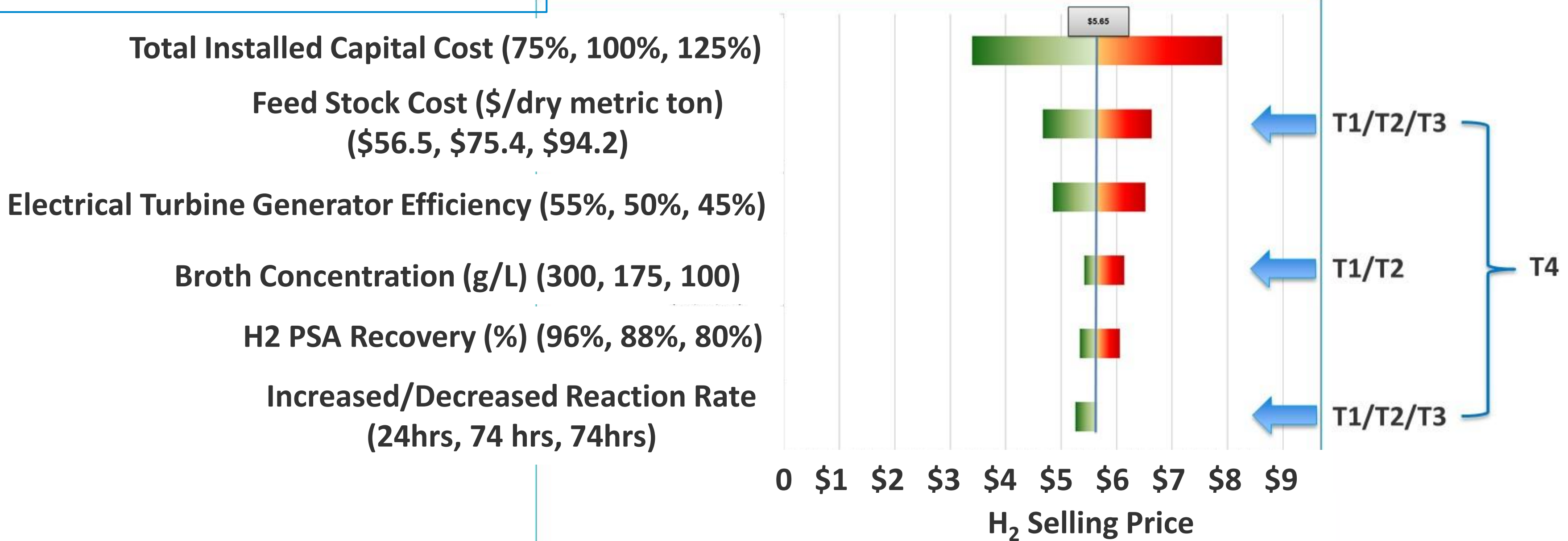
- TEA/LCA
- Process Design/Modeling
- System Integration

Task 2 (LBNL) HIGH-SOLID BIOREACTOR

- High-solid Fermentation
- Reactor Design/Optimization
- Co-culture Synergism
- Scalability

Task 3 (PNNL) MEC

- Electrode Development
- Reactor Geometry
- Strain Selection
- Cell-electrode Interface

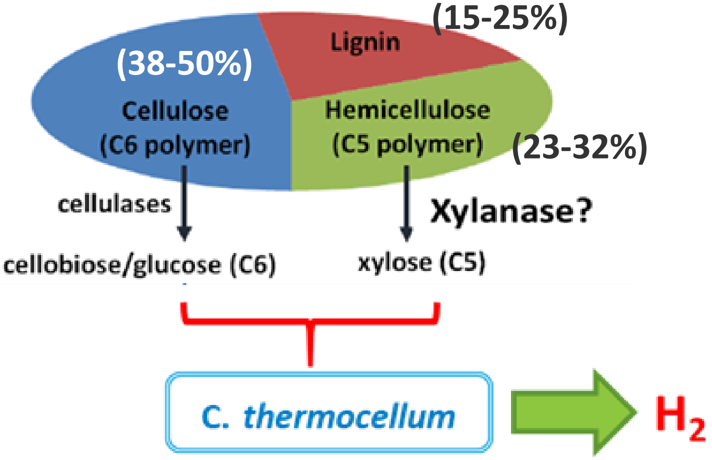


Approach
Task 1: Strain Development and Improvement (NREL)

Approach: Via targeted engineering and adaptive laboratory evolution, we aim to improve hemicellulose (five-carbon xylose polymer) utilization. Cellulose-hemicellulose co-utilization will lower the cost of biomass feedstock.

- *C. thermocellum* naturally can degrade cellulose. We have engineered it to also co-utilize xylose (*xylAB* strain), which doubled the output of H_2 when both substrates are present (2017 AMR Results). The engineered strain was further evolved in xylose for improved growth on hemicellulose and improved H_2 production rate (2018-2019 AMR results)
- Yet <u>hemicellulose</u> hydrolysis is still a rate-limiting step, and overcoming it is an FY19-20 goal.

