BioHydrogen (BioH2) Consortium to Advance Fermentative H_2 Production

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P179

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Overview

Timeline and Budget

- Project start date: 10/1/2018
- FY19 DOE Funding: $1.2M
- Total DOE funds received to date: $952K
  - NREL: $365K
  - PNNL: $200K
  - LBNL: $200K
  - ANL: $187K

Barriers

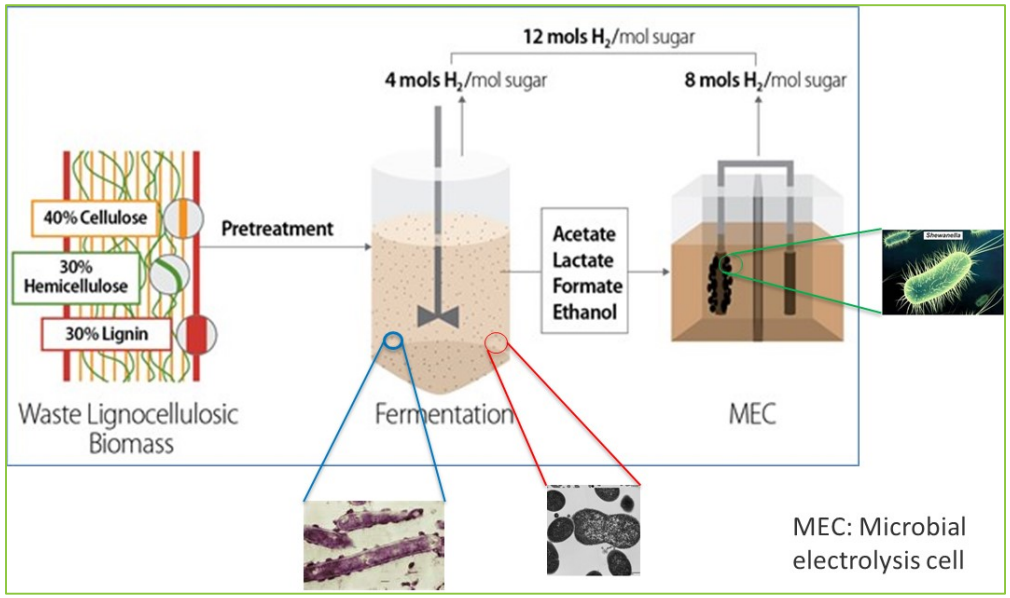
- H_2 molar yield (AX)
- Feedstock cost (AY)
- System engineering (AZ)

Consortium Partners

- Dr. Alex Beliaev, Pacific Northwest National Lab (PNNL)
- Drs. Eric Sundstrom and Steven Singer, Lawrence Berkeley National Lab (LBNL)
- 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 innovatively integrate it with microbial electrolysis cell (MEC) to meet DOE H_2 production cost goal.

