



BioHydrogen (BioH₂) Consortium to Advance Fermentative H₂ Production

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National Renewable Energy Laboratory
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Project ID: P179

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Project Goal

Overall Objective: Develop a high-solids microbial fermentation technology to convert renewable lignocellulosic biomass resources into H_2 via strain engineering and integrate microbial electrolysis cell (MEC) to meet DOE H_2 production cost goal of $< \$2/\text{kg-}H_2$.

Current Project Year Objectives (Oct 2020 – March 2021)

Task 1. Strain Development and Improvement (NREL Lead)

- Lower feedstock cost and improve the conversion of cellulose (hexose) hemicellulose (5-carbon sugars) to H_2 via genetic engineering of *Clostridium thermocellum* which is capable of direct cellulose fermentation for H_2 production.

Task 2. High-solids Bioreactor Development (LBNL)

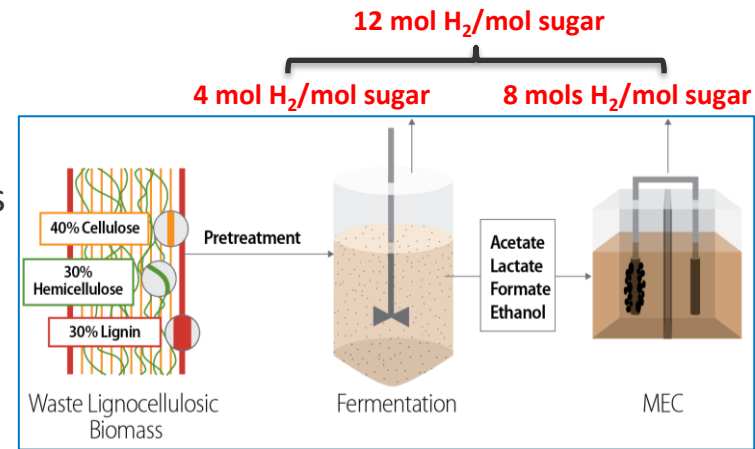
- Development and characterization of a high intensity fermentation system for H_2 production at high solids loading, using new mixing geometries and improved operational conditions

Task 3. Microbial Electrolysis Cell (PNNL)

- Design MEC process integrated with dark fermentation (Tasks 1 & 2) for conversion of the fermentation effluent to H_2 using robust exo-electrogenic microbes and co-cultures to increase H_2 production and yield.

Task 4. System Integration, Techno-economic Analysis (TEA), and Life Cycle Analysis (LCA) (ANL)

- Design a conceptual, large-scale system to integrate the dark fermentation (DF) and MEC for bio H_2 production. Model the overall system with Aspen Plus, evaluate economics with H2A model, analyzes CO_2 emissions with GREET model.



Overview

Timeline and Budget

- Project start date: 10/1/2018
- FY21 DOE funding: \$1.08M
- FY22 Planned DOE funding:

	FY19	FY20	FY21	FY22
NREL	\$485K	\$600K	\$600K	\$300K
LBNL	\$200K	\$200K	\$150K	\$150K
PNNL	\$200K	\$200K	\$200K	\$150K
ANL	\$200K	\$125K	\$125K	\$ 75K
Total	\$1.08M	\$1.13M	\$1.08M	\$675K

- Total DOE Funds Received to Date*\$3.95M

*for the consortium since the project started

Barriers

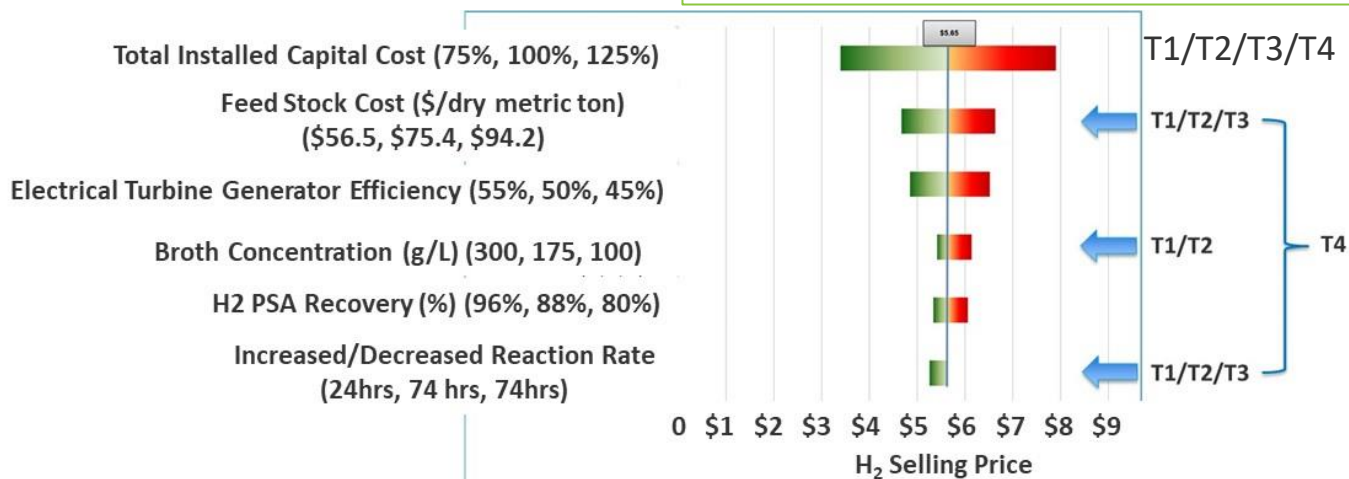
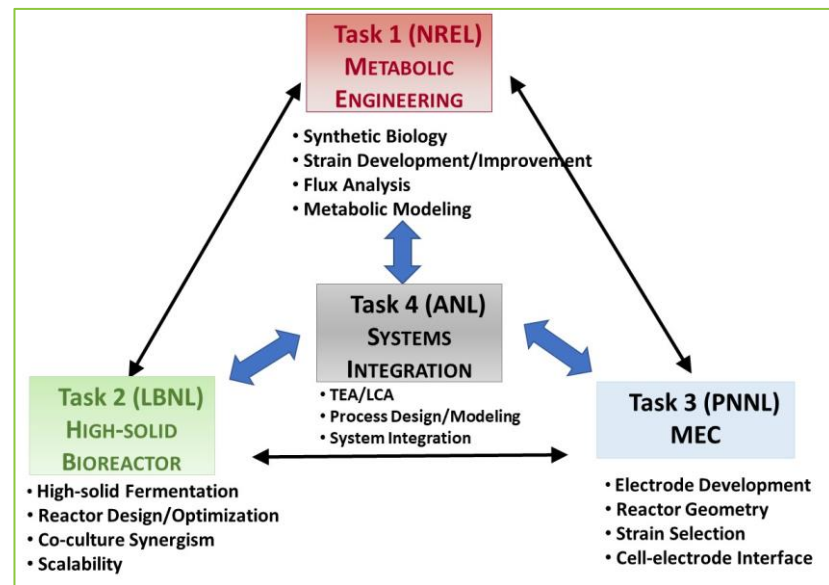
- H₂ molar yield (AX)
- Feedstock cost (AY)
- System engineering (AZ)