<u>Engineer Cellulose-Degrading Microbe to Co-metabolize C5 Sugars</u>



C. thermocellum utilizes cellulose (C6), but not hemicellulose (C5 sugars)

1926 – 2016

NREL genetically modified strain to enable C5 (xylose) co-utilization

2017 – 2018

2018 - 2019

NREL evolved strains for improved growth and H_2 production rate on **hemicellulose**

2019 – 2020

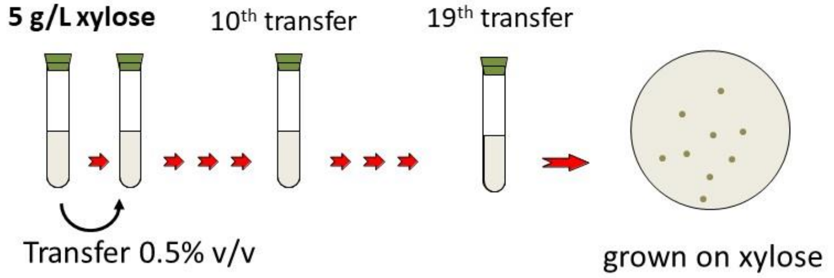
Identified key rate-limiting step

Further optimization for C5/C6 utilization

Ferment all the sugars to H_2 in <u>one</u> bioreactor: lowering both feedstock and reactor cost.

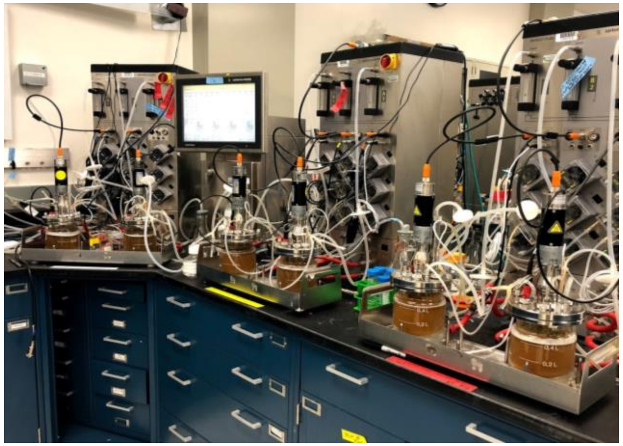
Task 1. Accomplishments: 24% increase in H_2 production from current baseline via better hemicellulose utilization (NREL)

FY19 Q4 Go-No-Go	Increase H_2 production by 20% in bioreactors from the current baseline of 2.5 L/L/d to 3 L/L/d via improving xylan utilization, either with an engineered *C. thermocellum* strain or with a co-culture in a consolidated bioprocessing configuration	9/2019	**Complete**



Start with an engineered strain then evolve in lab

test for growth on hemicellulose



Test on pretreated biomass

	24 hr H_2 production rate (L/L/hr)
Δhpt	**2.21 ± 0.54**
19-9	**2.75 ± 0.27**

24% Higher

- Generated a H_2 production baseline using *C. thermocellum Δhpt* (wild-type, derived from DSM 1313) and compared it with the **19-9** strain (engineered and evolved for better C5 sugar utilization).
- Previous baseline (2.5 L/L/d) used a different strain (27405 vs. DSM1313). Only DSM 1313 can be genetically modified.
- We fed *C. thermocellum Δhpt* and 19-9 strain 38 g/L pretreated biomass (as cellulose) and measured H_2 and CO_2 online via a mass spec.

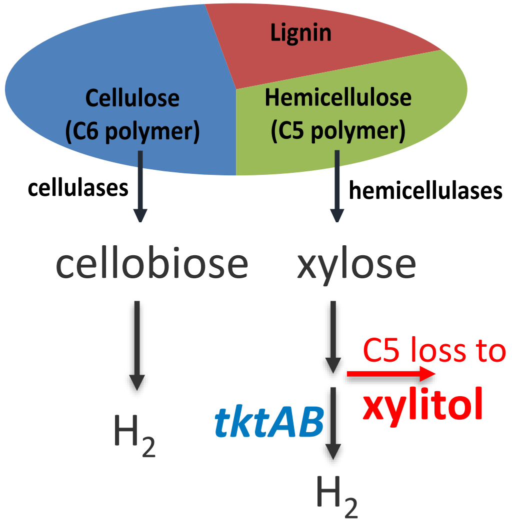
Strain 19-9 improved H_2 production rate by 24% in 24 h, over a baseline of 2.2 L/L/d.

Task 1. Accomplishments: (1) Better Utilization of C5 is feasible with C5 loss identified; (2) Improve growth by 3.8-fold via overcoming a rate-limiting step (NREL)

FY20 Q1 QPM	Determining if xylitol is produced and could be accounted for in the pentose sugar recovery for improved carbon balance (NREL)	12/2019	**Complete**

initial cellobiose (g/L)	0
initial xylose (g/L)	15
xylitol produced (g/L)	**3.1 ± 0.09**
xylose consumed (g/L)	**9.4 ± 0.85**
molar yield of xylitol/xylose	**0.30 ± 0.039**

- Xylitol is secreted during 15 g/L xylose-feeding, which accounts for a 30% loss of C5 sugar.
- Blocking xylitol production will redirect carbon and electron toward increased H_2 production.