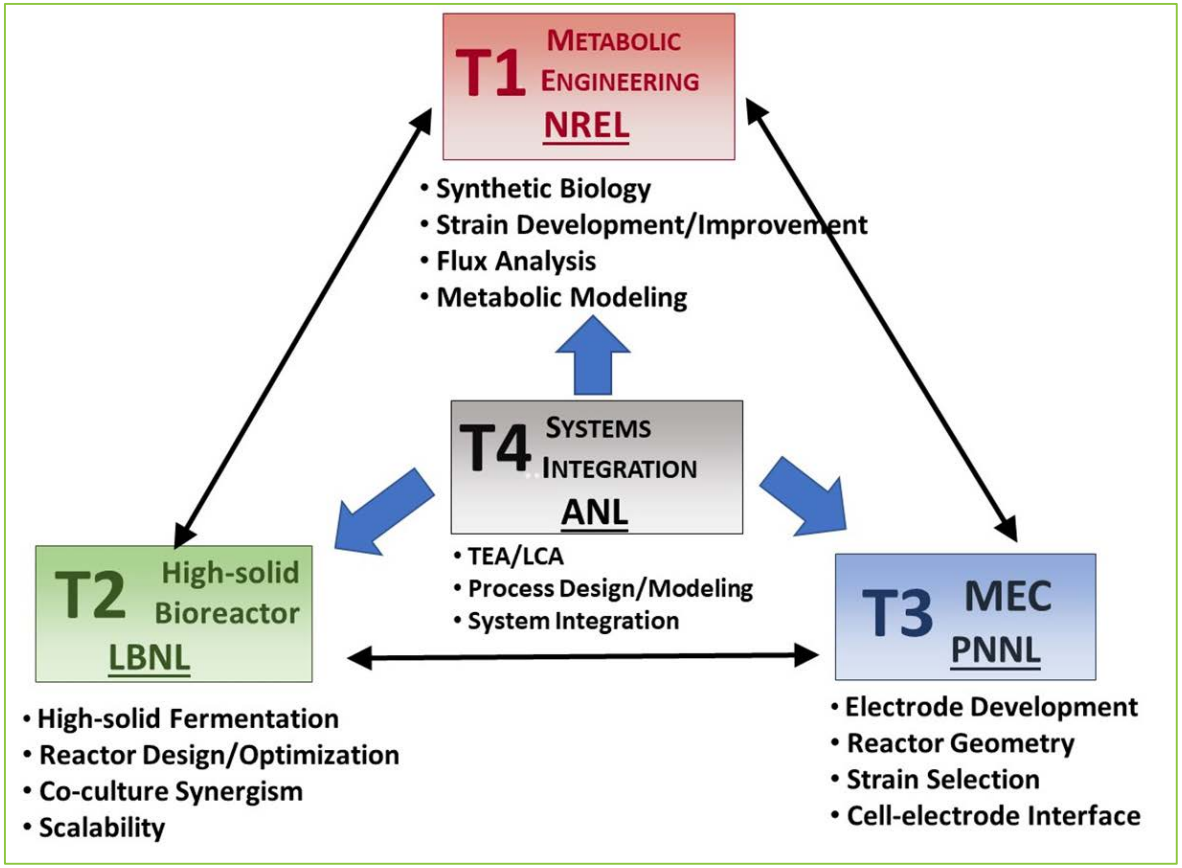


Current Project Year Objectives (October 2018 – March 2019)

- **Task 1. Strain Development ad 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 use cellulose.
- **Task 2. High-solids Bioreactor Development (LBNL Lead)**
  - Use a better impeller design to address the dynamics of high-solid loadings in bioreactors.
- **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)**
  - Address the systems engineering challenge for cost-effective implementation of dark fermentation technology with MEC in an integrated system, with input from Tasks 1-3.

Relevance –BioH2 Consortium Synergy

Rationale: In this Consortium 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.

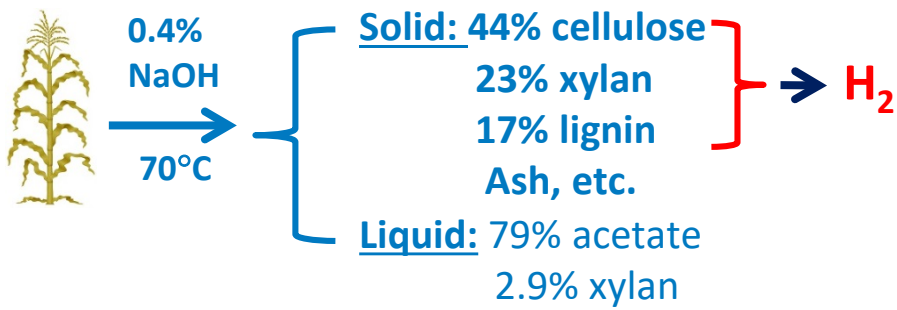


Approach
Task 1: Strain Development and Improvement (NREL)

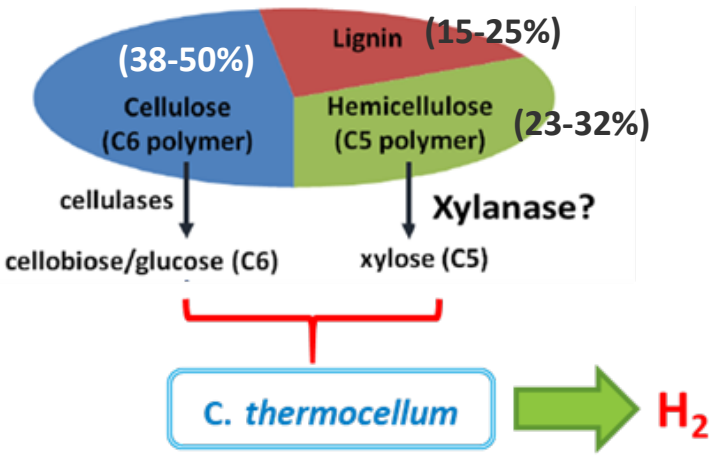
Approach: Via targeted engineering and adaptive evolution, we aim to improve xylan (hemicellulose; a xylose polymer) utilization. Cellulose-hemicellulose co-utilization will lower the cost of biomass feedstock.