Partners

- Dr. Katherine Chou (PI, NREL)
- Co-PIs: Drs. Steve Singer (LBNL), Alex Beliaev (PNNL), Amgad Elgowainy (ANL)
- Lawrence Berkeley National Lab (LBNL), Pacific Northwest National Lab (PNNL), Argonne National Lab (ANL)

Relevance – BioH2 Consortium & Synergies

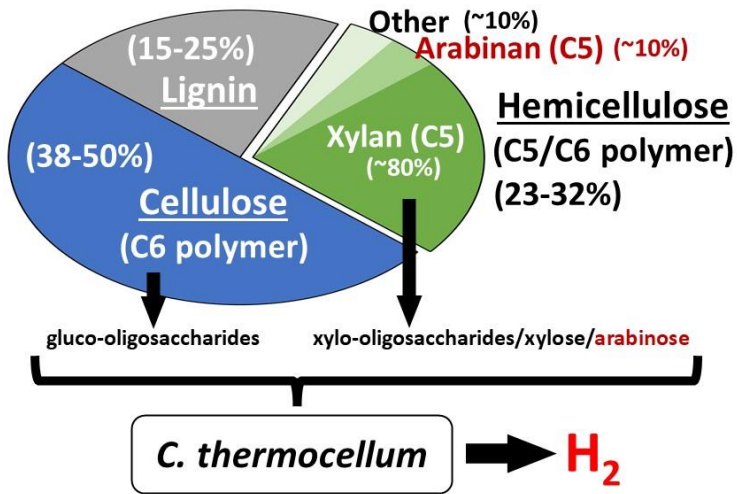
Rationale: We assembled a highly productive and collaborative team of scientists from **four** National Labs whose research accomplishments and expertise lay down a strong foundation in addressing knowledge gaps and technical barriers for long-term success toward meeting the HFTO H₂ production cost goal (\$2/kg H₂).



Approach: Task 1. Strain improvement (NREL)

Combined laboratory evolution & targeted genetic engineering (FY18-22), we continue to improve hemicellulose (five-carbon sugar polymer) utilization. Cellulose-hemicellulose co-utilization will lower biomass feedstock costs.

Engineer Cellulose-Degrading Microbe to Co-metabolize C5 Sugars



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

1926 – 2016

NREL genetically modified strain (*xy/AB*) to co-utilize C5 sugar (xylose)

2017 – 2018

NREL evolved strains (created strain **19-9**) for improved growth and H₂ production rate on **hemicellulose sugars**

2018 - 2019

2020 – 2021 Co-utilizing hemi-/cellulose for H₂ production

Enabled the co-utilization of hemi-/cellulose (**BX**)

2021 – 2022 Engineering strain to use other hemicellulose sugars

Genetically modified 19-9 to co-utilize arabinose

Ferment all the sugars to H₂ in one bioreactor: lowering both feedstock and reactor cost.

Task 1. Accomplishments: Greater than 80% assimilation of arabinose loaded at 5 g/L by genetically modified *C. thermocellum* strain (NREL)

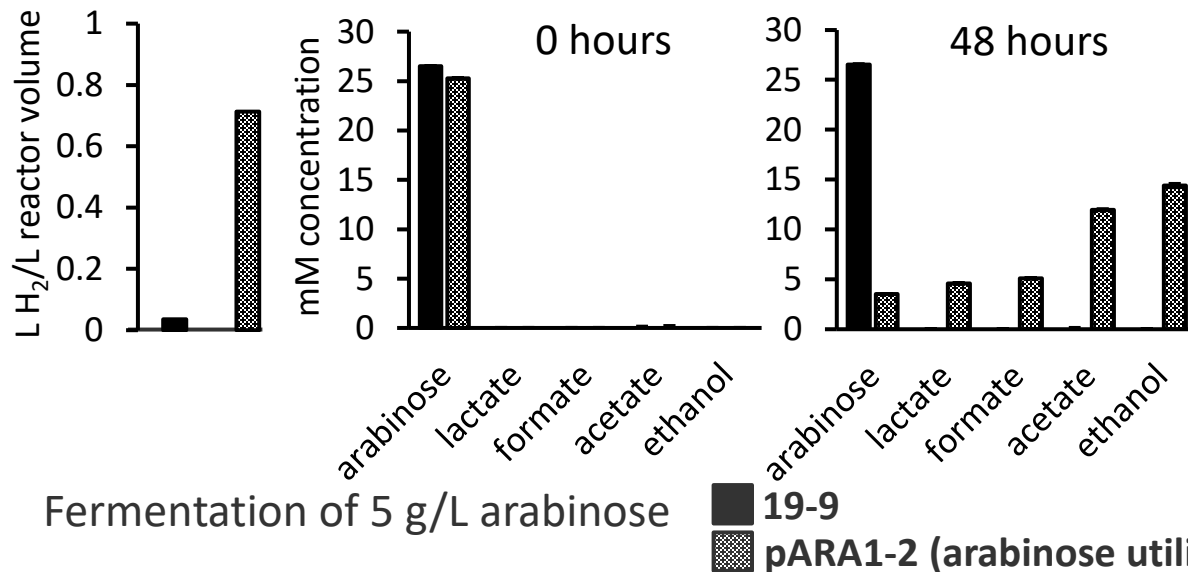
FY22 Q1 Milestone: Arabinose Utilization

Improve hemicellulose utilization by genetically expressing 3 arabinose-catabolizing genes in *C. thermocellum* to consume over 50% of the L-arabinose (pentose sugar which is embedded in hemicellulose) loaded at 5 g/L for H₂ production (SMART; NREL)