Lignin

Cellulose (C6 polymer)

Hemicellulose (C5 polymer)

cellulases

hemicellulases

cellobiose

xylose

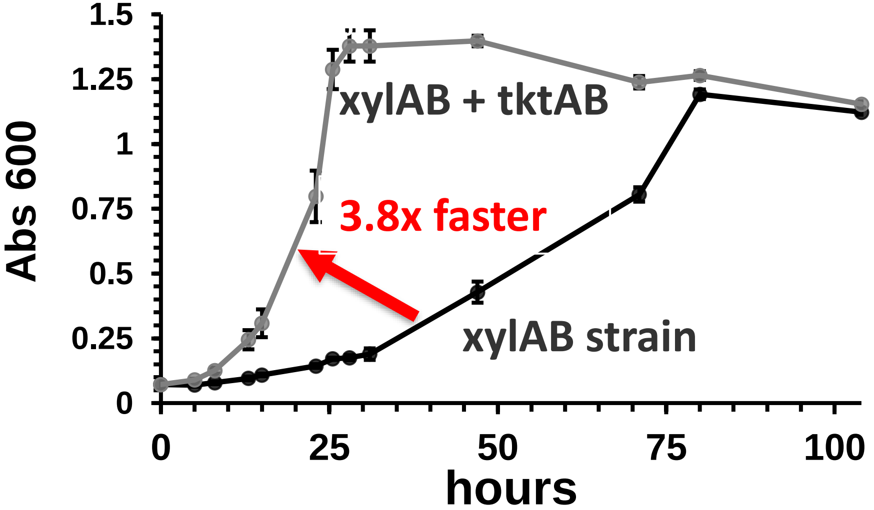
C5 loss to **xylitol**

H_2

tktAB

H_2

Growth on xylose



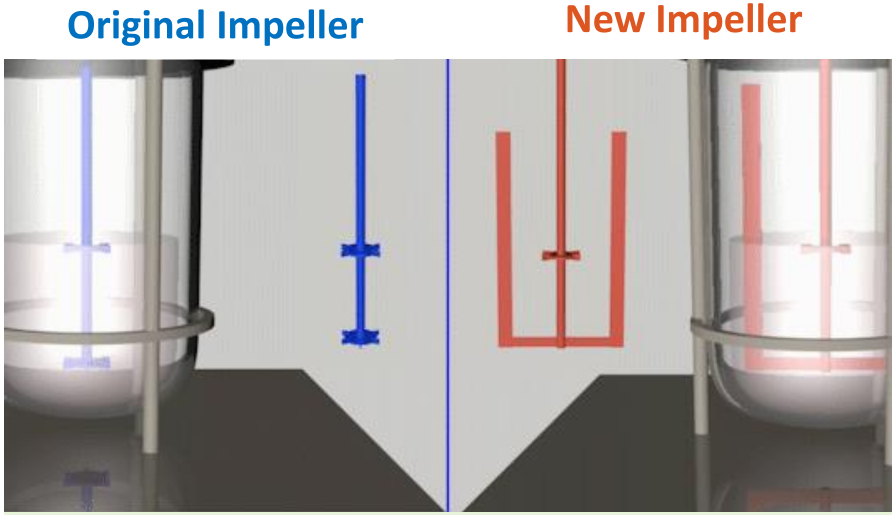
- Identified transketolase (*tktAB* genes) as rate-limiting in hemicellulose utilization via global gene expression profiles.

Over expression of *tktAB* genes indeed improves growth on xylose by 3.8-fold

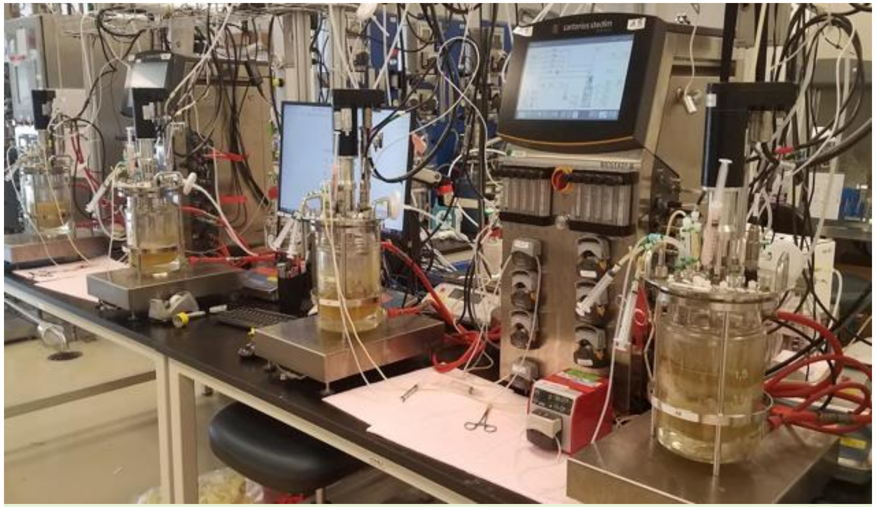
Approach
Task 2: High-solid Bioreactor Development (LBNL)

Approach: Leverage new impeller designs to optimize substrate availability, inorganic carbon supply, and gas removal in bioreactor fermentations for enhanced H_2 production at high solids loading

- Optimize bioreactor parameters for *C. thermocellum,* both wild type and engineered strains, under high solids conditions (targeting 175 g/L biomass)
- Evaluate new impeller designs to improve mixing and enhance gas-liquid mass transfer for high-viscosity fermentations



Anchor-style impeller: Eliminates dead zones at the bottom and sides of the bioreactor during high-viscosity mixing