- *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).
- Yet xylan hydrolysis is still a rate-limiting step, and overcoming it is an FY19 goal.

Pretreatment – De-acetylated and Mechanically Refined (DMR): leveraging BETO funding.



Engineer Cellulose-Degrading Microbe to Co-metabolize C5 Sugars



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

Tasks 1-4. Accomplishments and Progress: Conducted the Consortium Kick-off Meeting

FY19 Q1 Milestone	Organize the project team kickoff meeting and discuss future meetings, establishing inter-lab MTAs, and reporting logistics (all team members)	12/2018	**Complete**

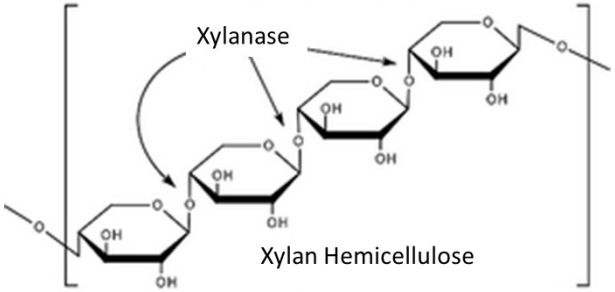
- NREL/LBNL/PNNL/ANL conducted a kick-off meeting via WebEx with DOE on November 16, 2018, during which inter-lab material transfer agreement/non-disclosure agreement (MTA/NDA), project overview, and lab task responsibilities were updated by National Labs key personnel.
- NREL sent LBNL the *C. thermocellum* wild type strain, protocols, and growth medium for culture growth at LBNL.

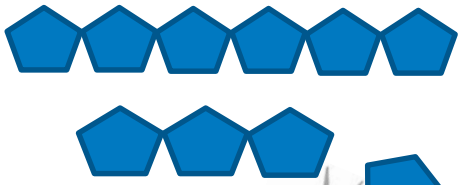
Task 1. Accomplishments and Progress: Identified Three Potential β-xylosidases to Improve Xylan Utilization

FY19 Q2 Milestone	Identify one key gene or mechanism contributing to xylan degradation in *C. thermocellum* (NREL);	3/2019	**Complete**

- The *xylAB* strain can degrade xylose alone or xylan with a small amount of xylose present, indicating likely a need for "activation" to utilize xylan.
- One goal is to enable the bacteria to utilize xylan when cellulose is present.
- One promising approach is via targeted approach to express foreign **β-xylosidase**:
  - β-Xylosidase (EC 3.2.1.37) cleaves xylose residues from the non-reducing ends.

- We identified three β-xylosidase from a thermophilic anaerobic xylan-degrading bacterium *Thermoanaerobacterium saccharolyticum* (*Tsac*):
  - XylA: responsible for growth of *Tsac* in xylan
  - XylB: unknown activity/function
  - XylC: demonstrated high tolerance for xylose and broad substrate range (both xylobiose and xylotriose).
- These genes are being cloned for expression in *C. thermocellum.*





β-xylosidase cuts

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

Approach: Increase loading of high solids (ultimate goal of 175 g/L) by improving mechanical design (e.g., impeller, baffles) to enhance microbe-substrate interface and mass transfer.

- Optimizing bioreactor conditions for *C. thermocellum*, both wild type and mutant strains developed in Task 1.
- Batch, fed-batch, sequencing fed-batch modes
- Mass balance closure (H_2, CO_2, feed, microbial biomass, etc.) to guide process optimization – lowering both CapEx and OpEx.