Dec 2021

Complete

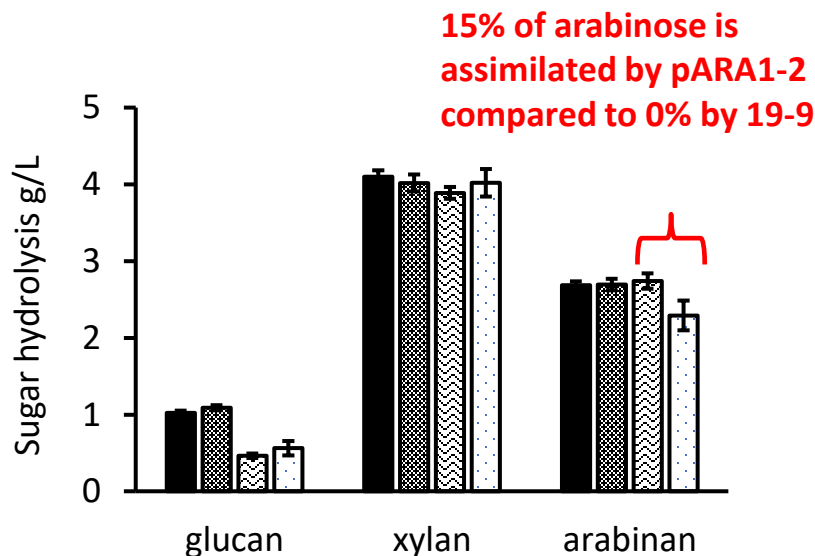
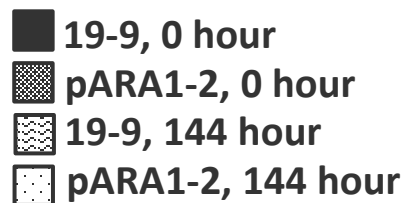
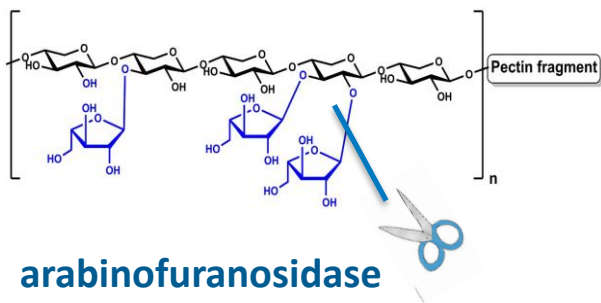
- **pARA1-2** consumed 86% of the arabinose compared to 0% by strain **19-9**
- H₂ is produced by **pARA1-2** from arabinose fermentation, but not much from **19-9**



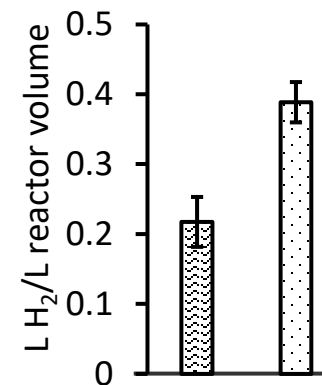
- 2 strains compared for arabinose utilization:
 - **19-9**: engineered and evolved in lab for xylose utilization
 - **pARA1-2**: 19-9 further expresses enzymes for arabinose utilization, and further adapted for growth on arabinose by adaptive laboratory evolution

Task 1. Accomplishments: Addition of arabinose utilization genes permits assimilation of arabinose from more complicated biomass substrates (NREL)

- C. thermocellum* has arabinofuranosidases to cleave arabinose from arabinoxylan in hemicellulose
- Both 19-9 and pARA1-2 assimilate glucan sugars from arabinoxylan with pectin fragments, but the pARA1-2 strain can also assimilate arabinose from this substrate.



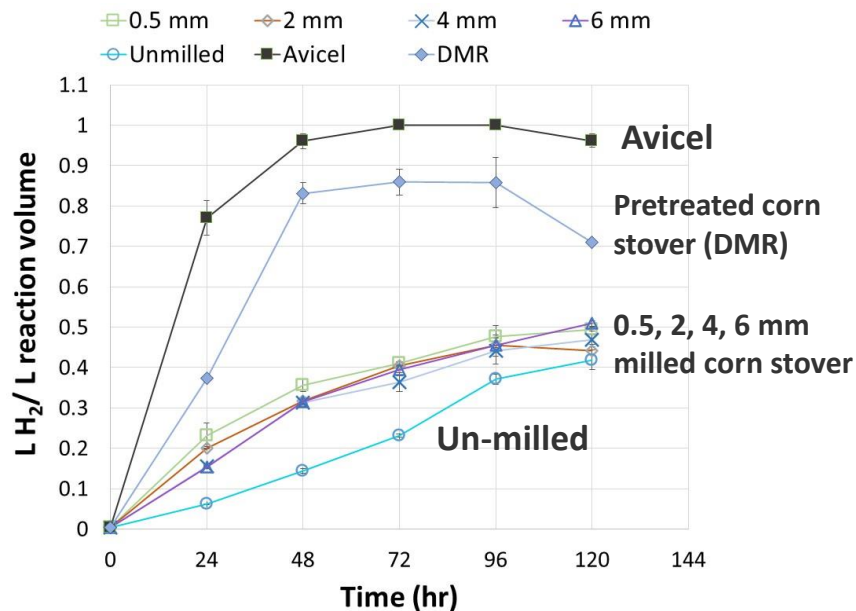
78% more hydrogen produced by pARA1-2 from the 15% arabinose assimilated



Data from fermentation of 10 g/L arabinoxylan in 10 ml of rich media

Task 1. Accomplishments: Demonstrate H₂ production from fermenting untreated, milled biomass, meeting GNG milestone (NREL)

Name	Description	Criteria	Date	Status
FY22, Q2 Go/No-go	H ₂ production on four different milled corn stover particle sizes of 0.5, 2, 4, and 6 millimeters loaded at 10 g/L will be tested and compared using equivalent amount of cellulose fermentation as baseline. We anticipate approximately 3 g of cellulose embedded in 10 g of milled corn stover.	Demonstrate at least 25% of the expected H ₂ production from equivalent amount of crystalline cellulose (i.e., Avicel) using milled corn stover as the feedstock.	March, 2022	Complete



Corn Stover Source	0.5 mm	2 mm	4 mm	6 mm	Unmilled	DMR	Avicel
% H ₂ produced	48	45	44	46	37	86	100

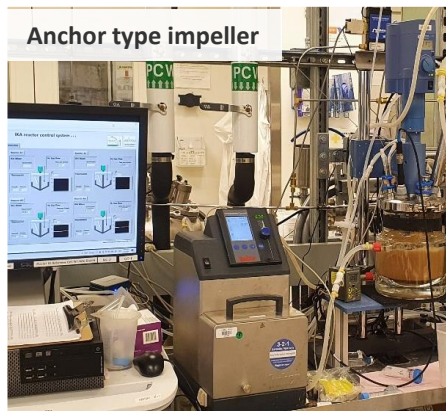
Untreated, milled corn stover were 42% (w/w) cellulose on average.

- Using Avicel (4 g/L) as baseline (1.0 L H₂/L_{reactor}), 44-48% of H₂ was produced in milled corn stover of varying sizes, exceeding 25% and meeting FY22 Q2 GNG milestone.
- Minimally engineered *C. thermocellum* Δhpt strain is capable of fermenting untreated corn stover for H₂ production
- H₂ production rate is the slowest in un-milled biomass, suggesting milling improves access to the sugars by the bacteria.