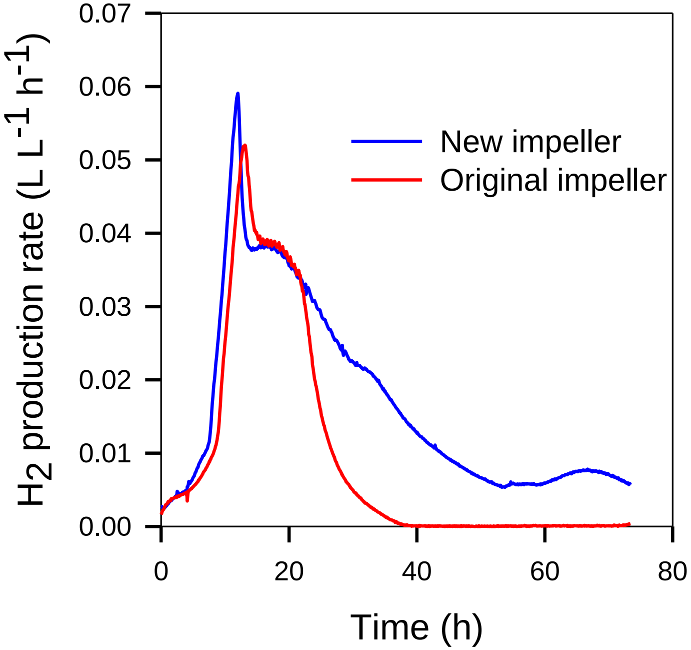
ABPDU fermentation suite: 4 x 2L bioreactors, process mass spectrometer

Task 2. Accomplishments and Progress: Evaluated gas removal strategies and new impeller designs at 45 g/L Solid Loading (LBNL)

FY20 Milestones	Q1: Evaluate productivity with Avicel at or above 45 g/L to identify factors that limit hydrogen production Q2: Evaluate new impeller designs for high solids mixing	12/2019 3/2020	**Complete**

Key results

- Documented significant H_2 productivity boost with CO_2 supplementation, up to 2.84 L L^{-1} d^{-1}
- Fabricated and deployed anchor-style impellers for improved high-viscosity mixing
- New impellers result in improved performance with 45 g/L Avicel



Preliminary data with new impellers reveals 82% increase in H_2 production output at 45 g/L Avicel

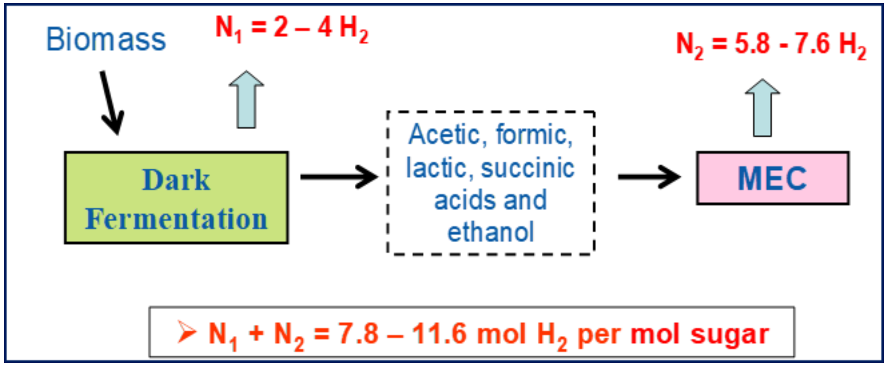
Continuous N_2 and CO_2 sparging increases H_2 production by 74% compared to bicarbonate alone

Gas supply condition	Hydrogen production rate (L L^{-1} d^{-1})	Maximum H_2 production rate (L L^{-1} h^{-1})
N_2 sparging / CO_2 sparging	**2.84**	**0.058**
N_2 sparging / bolus $NaHCO_3$	1.63	0.033
N_2 overlay / CO_2 sparging	1.59	0.032
N_2 overlay / bolus $NaHCO_3$	1.08	0.019

Approach
Task 3: Microbial Electrolysis Cell (PNNL)

Approach: Design MEC–driven process integrated with biomass fermentation (Tasks 1 & 2) for improved conversion of the fermentation effluent to H_2 using defined exoelectrogenic microbes and consortia

- Identify and characterize genetically tractable exoelectrogenic strains with complementary metabolic capacities and high extracellular electron transfer (EET) rates to increase current/H_2 production in MEC through genome engineering. Previous approach employed complex MEC consortia of undefined species, which are challenging to optimize (i.e., genetically engineer)
- Rationally design defined microbial consortia capable of using different fermentation by-products (e.g., organic acids, alcohols, proteins) to increase the effluent utilization and Coulombic (C_E) efficiencies. Previous approach was mostly geared towards acetate conversion
- Apply accelerated strain evolution and process optimization to reduce inhibitory effects of effluent components (lignin, humic acids) on current/H_2 production