Potential impeller design to be used for high solids bioprocess

Task 2 Accomplishments and Progress: Obtained High Rate of H_2 Production with 15 g/L Solid Loading

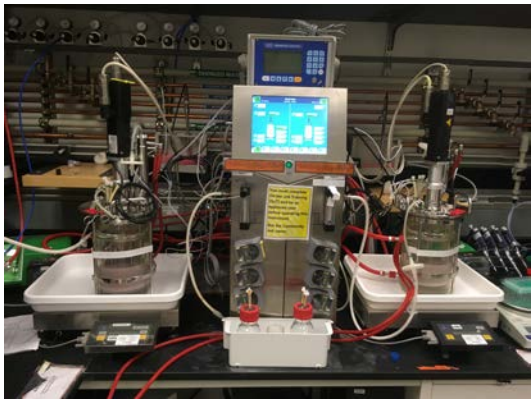
FY19 Q2 Milestone	Culturing of *C. thermocellum* in high-solid reactor with 15 g/L cellulose loading (LBNL);	3/2019	**Complete**

Successful NREL-LBNL tech transfer and test 15 g/L cellulose loading

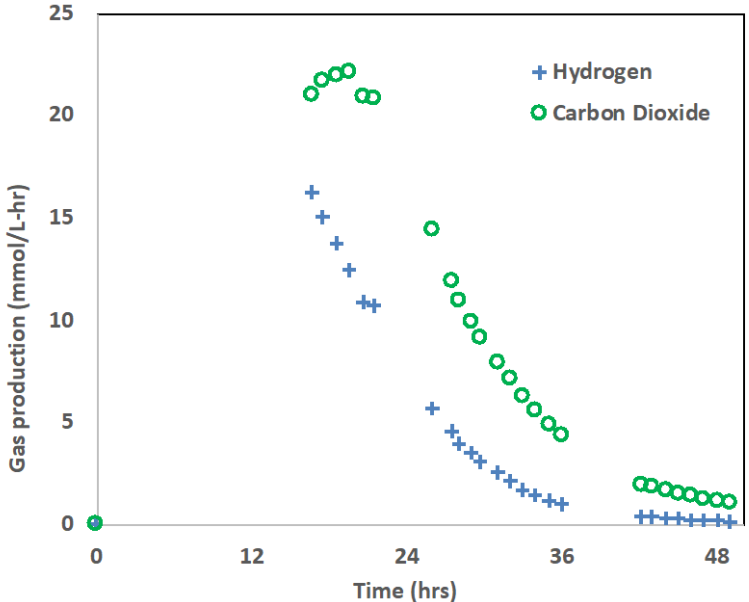
- Up to 16 mmol/L-hr H_2 production recorded in 1-L fermentation
- Significant formate and acetate accumulation

Next steps

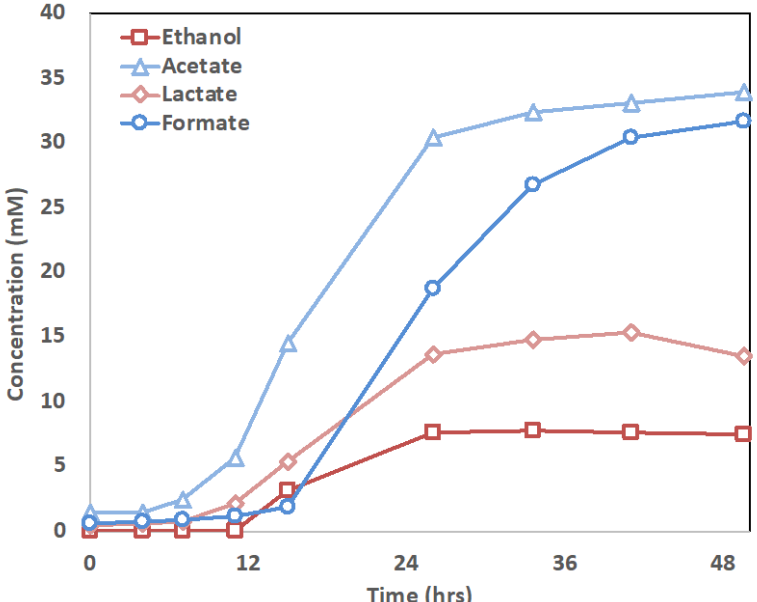
- Improve gas analysis hardware to better close mass balance
- Interrogate current process bottlenecks
- Move towards higher solids loading and pretreated biomass substrates