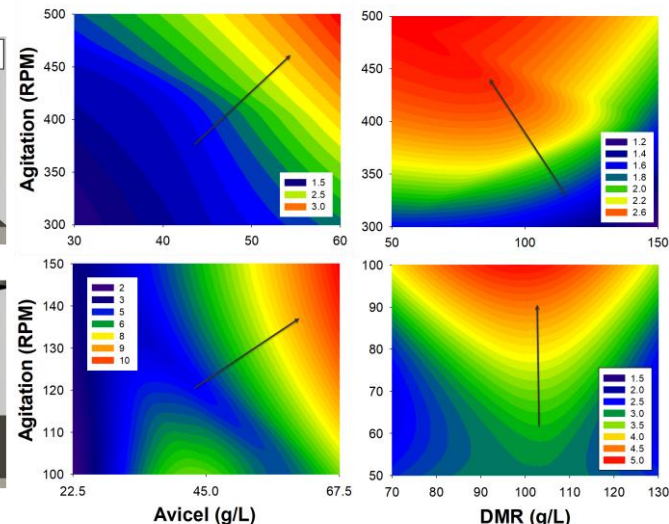
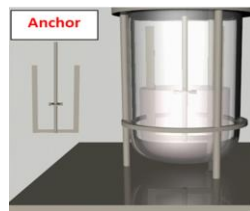
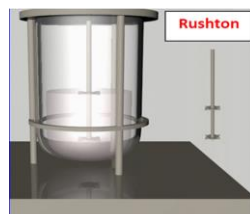
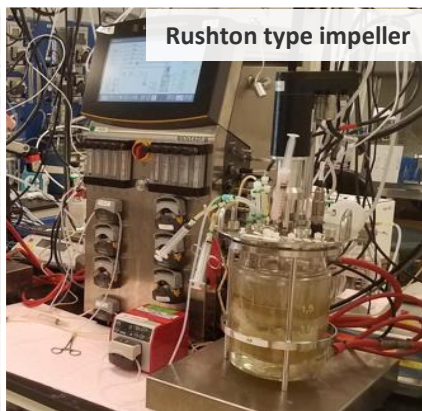
Approach: Task 2. High-solids Bioreactor Development (LBNL)

Approach: Development and characterization of a high intensity fermentation system for H_2 production at high solids loading, using new mixing geometries and improved operational conditions

- Optimization of bioreactor parameters for the engineered strain *C. thermocellum* 19-9 under high solids conditions
- Improvements to impeller geometry, co-optimized mixing conditions (agitation and gas sparging), and use of fed-batch operation to enhance gas-liquid mass transfer for high-viscosity fermentations
- Analysis of major metabolites and biomass decomposition products in effluent from DMR corn stover fermentation



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



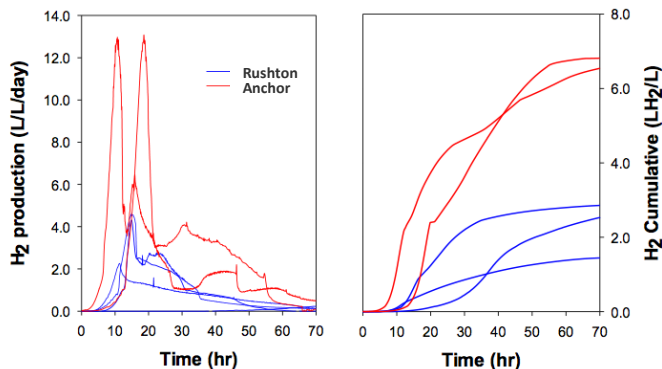
Design of experiments approach for co-optimization of agitation speed, impeller geometry, and substrate supply

Task 2. Accomplishments and Progress: Evaluated impeller design, fed-batch operation, biomass depolymerization, and metabolite profiles with *C. thermocellum* 19-9 (LBNL)

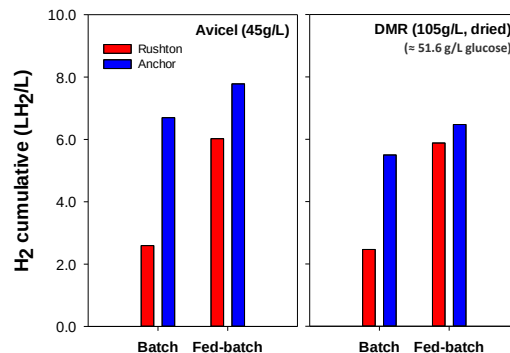
FY21 - FY22 Milestones

- Achieve 15% increase in baseline production of H_2 from DMR corn stover by combining improvement in gas sparging and mixing; Measure H_2 production from DMR corn stover at 100 g/L (45 g/L cellulose) (FY21 Annual)
- Evaluate combined effects of mixing energy and solids loading on biomass depolymerization and H_2 production (FY22 Q1)
- Use a combination of HPLC and LC-MS to identify major metabolites in effluent from DMR corn stover fermentation. The focus will be on aromatics from lignin and amino acids. We will also compare DMR effluent from relatively high vs low solids fermentation (FY22 Q2)

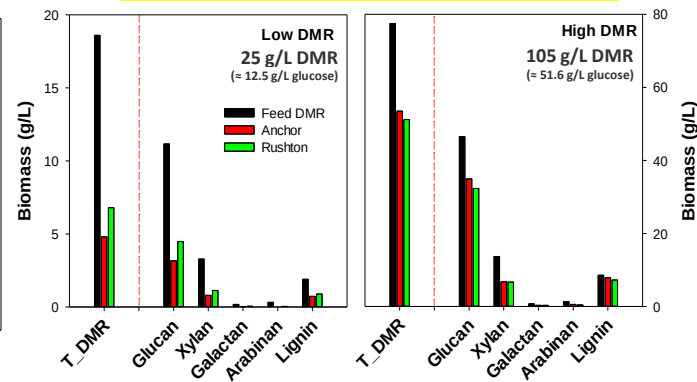
❖ Deployment of new impellers



❖ Fed-batch bioreactor operation



❖ Analysis of lignin solubilization



- Successful deployment of customized bioreactors featuring anchor-style impellers
- Anchor impellers and fed-batch operation significantly enhance overall hydrogen production
- Maximum H_2 titer increased from 4.66 L H_2 / L in the FY21 AMR to 7.78 L H_2 / L in the FY22 AMR, a 67% increase

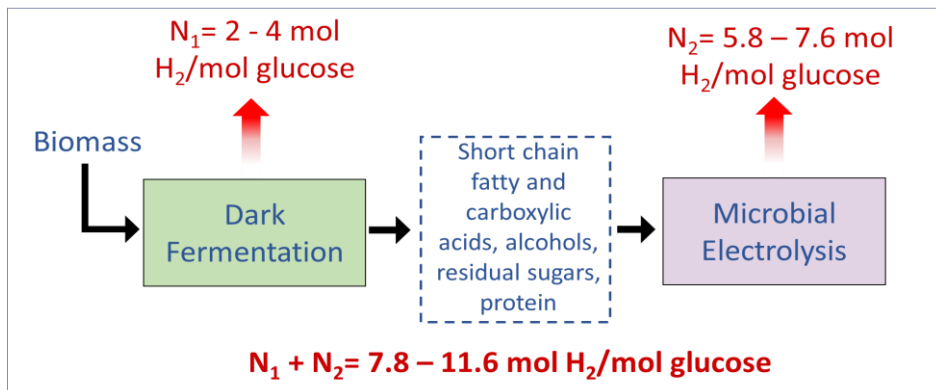
		Total H_2 titer (L- H_2 /L)	
		Batch	Fed Batch
Avicel (45 g/L)	Rushton	2.59	6.02
	Anchor	6.70	7.78
DMR (105 g/L)	Rushton	2.47	5.88
	Anchor	5.50	6.47

- Lower biomass loading is associated with enhanced glucan, xylan, and lignin depolymerization
- Future efforts will target efficiency improvements under fed-batch and continuous conditions

Task 3. Microbial Electrolysis Cell (PNNL)

Approach: Design MEC process integrated with dark fermentation (Tasks 1 & 2) for conversion of the fermentation effluent to H_2 using robust exoelectrogenic microbes and co-cultures

- Deploy robust and controllable exo-electrogenic cultures with broad metabolic capacity to increase H_2 production from fermentation effluent.
- Rationally design continuous MEC process for conversion of lignocellulosic fermentation effluent (e.g., organic acids, alcohols, proteins, sugars) to H_2 with increased efficiencies and productivities.