Biomass

$N_1 = 2 – 4\ H_2$

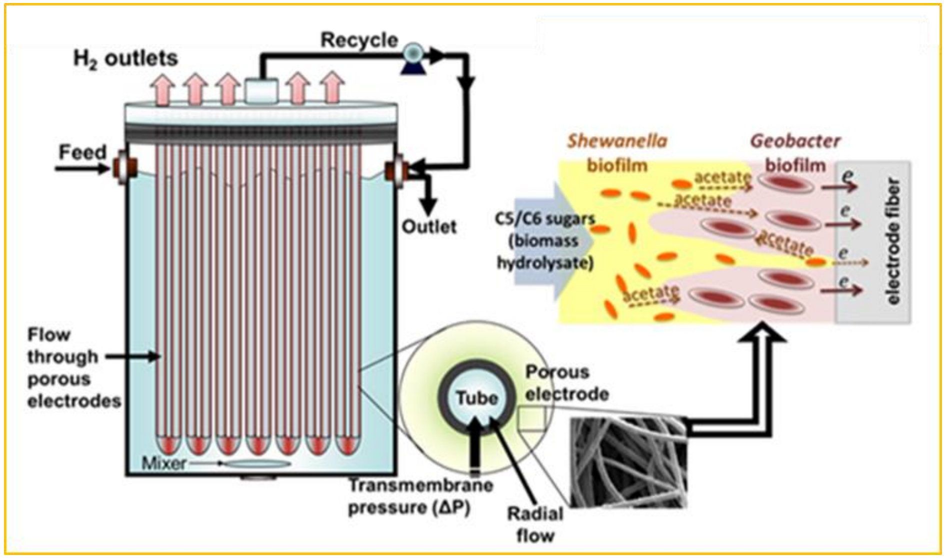
$N_2 = 5.8 - 7.6\ H_2$

Dark Fermentation

Acetic, formic, lactic, succinic acids and ethanol

MEC

➢ $N_1 + N_2 = 7.8 – 11.6$ mol H_2 per mol sugar



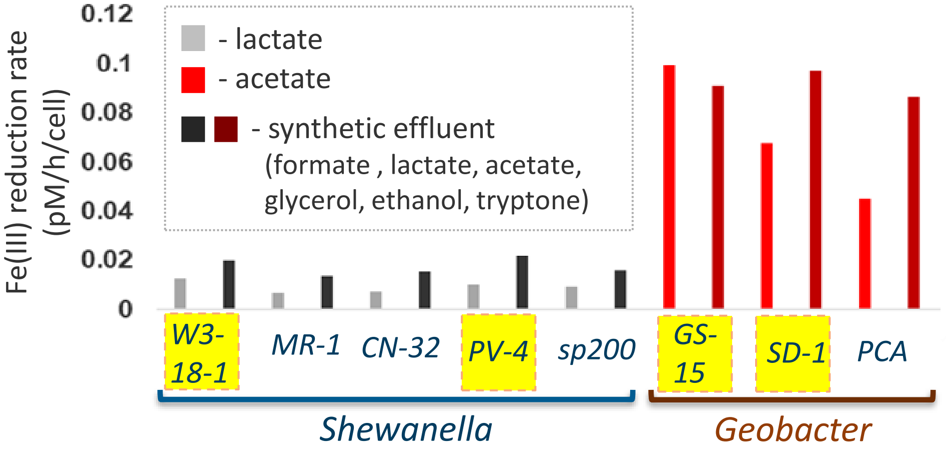
Task 3 Accomplishments and Progress: Identified Four Promising MEC Microbes (PNNL)

FY20 Q1 Milestone	Characterized the effluent conversion capacity of each exoelectrogenic strain as it relates to current production potential in MFC and identified co-culture pairing	12/2019	**Complete**

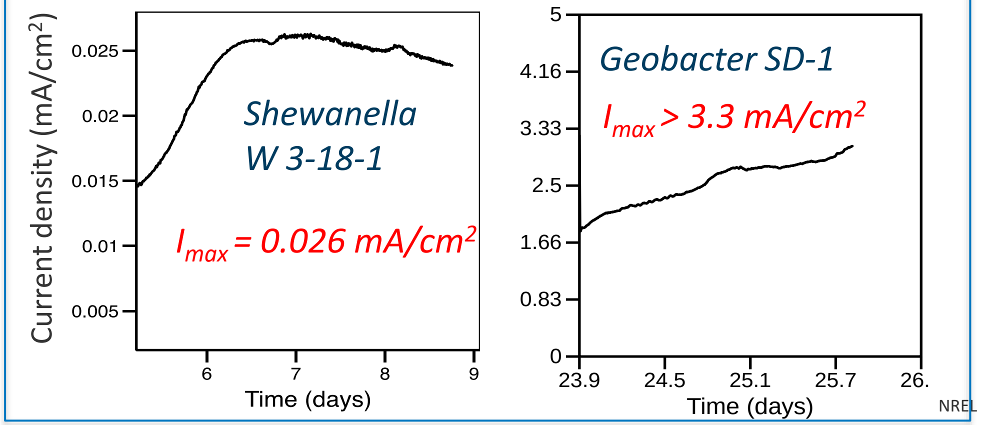
Key results

- Characterized and selected 4 most promising exo-electrogenic strains (*Shewanella* PV-4 and W3-18-1, *Geobacter* SD-1 and GS-15)
- Quantified rates of fermentation by-products consumption and current production on simulated effluent
- Tests on Avicel and DMR effluent showed consumption of key fermentation by-product but also indicated that current production is inhibited in the latter (likely by aromatics, protein)
- *Shewanella* W3-18-1 and *Geobacter* SD-1 co-culture was selected for development of MEC-driven process