Gas production – 15 g/L avicel



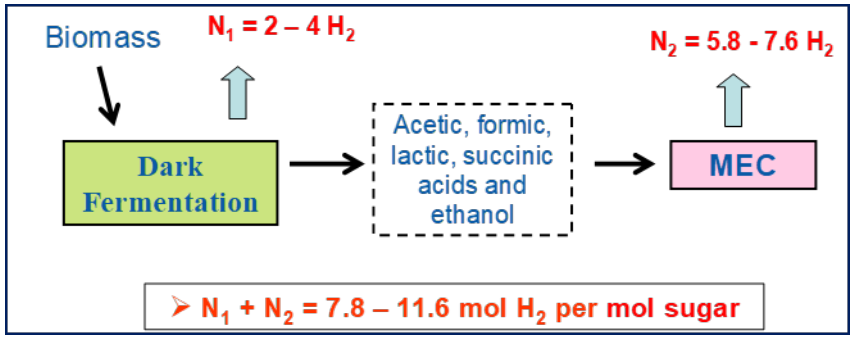
Organic acid production – 15 g/L avicel

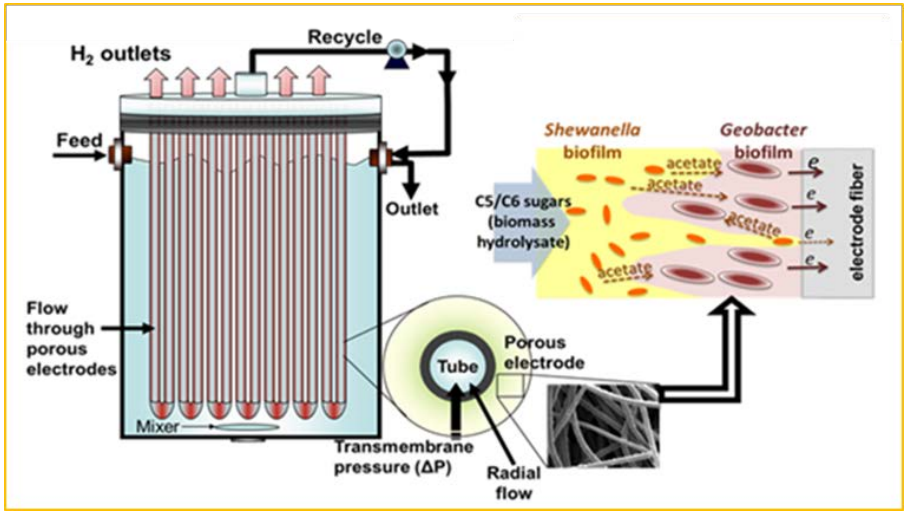


Approach
Task 3: Microbial Electrolysis Cell (PNNL)

Approach: Integrate closely with Tasks 1 & 2 to convert fermentation waste organic compounds to more H_2 by engineering exoelectrogenic microbes and improving electrode materials.

- Increase rate of electron transfer (current density) on the microbe-electrode interface.
- Engineer exoelectrogenic microbes to expedite waste conversion to H_2.
- Improve electrode materials, macro/micro-nutrients, other external factors
- Improve ion transport and develop new reactor geometries, 3D flow-through or radial-flow high-surface area electrodes
- Effect of proteins/lignin in fermentation effluent on H_2 production.





Task 3 Accomplishments and Progress: XXX

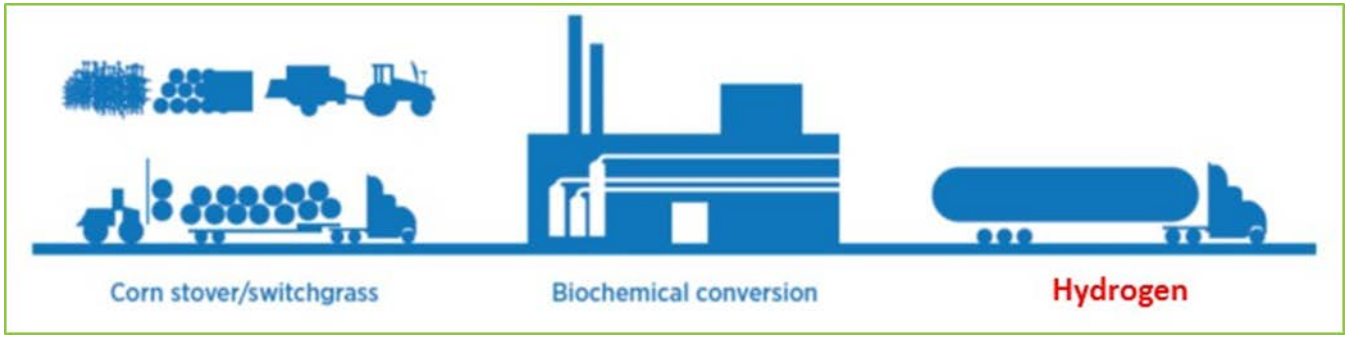
FY19 Q2 Milestone	Identify the most promising exoelectrogenic strains of *Shewanella* and *Geobacter* for MEC cultivation	3/2019	**Complete**