Process flow diagram of the integrated fermentation-MEC process for H_2 production from waste biomass



H_2 production in an H-type MEC using effluent derived from high-solid loading Avicel fermentation

Task 3: Accomplishments and Progress: Sustained Current Production (PNNL)

FY21 Q4 Milestone	Demonstrated continuous MECs utilization of Avicel effluent (>1000 hs) achieving >15 A/m ² peak current densities and ~4 L/d/L reactor peak H ₂ production rates (PNNL)	Sept, 2021	Complete
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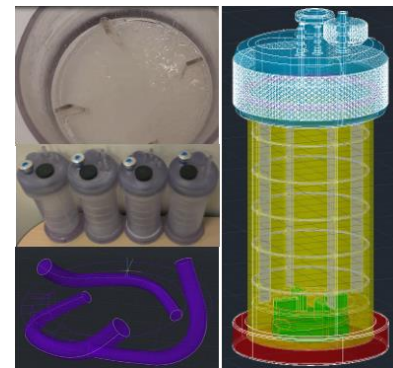
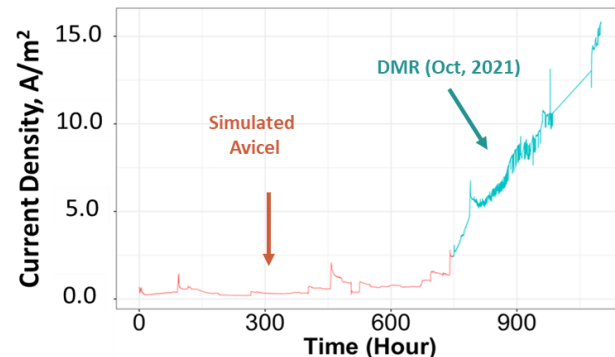
- Current efforts: improving current densities and H₂ yields from DMR effluent

	Pyruvate mM	Lactate mM	Formate mM	Acetate mM	Ethanol mM
DMR Start	18.334	77.957	39.7	155.966	392.233
DMR End	0.942	10.403	91.537	0	363.821
Difference	17.391	67.553	-51.837	155.966	28.412

- *In FY22, we demonstrated utilization of high-load DMR effluent and achieved sustainable current production reaching 10 A/m²*

- Planned activities: improving current densities through new MEC geometry (3D-printed)

- *Improved mass-transfer & mixing efficiency*
- *Close proximity of anode and cathode for decreased resistance*
- *Physical gathering of pure H₂ from cathode with electrode cages*

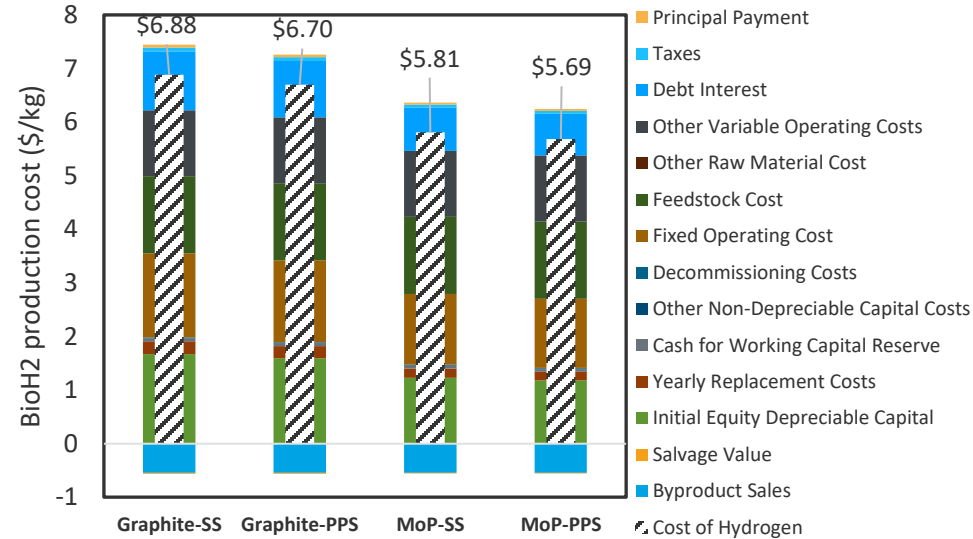


Approach

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

Approach: Use TEA (Aspen Plus) and LCA (GREET) to set research targets, guide research directions and suggest system design to achieve cost targets and reduce life cycle greenhouse gas emission.

- Using literature data, evaluated various MEC electrode and membrane options to balance between high current density and capital cost.
- Recommended electrode materials to experimental collaborators to improve current density and suggested MEC system design and engineering control to improve mass transfer.
- With high purity CO₂ produced from DF and MEC, we included by-product CO₂ capture & sequestration for potential tax credit, to reduce H₂ production cost.
- Develop LCA model to identify the CO₂ emission contributors for the bioH₂ production.

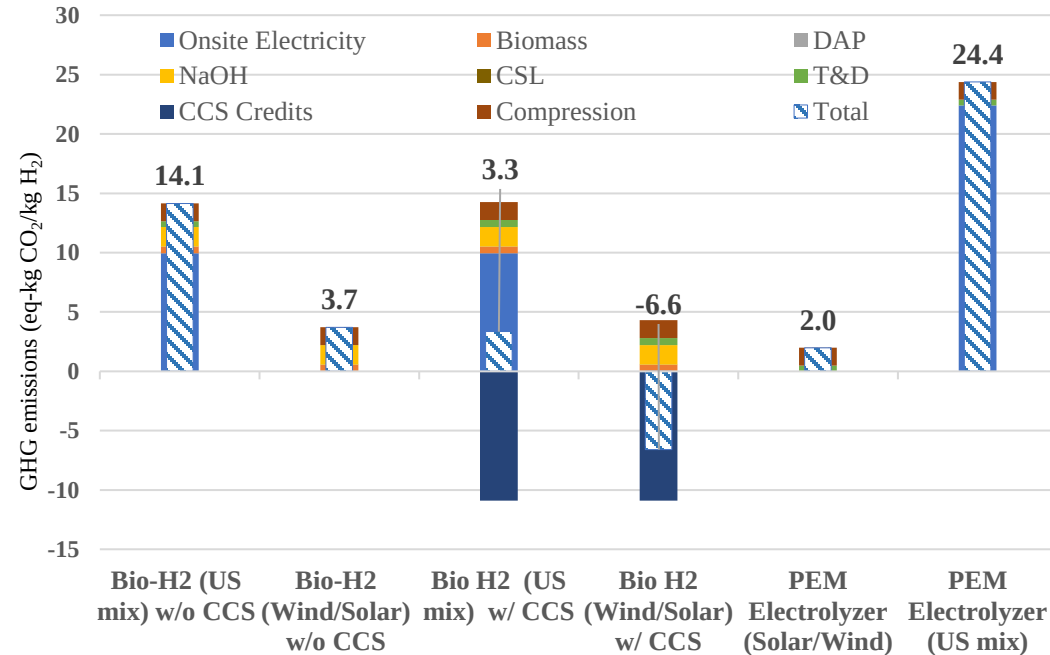


MoP: a current density of 100 A/m²; Graphite: a current density of 30 A/m²
SS: stainless steel; PPS: polyphenylene sulfide with 40% glass fiber filler