Fe(III) reduction assay as a proxy for current production



Current production by synthetic effluent

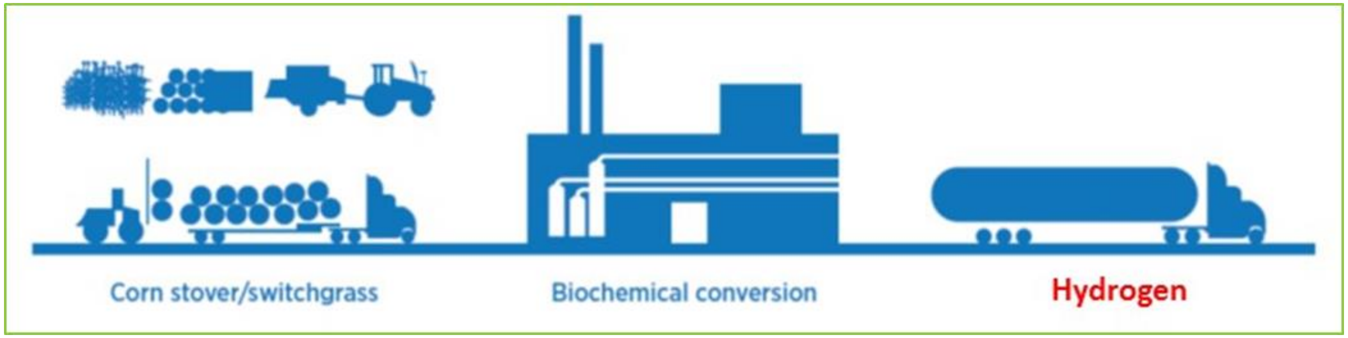


Approach

Task 4: System Integration, Techno-economic Analysis and Life Cycle Analysis (ANL)

Approach: Use TEA/LCA to set research targets and guide research directions by addressing system engineering challenges to cost effectively implement fermentation with MEC in an integrated system.

- Capital cost of components
- Feedstock and material costs
- H_2 collection and onsite compression/storage needs
- Incorporate design and operation parameters into TEA model, conduct sensitivity analyses to above parameters
- Develop LCA model for production process, mass and energy balance to calculate energy use and emission associated with H_2 production and all process input (feedstock, materials, electricity, process heat, etc.)
- TEA/LCA set research targets and guide future research directions



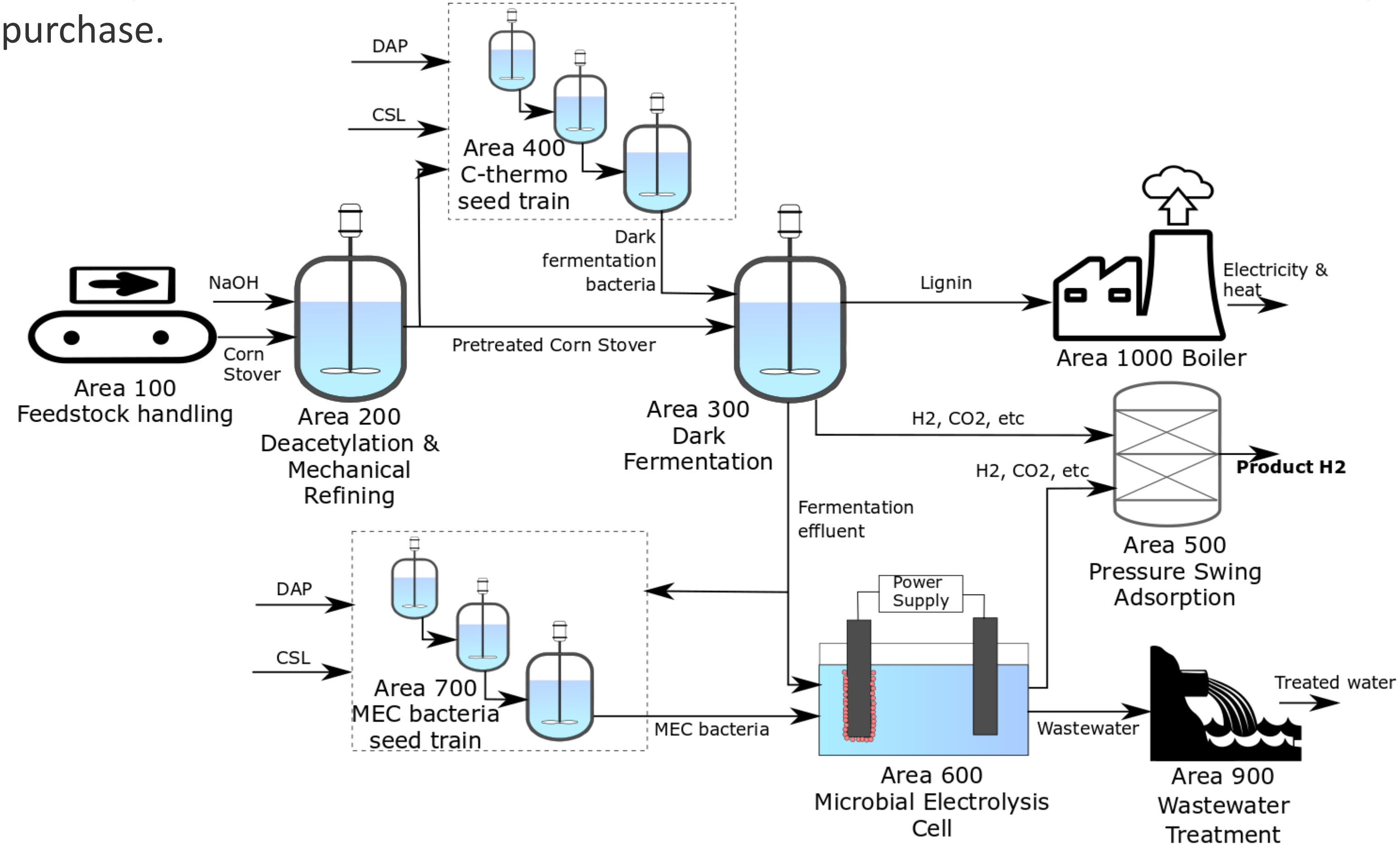
Task 4 Accomplishments: Develop TEA Framework (ANL)

FY20 Q2 Milestone	Update H2A model with refined cost estimates for the dark-fermentation (DF) process.	3/2020	**Complete**