- Improvements in MEC-driven H_2 production depend on the efficient recovery of reductant from the byproducts of the lignocellulosic fermentation. This will be achieved using binary co-cultures that will breakdown the organic by-products into low-molecular fatty acids which will subsequently be oxidized on the anode to generate electricity
  - We identified 3 *Shewanella* strains (*S. loihica* PV-4, *S. putrefaciens* strain 200, *S. amazonensis* strain SB2B) for their exceptional anaerobic growth rates and ability to use a broad range of organic electron acceptors.
  - We identified *Geobacter sulferreducens* strain PCA and *Anaeromyxobacter dehalogenans* 2CP-2 as the most promising exoelectrogens for acetate conversion into electricity and H_2
  - The identified strains will be tested on a simulated and actual fermentation effluents for anaerobic growth and current production.

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 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

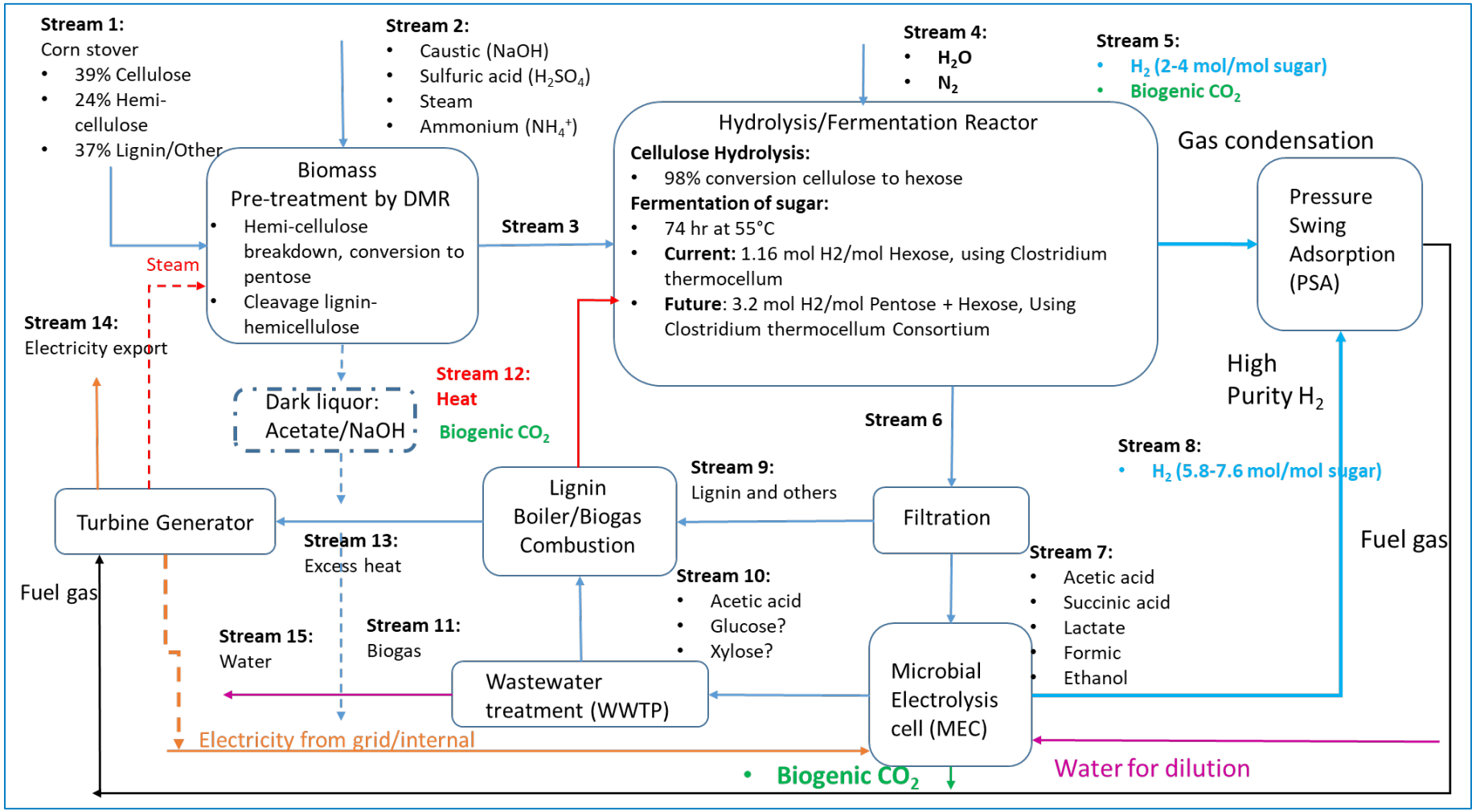


Task 4 Accomplishments: Develop TEA Framework

FY19 Q2 Milestone	Develop TEA framework with place holders for cost and performance information (ANL)	3/2019	**Complete**

- A preliminary process design based on recent reports was constructed for the integrated dark fermentation-MEC process.
- The process design will involve technology experts in various experimental research teams in NREL, PNNL, and LBNL to check feasibility, identify potential research goals, and improve the integrated process efficiency.
- ANL identified that Aspen modeling will be necessary to model the process design, calculate mass and energy balance, and track resources usage (e.g. utility, water). A solid system modeling is the foundation of accurate TEA-LCA analyses.
- The Aspen modeling work is initiated.

Task 4 Accomplishments: Mass and Energy Flow for TEA Framework



Accomplishments and Progress: Responses to Previous Year Reviewers' Comments

None - a new FY19 project with a start-up date of October 2018.

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-solid 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 xylan both in the presence and absence of cellulose.
  - Test expressing various xylanases and enzymes to hydrolyze xylan or xylo-oligomers to xylose and profile the genes (or the lack of genes) responsible for the bacteria to degrade xylan via transcriptomic analyses