- The selection of electrode and membrane has a large impact on bioH₂ production cost. NREL | 13

Task 4 Accomplishments: Explore impacts of various DF-MEC design parameters on H₂ production cost and lifecycle GHG emissions

FY21 Q4 Milestones	Update the LCA results based on the new MEC current density by using various electrode materials, as well as on other updated energy consumption information. For the integrated system, the leading CO ₂ emission source is electricity consumption, which is in turn dominated by MEC energy use (ANL).	9/2021	Complete
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- Carbon intensity of the electricity source has a large impact on the bioH₂ life cycle CO₂ emission.
- CCS can reduce onsite CO₂ emission greatly.
- Using renewable electricity and employing CCS, bioH₂ production can lead to zero or negative fossil CO₂ emission.

CCS = carbon capture and sequestration

Accomplishments and Progress: Responses to Previous Year Reviewers' Comments (addressed via emails)

Reviewer Comments:

I believe in previous quarters, PNNL has indicated that a component of the DMR fermentation effluent is poisoning or restricting their microbial colony, and have continued their reactor design using avicel. The TEA reports on the promise of the fermentation + MEC system to be able to produce H_2 at a cost of \$5.60 to \$6.60 per kg H_2 without the sale of the CO_2 . Is that assuming 175 g/L loading? There is a considerable amount of effort is needed for the MEC to accept the fermentation effluent and achieving a current density of 100 A/m². Is it possible for you to consider the steps that would be needed to move away from the DMR and use the mill biomass while meeting the same (or improved) H_2 yield as you prepare your writeup for future scope options? Would the effluent then expected to be compatible with the MEC reactor, or negatively impact the solid loading work? How would the mill biomass as a feedstock affect the predicted cost of hydrogen?

Responses to Reviewers Comments:

For your first question, yes, 175 g/L loading was part of the assumption made for H_2 production at \$5.6 to \$6.6/kg H_2 for the combined (fermentation + MEC) system. This number (175 g/L) first came from a TEA model developed by Strategic Analysis (SA). This TEA model was for dark fermentation alone and concluded that the solid loading needs to be 175 g/L to be economical. That said, as we continue to improve the state of the technology of (fermentation + MEC) and analyze the system as an integrated technology and not stand-alone technologies, it could be beneficial to revisit this assumption and the integrated system's sensitivity to this particular target loading at a later point.

One big incentive for us to use milled biomass and not DMR biomass is the \$1 saved from the cost of \$5.6 – 6.6/kg H_2 . Based on ANL's TEA model, \$1 of that 5.6 – 6.6/kg H_2 comes from the cost of NaOH used to pre-treat and remove acetate and lignin from the milled biomass.

As you probably saw too, even if we can demonstrate successful fermentative H_2 production directly from milled biomass in FY22, we would expect additional benefits and certain challenges associated with milled biomass, which we would need to address beyond FY22.

One benefit is that we actually attain more acetate from biomass for MEC. Currently the acetate in the fermentation effluent is sourced from the breakdown of hexose and pentose sugars (cellulose/hemicellulose) by the bacteria, so the acetate removed during chemical pretreatment to attain DMR biomass will be retained and that provides additional acetate to MEC.

Some of the challenges of milled biomass could be toxicity from lignin on fermentation and/or MEC, or other unforeseen issues to resolve. We would likely need to continuously assess what the issues may arise and identify potential remedies. We would also need to take into considerations the cost of the proposed remedies in comparison to the \$1 saved. Currently lignin is burned for energy production in our TEA model, which is commonly the case across what people do in practice with lignin, as it's not readily used by microbes. I believe BETO is investing in R&D to valorize lignin, and we can see what we could leverage from their investment for H_2 production as needed.

Before we know milled-biomass is feasible, NREL can set one milestone in FY22 to assess the feasibility of H_2 production from fermenting milled-biomass in lower loading and smaller scale pilot experiments. In the meanwhile, PNNL and LBNL continue to use DMR biomass in FY22 as the feedstock to optimize their systems. LBNL can continue with co-optimizing fed-batch fermentation with wall-scraping impeller at high solids fermentation. PNNL can re-assess and troubleshoot DMR effluent on their MEC system, optimize their MEC parameters using DMR biomass to establish baseline, and continue to improve current density. After all, DMR remains an excellent feedstock to fall back on (and for establishing baseline and parameter optimization) if milled biomass creates unsolvable problems.

Collaboration and Coordination

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

- Dr. Katherine Chou (PI) and team develop and test strains to improve H₂ production and send the strains to LBNL for testing in high solids fermentation for Task 2.
- NREL assumes the leadership of setting & coordinating directions & efforts between labs.
- NREL leverages BETO investment in biomass pretreatment and Office of Science BER investments in understanding *C. thermocellum*. Collaboration with UCLA (BER support) laid the foundation to supply CO₂ for improved bacterial culturing.

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

Drs. Eric Sundstrom and Steve Singer (LBNL) develop and optimize bioreactors for high solid loadings and supply fermentation effluent to PNNL for Task 3.

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

Dr. Alex Beliaev and the PNNL team are optimizing MEC-driven effluent conversion to address the H₂ molar yield, biological system performance and capital cost barriers

- **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)

- Continue to reduce biomass feedstock cost by minimizing pretreatments, and enable stable co-utilization of both cellulose and hemicellulose components of biomass
 - Access to arabinose side chains in hemicellulose via arabinofuranosidase activity
 - Cleavage and assimilation of the 3rd most abundant sugar (acid) of hemicellulose, glucuronic acid

Task 2. High-solid Bioreactor Development (LBNL)

- Overall conversion efficiency declines at high solids loading due to high bulk viscosity, production of overflow metabolites/inhibitory metabolites, and release of inhibitory compounds from biomass. Additional research must target these barriers to maximize H₂ yields.
- Current production relies on batch or fed-batch experimentation, for commercial deployment a continuous process would offer operational simplicity while maximizing volumetric H₂ production.