- The process modeling using Aspen Plus was completed to simulate the integrated DF-MEC system, for a conceptual, large-scale bio-H_2 facility with the capacity of 50,000 kg/day. Given the absence of MEC unit in Aspen model, it was modeled using an equilibrium reactor.
- The process model was based on up-to-date laboratory test results from collaborators (NREL, LBNL, PNNL), as well as assumptions derived from previous studies.
- The process modeling results (e.g., flow rate of various streams) were incorporated in the H2A model framework to size all equipment and calculate capital cost.
- The MEC process is identified as a major cost driver for the overall system, while upscaling its design is challenging, due to lack of scaled design and cost information. We estimated design scale up information based on the fundamentals of MEC and chemical engineering practices.
- The developed methodology for MEC scale up consists of sizing four elements individually: reactor tank volume, cathode surface area, anode surface area, anode bacterial loading.
- For the MEC scale up design, we adopted the stack design of proton exchange membrane (PEM) water electrolyzer. The cost of various electrode materials and tank materials have been collected based on available market prices.

Task 4 Accomplishments: Mass and Energy Flows for TEA Framework (ANL)

- MEC is studied and designed in great details, to ensure process feasibility.
- Wastewater treatment plant (WWTP) cost is reduced largely by eliminating anaerobic digester (AD), as MEC carries out a function of wastewater treatment while producing high value H_2, instead of low value CH_4 from AD.
- The current design uses intermediate streams to culture bacteria for DF and MEC, reducing cost for material purchase.



Accomplishments and Progress: Responses to Previous Year Reviewers' Comments

None - a new start in FY19. A poster was presented in 2019 AMR but not reviewed

Collaboration and Coordination

- **Task 1 (Strain Development and Improvement)**

  Drs. Ali Mohagheghi and Melvin Tucker, National Bioenergy Center at NREL: provide DMR pretreated corn stover and their characterizations - leveraging DOE BETO funding.

- **Task 2 (High-solids Bioreactor Development)**

  Drs. Eric Sundstrom and Steve Singer (LBNL) will develop and optimize bioreactor for high solid loadings (increased broth concentration) to reduce CapEX and OpEx challenges.

- **Task 3 (Microbial Electrolysis Cell)**

  Dr. Alex Beliaev will optimize MEC to increase rate and yield of H_2 – addressing H_2 molar yield barrier.

- **Task 4 (System Integration, TEA and LCA)**

  Dr. Amgad Elgowainy will use TEA/LCA to set research targets and guide research directions, working closely with all the tasks.

Remaining Challenges and Barriers

Task 1 Strain Development and Improvement (NREL)

- Need to improve bacteria's ability to utilize hemicellulose both in the presence and absence of cellulose.
  - Test, identify, and express various xylan degrading enzymes to hydrolyze xylan or xylo-oligomers for improved substrate utilization and H_2 production

Task 2. High-solid Bioreactor Development (LBNL)

- High solid-substrate loading (175 g/L) is needed to lower H_2 selling price.
  - Continue to assess impeller designs to improve high viscosity mixing

Task 3. Microbial Electrolysis Cell (PNNL)

- Components in the actual fermentation effluent inhibit electrochemical activity indicating a need for pre-treatment to improve current production

Task 4. System Integration, TEA and LCA (ANL)

- The selections of equipment and the calculation of power demand in H2A model must be practical. The translation of lab results to large-scale design needs to have solid foundation and rationale.
  - The process modeling and TEA of MEC is challenging, given the absence of large-scale information to guide scale up design.

Proposed Future Work

Any proposed future work is subject to change based on funding levels

Task 1 (NREL)

- Construct and express the various *Tsac* xylanases genes (*xylA, B, C*) in *C. thermocellum* (FY20/21); use adaptive evolution to improve xylan utilization leading to increased H_2 (FY20).
- Identify other rate-limiting mechanisms to degrade hemicellulose (FY20/21).

Task 2 (LBNL)

- Evaluate higher solids loading with new impellers, including fed-batch configurations (FY20/21)
- Optimize strategies to improve H_2 removal in high-viscosity conditions and reduce feedback inhibition (FY20/21).

Task 3 (PNNL)

- Evaluate different approaches to reduce or eliminate inhibitory effects on current production using fermentation effluent components (i.e., lignin, proteins) from real biomass (i.e., DMR biomass) in FY20/21.

Task 4 (ANL):

- Continue to evaluate and incorporate inputs from project team, and update the Aspen process, H2A and GREET models based on progress from experimental work (FY20/21).

Technology Transfer Activities

Technology-to-market or technology transfer plan or strategy

- Visolis is interested in using the bio-based H_2 for fuel/product upgrade generated from their proprietary processes.

Plans for future funding

- Pursue opportunities to collaborate with industry for potential future funding support.
- Network with biofuels industry to expand the use of H_2.
- Advocate the advantages of "green" H_2 rather than fossil-fuel derived H_2.

Patents, licensing

- A patent application is accepted by USPTO on a genetic device developed by NREL team to enable "tunable gene regulatory control in thermophilic bacteria."
- A Record of Invention (ROI-14-70) is filed for developing the proprietary genetic tools tailored for *C. thermocellum*.
- A second ROI-15-42 has been filed for generating xylose-metabolizing strain, leading to enhanced biomass utilization.