Task 2. High-solid Bioreactor Development (LBNL)

- High solid-substrate loading (175 g/L) is needed to lower H_2 selling price, which might present a challenge to ensure sufficient mixing.
  - Require better impeller design to ensure microbe-substrate interface.

Task 3. Microbial Electrolysis Cell (PNNL)

- Improve MEC microbes and configurations to increase rate and yield of H_2.
  - Efficient conversion of complex fermentation effluent stream require development of multi-organism biofilm consortia.

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

- Evaluate integrated dark fermentation-MEC system for technical and economical feasibility.
  - Evaluation involves progress of the entire team, and any design or yield changes could lead to the resize of many, if not all, equipment.

Proposed Future Work

Task 1 (NREL)

- Construct and express the various *Tsac* xylanases genes (*xylA, B, C*) in *C. thermocellum* (FY19); use adaptive evolution to improve xylan utilization leading to increased H_2 (FY19/20).
- Refine and improve method to quantify DMR biomass constituents (FY19).

Task 2 (LBNL)

- Improve gas analysis hardware to better close mass balance (FY19).
- Test *C. thermocellum* strain in bioreactor with higher solid loading (>15 g/L) (FY19/20)

Task 3 (PNNL)

- Test anaerobic respiration rates of *Shewanella* and *Geobacter* spp. on major fermentation by-products (FY19) and identify at least 2 fast-growing strains from each genera with overlapping growth optima for follow-up co-cultivation and MEC experiments (FY19/20)

Task 4 (ANL):

- Develop system boundary, analysis steps, and acquire available design and cost data from the team for TEA/LCA (FY19/20).

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 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

- Evaluated various promising candidate xylanases genes for expression in *C. thermocellum* to afford xylan utilization.
- Cloning of these genes is underway for heterologous expression in *C. thermocellum*.

Task 2

- Set up H_2-CO_2 online gas analysis, completed initial fermentation, and analytical chemistry technology transfer from NREL to LBNL.
- Obtained high rate of H_2 production, up to 16 mM H_2/L-h, with troubleshooting on going.