Task 3. Microbial Electrolysis Cell (PNNL)

- Optimize MEC operation on DMR fermentation effluent at high-solids loading to achieve current density of $\geq 30 \text{ A/m}^2$ and H₂ production rate greater than 4 L H₂/ L reactor/day
- Reduce electrode costs using high-performance cathode materials

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

- The absence of design parameters for large scale MEC system, and the information gap for MEC assembly cost.

Proposed Future Work

Task 1 (NREL)

- Through genetic manipulation we will tackle both enzyme deconstruction (arabinofurnosidae activity, etc.) and glucuronic acid assimilation, which will result in a robust cellulose/hemicellulose eater for H₂ production (FY22/23)
- Improve accessibility of sugar for H₂ from fermenting untreated milled corn stover (FY22/23).

Task 2 (LBNL)

- Apply lessons learned for development of a continuous process using fed-batch higher solids loading, combining advances in gas sparging and H₂ removal, impeller geometry, and fed-batch operation (FY21/22).
- Leverage analytical techniques to reduce overflow metabolites, enhance biomass depolymerization, and improve overall yields of hydrogen from biomass.

Task 3 (PNNL)

- Test new reactor geometries and electrode support structures which increase current density while maintaining H₂ purity

Task 4 (ANL):

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

Summary

Task 1 (NREL)

- Demonstrated 86% utilization of arabinose (5 g/L), a hemicellulose sugar for H₂ production, thereby lower the cost of H₂ production by using more sugars in the feedstock for higher amount of H₂ that can be produced
- Meeting Go/No-Go Milestone: Demonstrated H₂ production from fermenting untreated, milled corn stover using equivalent loading of crystalline cellulose as the baseline.

Task 2 (LBNL)

- Enhanced H₂ production at high loading by shifting to fed-batch operation, increasing H₂ production by 67% from the FY21 AMR, to a maximum of 7.78 L H₂ / L in fed-batch operation with avicel, and 6.47 L H₂ / L in fed-batch fermentation with DMR-pretreated biomass.
- Conducted a full analysis of effluent from DMR corn stover fermentation with *C. thermocellum* 19-9 under varying operational conditions, including biomass depolymerization, lignin decomposition and solubilization, major metabolites, and solubilized amino acids.

Task 3 (PNNL)

- Achieved ~4 L H₂/L reactor/day and ~10 A/m² current densities using Avicel fermentation effluent
- Obtained 10 A/m² current densities on DMR fermentation effluent

Task 4 (ANL)

- Explore impacts of various DF-MEC design parameters on H₂ production cost and lifecycle GHG emissions.
- Further cost reduction opportunities include the continuous enhancement of current density, the use of zero-cost waste feedstock, the reduction of electricity use in MEC, and the reduction of NaOH use in biomass pretreatment.

Thank You

www.nrel.gov

Publication Number

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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.)

Technology Transfer Activities

Technology-to-market or technology transfer plan or strategy

- Co-localize biohydrogen refinery to the source of feedstock and expand the use of H₂ to current biorefinery

Plans for future funding

- Expansion of feedstock portfolio beyond terrestrial biomass to potentially include waste
- Pursue opportunities to collaborate with industry to convert waste to H₂.
- Network with biofuels industry to expand the use of H₂.
- Advocate the advantages of “green” H₂ rather than fossil-fuel derived H₂.

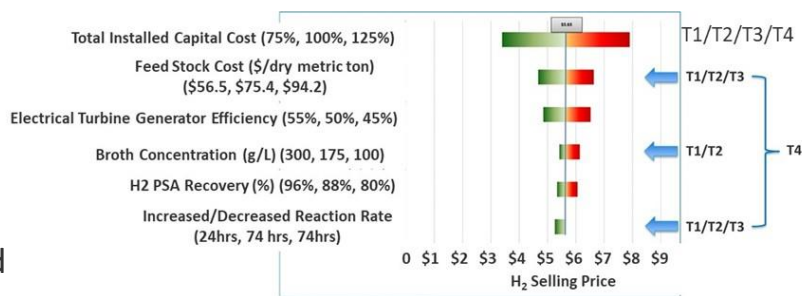
Patents, licensing

- A patent application is filed in Dec, 2021 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.
- A patent application on creating *C. thermocellum* strains capable of co-utilizing cellulose and hemicellulose is underway. The patent application will also cover genetically modified strain recently developed to utilize arabinose.

Progress toward DOE Targets or Milestones

The BioH2 consortium is setup to directly address technical barriers:

- Feedstock Cost (AY): by fully utilizing all sugars in biomass derived from both cellulose and hemicellulose;
- MEC current density and capital cost



Addressed Barrier/ Technical Parameter	2019 status at project start	2021 Target	2021 Status by Q2
Feedstock cost (AY)/ Utilization of biomass (NREL)	Utilize mainly cellulose to produce H ₂	Ferment both cellulose and hemicellulose for H ₂ production	Co-utilize cellulose & hemicellulose, which led to 33% increase in H ₂ production per unit of real DMR biomass by weight (FY21 Q2 G/NG milestone completed)
H ₂ productivity (Dark Fermentation) (NREL)	2.2 L H ₂ /L /day	3.0 L H ₂ /L /day	3.1 L H ₂ /L /day (FY21 Q2 Go/No-Go milestone completed)
High solids fermentation (LBNL)	30 g/L crystalline cellulose as model substrate	Ferment 67.5 g/L as cellulose in 150 g/L real DMR biomass (Q3-4)	Ferment 45 g/L as cellulose in 100 g/L real DMR biomass (FY21 Q2)
MEC Current Density (PNNL)	4 A/m ² (simulated effluent, acetate)	5-10 A/m ² (actual Avicel effluent, Q3-4)	2.5 A/m ² (actual Avicel effluent, high solid load fermentation, FY21 Q1-2)
H ₂ production cost (ANL)	\$58/kg-H ₂ (for Dark Fermentation only)	\$9-15/kg-H ₂ * *Assumptions: Lower bound of H₂ cost assumes the use of Zirfon as separator/diaphragm, while the higher bound assumes the use of Nafion as membrane	\$15-38/kg-H ₂ *

Special Recognitions and Awards

- NREL accomplishment in improving H₂ production by 190% by a *C. thermocellum* strain engineered to co-ferment Avicel and Xylan is selected as one of the top 10 NREL accomplishments by the HFTO program in FY20 PEMP (Performance Evaluation and Measurement Plan)
- Dr. Katherine Chou (Project PI) is nominated and selected to be a US Representative for International Energy Agency Hydrogen Implementing Agreement (IEA H2) Task 34: Biological Hydrogen For Energy and Environment. This organization pursues collaborative hydrogen R&D and information exchange among its member countries.