Summary

Task 1 (NREL)

- **Meeting Go/No-Go Milestone:** Increased H_2 production rate by 24% over the current baseline in bioreactor loaded with 38 g/L real biomass (as cellulose) via better hemicellulose utilization
- Identified an enzymatic rate-limiting step and improved bacterial growth on xylose by 3.8-fold by overcoming the limitation.
- Identified loss of carbon to xylitol which will guide future engineering of C. thermocellum for improved hemicellulose utilization.

Task 2 (LBNL)

- Improved H_2 production rate by 74% via sparging bioreactor with both N_2 and CO_2 gases.
- Evaluated new impeller design to improve high-solids loading, prolonged H2 production leading to 82% increase in total H2 molar output.

Task 3 (PNNL)

- Based on electrochemical activity measurements and complementing metabolic capacities, one *Shewanella* (W3-18-1) and one *Geobacter* (SD-1) species was selected for development of a defined-species co-culture, MEC-driven process

Task 4 (ANL)

- Completed the process modeling using Aspen Plus.
- Incorporated Aspen process model results in H2A model framework for equipment sizing and cost estimation.
- Developed a methodology to scale up MEC design and collected cost information for various electrode materials.

Thank You

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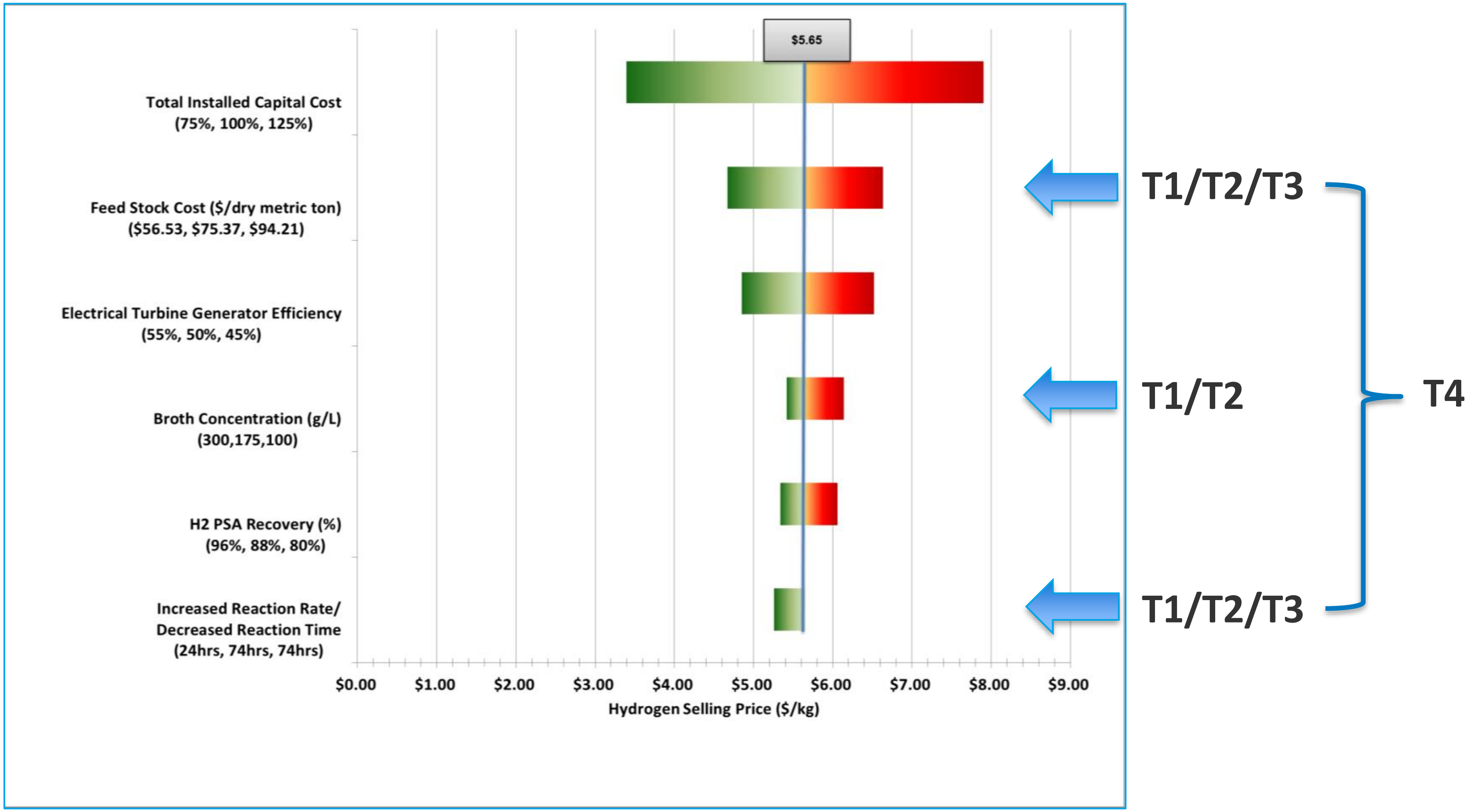
Publication Number

Technical Back-Up Slides

(Include this "divider" slide if you are including back-up technical slides [maximum of five]. These back-up technical slides will be available for your presentation and will be included in Web PDF files released to the public.)

Relevance: Research Directions are guided by a Cost Analysis from Strategic Analysis, Inc.

Tornado chart showing parameter sensitivities for the future central fermentation case (2025 goal), which guides research direction.



Case Study	Low Value ($/kg H_2)	Baseline ($/kg H_2)	High Value ($/kg H_2)
Current Case (2014)	$48.49	**$58.53**	$68.57
Future Case(2025)	$3.39	**$5.65**	$7.90