Task 3

- Identified 5 promising exoelectrogenic strains for anaerobic oxidation of organic waste by-products in the fermentation effluent.
- Quantitative growth and respiration characterization will enable the development of optimal co-cultures for MECs thus increasing the hydrogen molar yields.

Task 4

- Examined preliminary design for the integrated system and identified sensitive factors for key economic and environmental metrics, and provide feedbacks to collaborators.
- Adopted Aspen model to model the process and acquire mass and energy flows for TEA and LCA.

Thank You

www.nrel.gov

Publication Number

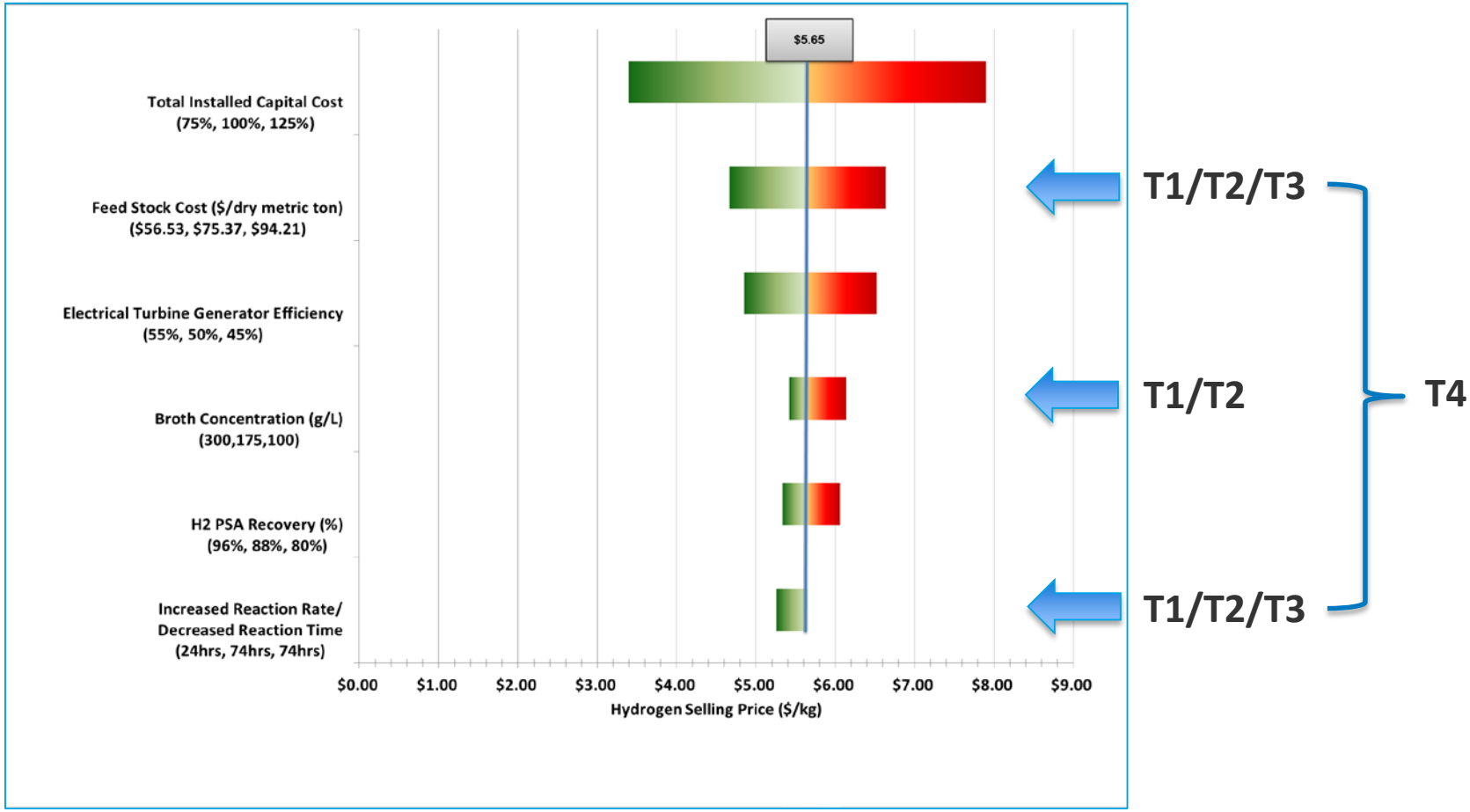
NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

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