Publications and Presentations

Publications

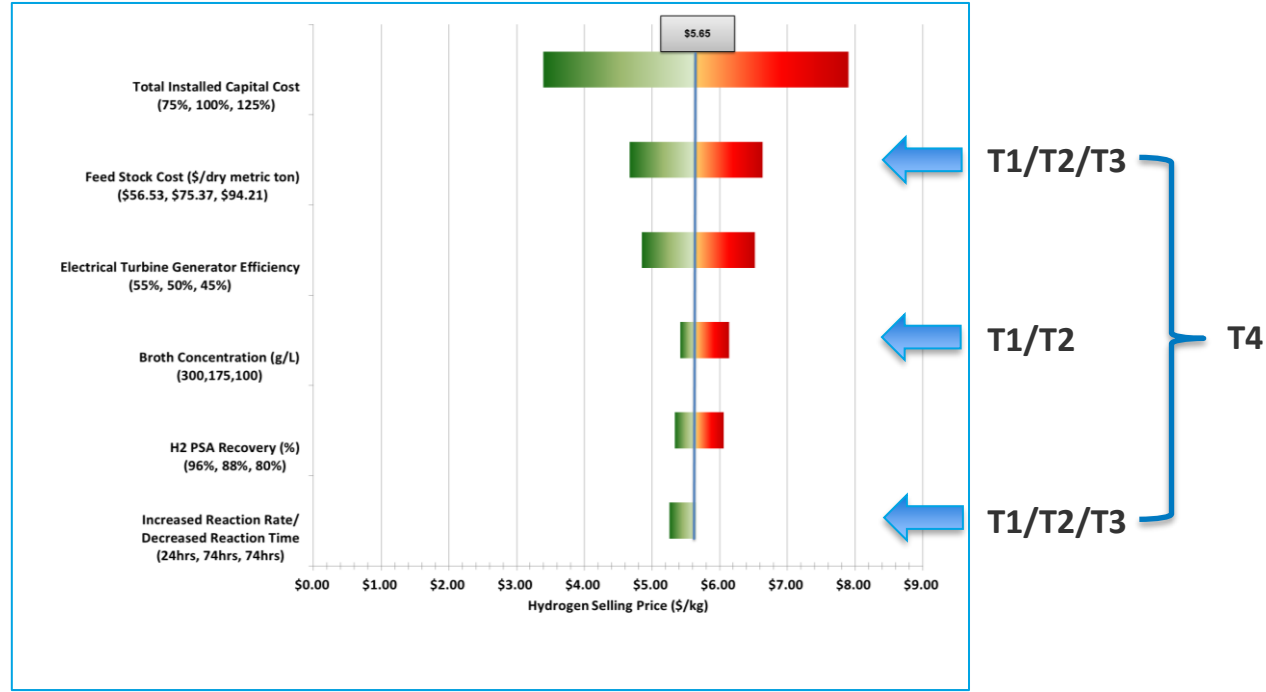
- ***Developing riboswitch-mediated gene regulatory controls in thermophilic bacteria.*** Marcano, J. G., J. Lo, A. Nag, P. C. Maness, K. C. Chou*. ACS Synthetic Biology. 2019, 8, 4, 633-640. DOI: 10. 1021/acssynbio.8b00487
- ***Integrated thermodynamic analysis of electron bifurcating [FeFe]-hydrogenase to inform anaerobic metabolism and H₂ production.*** Jay, Z., Hunt, K.A., Chou, K.J. Schut, G.J., Maness, P.C., Adams, M.W.W., Carlson, R.C. 2020. BBA Bioenergetics. 2020 Jan 1;1861(1):148087. DOI: 10.1016/j.bbabi.2019.148087
- ***Transcriptomic analysis of a Clostridium thermocellum strain engineered to utilize xylose: responses to xylose versus cellobiose feeding.*** Rangel, A.E.T., Croft, T.J., Barrios, A.G., Reyes, L.H., Maness, P.C.*, Chou, K.J.* Scientific Reports, in review.
- ***Renewable Hydrogen from Biomass Fermentation.*** Chou, K.J., Magnusson, L.R., Seibert, M., Maness, Pin-Ching. A book chapter for Encyclopedia of Biological Chemistry. Manuscript accepted and in print by Elsevier, Aug. 2021

Presentations

- Maness P.C., “**BioHydrogen Consortium to Advance Fermentative H₂ Production,**” 2019 HFTO AMR. April 30, 2019.
- Chou, K. J., “**Engineering a cellulolytic and thermophilic bacterium Clostridium thermocellum for biofuel production,**” Invited Presentation at UCLA Chemistry and Biochemistry Departmental Seminar. Jan. 10, 2020
- Chou, K.J., “**Discovery and Genetic Engineering of a Thermophilic bacterium Clostridium thermocellum for Consolidated BioProcessing,**” Invite Virtual Presentation at Boise State Chemistry and Biochemistry Departmental Seminar: Nov. 10, 2020
- Chou, K.J., “**Biohydrogen form Waste Lignocellulosic Biomass through Consolidated Bioprocessing,**” Invited virtually presentation at the “2nd Forum of Revolutions in Renewable Energy in 21st Century” (FOREN-2022) on March 22, 2022, which physically took place at Budapest, Hungary.

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

Reviewer-Only Slides

(Include this “divider” slide between those to be presented and the “Reviewer-Only” slides. These slides will be removed from the presentation file and the Web PDF files.)

Data Management Plan

- This project will maintain compliance with data management requirements of the Department of Energy and abide by the Office of Energy Efficiency and Renewable Energy data sharing and preservation requirements.
- To the greatest extent and with the fewest constraints possible, this project will make digital research data available to, and useful for, the broader scientific community, industry, and the public.
- Technical reports, journal article accepted manuscripts, software, and scientific research datasets will be submitted to OSTI through the DOE Energy Link System. Data from this project deemed appropriate for public access will be made available through the NREL Data Catalog, accessible at <https://data.nrel.gov>.
- Data in this public release will be in a machine-readable digital format (e.g., comma-delimited).
- This project will not generate or use Personally Identifiable Information (PII). Any data containing national security implications, business confidentiality, or intellectual property will not be released in accordance with all laws and DOE regulations, orders, and policies.

Critical Assumptions and Issues

The feedstock cost of lignocellulosic biomass will be reduced significantly to decrease the selling price of H₂ when produced via biomass fermentation.

- The DOE Bioenergy Technologies Office (BETO) is funding research to improve yields of high-energy crops, increasing cellulose contents, reducing recalcitrance, and improving pretreatment technologies. NREL has yielded data supporting the direct fermentation of untreated or mildly treated (DMR pretreatment) corn stover to H₂ without adding the costly enzyme cocktail, which should lower the cost of biomass feedstock. HFTO will leverage investment of DOE BETO to expedite progress.

***Clostridium thermocellum* will redirect its metabolic pathway toward more H₂ production when competing pathways are blocked by metabolic pathway engineering.**

- NREL has developed genetic tools to block the competing pathways in *C. thermocellum*. Increases in both total H₂ and specific rate of H₂ production have been observed in pathway mutants which provided the proof-of-concept for metabolic flux redirection.

Better impeller design will afford high-solid loading (up to 175 g/L) in bioreactor to reduce CapEx and OpEx.

Microbial electrolysis cells can be scaled up with reduced cost of anode and cathode materials.

- Identify promising exoelectrogenic microbes that can degrade both organic acids and proteins in the fermentation effluent will increase H₂ output.

Issues: None